Characterization and Treatment of Chromatin Remodeling Protein Deficient DDEC

by

Mackenzie Leigh Coatham

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Medical Sciences - Obstetrics and Gynecology

University of Alberta

Abstract

Some of the most aggressive endometrial cancer cases display extensive heterogeneity within the same tumor in the form of mixed undifferentiated and well-differentiated phenotypes. The emergence of dedifferentiation in higher grade endometrial carcinomas presents an obstacle for both timely and accurate diagnosis and specific and effective treatment. Even though endometrial cancers of this nature are less common, we used a collection of patient tissue and developed cell line-based models of disease using gene editing technology to discover and interrogate many features of dedifferentiated endometrial cancer or DDEC. Firstly, utilizing targeted sequencing and gene expression profiling paired with immunohistochemistry, I discovered loss of chromatin remodellers and markers of epithelial differentiation to be a defining feature of the undifferentiated portions of DDEC neoplasms. By comparing the profiles of the undifferentiated regions to their well-differentiated predecessors within the same tumor, I also demonstrated the emergence of a population of undifferentiated cells within DDEC acquires cancer stem cell-like features. Secondly, three models of DDEC with absent SWI/SNF chromatin remodelling complex subunit SMARCA4 expression, were generated and extensively characterized. I thoroughly documented how *in vitro,* SMARCA4 loss results in endometrial cancer cells exhibiting senescence but *in vivo*, SMARCA4 deficiency leads to the formation of tumors that recapitulate clinical DDEC neoplasms. I propose dysregulation of transcription factors governing epithelial cell-related gene expression as a major driver of dedifferentiation in the context of DDEC. Finally, screening of one of the isogenic pairs of gene edited endometrial cancer cells, revealed several synthetic lethal vulnerabilities specific to cells with absent SMARCA4 expression. *In vitro,* I extensively validated many of the hits and found chemical inhibition of CDK4 and EGFR both alone and in combination to be potentially effective in targeting DDEC-like cells. Preclinical animal studies demonstrated the efficacy of targeting CDK4 and EGFR may be counteracted by *in vivo* transformation of the model and stromal contribution. In its totality, the work in this thesis provides one of the first comprehensive examinations of DDEC and my development of and findings with the cell line models of this malignancy constitute a noteworthy contribution to our overall understanding of SWI/SNF deficient cancers of the gynecological tract.

Preface

Each chapter represents either a conglomeration of published manuscripts or manuscripts in preparation for submission. Author contributions are described below:

Chapter 1: Introduction

This chapter consists of an extended version of my contributions to the following review article and book chapter.

1) **Epithelial-to-Mesenchymal Transition in the Female Reproductive Tract: From Normal Fuctioning to Disease Pathology.**

Olena Bilyk, **Mackenzie Coatham,** Michael Jewer, Lynne Postovit. *Front. Oncol.* July 2017. doi: 10.3389/fonc.2017.00145.

2) **Chapter 6: Preventing phenotypic plasticity in cancer to mitigate therapy resistance.**

Mackenzie Coatham, Michael Jewer, Lynne Postovit. *Biological Mechanisms and the Advancing Approaches to Overcoming Drug Resistance.* November 2020. doi: 10.1016/C2019-0-02626-X

As a co-first author on this review and book chapter, I contributed equally to reviewing the appropriate literature, deciding on the final content and flow of the papers, designing, and making figures and finally writing and editing the manuscripts.

Chapter 2: Examination of clinical cases of dedifferentiated endometrial cancer reveals absence of chromatin remodelling proteins and loss of gynecological differentiation markers

This chapter consists of an extended version of my contributions to the following three published papers:

3) **Loss of switch/sucrose non-fermenting complex protein expression is associated with dedifferentiation in endometrial carcinomas.**

Anthony Karnezis, Lien Hoang, **Mackenzie Coatham,** Sarah Ravn, Norrah Almadani, Basile Cloutier, Julie Irving, Bo Meng, Xiaodong Li, Christine Chow, Jessica McAlpine, Kuan-Ting Kuo, Tsui-Lien Mao, Bojana Djordjevic, Robert Soslow, David Huntsman, Blake Gilks, Martin Kobel, Cheng-Han Lee. *Mod. Pathol.* January 2016. doi: 10.1038/modpathol.2015.155.

4) **Concurrent ARID1A and ARID1B inactivation in endometrial and ovarian dedifferentiated carcinomas**

Mackenzie Coatham, Xiaodong Li, Anthony Karnezis, Lien Hoang, Basile Tessier-Cloutier, Bo Meng, Robert Soslow, Blake Gilks, David Huntsman, Colin Stewart, Lynne Postovit, Martin Kobel, Cheng-Han Lee. *Mod. Pathol.* August 2016. doi: 10.1038/modpathol.2016.156.

5) **Immunophenotypic features of dedifferentiated endometrial carcinoma: Insights from BRG1/INI-deficient tumors**

Lien Hoang, Yow-Shan Lee, Anthony Karnezis, Basile Tessier-Cloutier, Noorah Almadani, **Mackenzie Coatham**, Blake Gilks, Robert Soslow, Colin Steward, Martin Kobel, Cheng-Han Lee. *Histopathology.* April 2016. doi: 10.1111/his.12989.

As an author on these papers, I mainly aided in in the writing process and assisting Dr. Lee in formulating the layout of the paper content. I also was involved in the targeted sequencing process, aiding in the identification of mutations to SMARCA4 and ARID1B in dedifferentiated endometrial carcinomas.

Chapter 3: Characterization of cell-line based models with chromatin remodeling protein deficiencies that recapitulate dedifferentiated endometrial cancer

This chapter is comprised of a manuscript being prepared for submission with the following authors:

Mackenzie Coatham, Zhihua Xu, Xiaodong Li, Guihua Zhang, Jiahui Liu, Tyler Cooper, Dylan Dieters-Castator, Joaquin Lopez-Orozco, Alan Underhill, Gilles Lajoie, Cheng-Han Lee, Lynne Marie Postovit

As the first author, I conducted most experiments and a substantial portion of the data analysis. I prepared all figures and wrote the manuscript. Xiaodong Li performed the targeted sequencing on the commercial endometrial cancer cell lines. Zhihua Xu, with my oversight aided in several of the immunoblots in addition to the β-galactosidase staining experiments. Guihua Zhang and Jiahui Liu performed *in vivo* animal experiments with my assistance and protocols were approved by the Animal Use Subcommittee at the University of Alberta (AUP00002496). Dr. Dylan Dieters-Castator and Dr. Tyler Cooper performed the mass spectrometry analysis on conditioned media in the lab of Dr. Gilles Lajoie. Dr. Alan Underhill's laboratory performed the histone mark immunofluorescence. Dr. Joaquin Lopez-Orozco as coordinator of the High Content Analysis Core at the University of Alberta performed the 10X Genomics single cell sequencing experiments.

Chapter 4: Treatment of cell line models of DDEC with a synthetic lethality based approach

This chapter is comprised of a manuscript being prepared for submission with the following authors:

Mackenzie Coatham, Zhihua Xu, Guihua Zhang, Jiahui Liu, Michael Jewer, Gabrielle Siegers, Franco Vizeacoumar, Edmund Su, Martin Hirst, Cheng-Han Lee, Lynne Marie Postovit

As the first author, I performed or directed most experiments and completed much of the data analysis. I prepared all figures and wrote the manuscript. Zhihua Xu, contributed substantially to both cell viability experiments with single drugs or inhibitors in combination as well as clonogenic growth assays. In addition, she also aided with several immunoblots. Guihua Zhang and Jiahui Liu performed *in vivo* animal experiments with my assistance and protocols were approved by the Animal Use Subcommittee at the University of Alberta (AUP00002496). Dr. Michael Jewer and Dr. Gabrielle Siegers performed flow-cytometry experiments. Dr. Franco Vizeacoumar's laboratory performed the shRNA-based synthetic lethality screen. Dr. Martin Hirst's laboratory performed RNA and ChIP-Sequencing experiments and assisted in bioinformatics analysis.

Acknowledgments

My sincerest thanks to all the current and past members of the Postovit research laboratory at the University of Alberta and Queens University. I cannot thank enough the many members of the lab who contributed to the success of this project over the years. In particular the hard work of Xiaodong Li, Guihua Zhang, Jiahui Lui and Zhihua Xu was instrumental in the progression of this scientific study and their personal support also has meant the world to me. I also had the honor of working with several amazingly talented undergraduate (Sarah Wilette) and high school students (Sarah Donald and Abby Kerner) who made substantial contributions to this thesis and whose company during the summers was so enjoyable.

I also cannot thank Dr. Lynne Postovit enough for taking a chance on a MatCH rotation student and providing me with such a rich environment to explore, learn and discover all about the world of cancer research. You are such a role model to me and your reassurance and support has made my completion of this Ph.D degree possible. I am forever grateful for the opportunities you have allowed me to experience during my time in your lab and the doors you have opened for me in the future.

I want to thank both my co-supervisor Dr. Cheng Lee and my committee member Dr. Alan Underhill for all of their input over the years which has contributed to my continuous growth as a scientist.

I also need to thank all of the support I received during the course of this Ph.D. Firstly, I want to thank the Maternal and Child Health (MatCH) program for allowing me to rotate through several labs in the Faculty of Medicine and Dentistry when I arrived at the University of Alberta. Audrey Lin and Dr. Denise Hemmings were also instrumental in making my journey as the first Ph.D student in the Department of Obstetrics and Gynecology as smooth and wonderful as possible. I have to thank CIHR, AIHS, WCHRI and CRINA for their financial support that provided me with many valuable experiences during the course of my studies. I was also fortunate to receive ample donor support for my studies not only through the university but Alberta Cancer Foundation as well, for which I'm eternally grateful.

Finally, I cannot thank my parents and husband enough for their support. It has certainly been a challenge, but I think we are all better for it. To my mom and dad, I could not be where I am today without your understanding and encouragement. I am so grateful for all your contributions that have made me into to the person I am today. To Harland, I am so fortunate to have you in my life. I cannot imagine having a different partner. Your sacrifices have not gone unnoticed, your support and empathy was instrumental in my success and your love has been unending. You were pivotal when I achieved my M.Sc degree and I'm so incredibly thankful you are now my husband as I complete this Ph.D.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AMF - autocrine motility factor
ANOVA – analysis of variance
ArchR - Analysis of Regulatory Chromatin in R
ARID1A/B - AT-rich interactive domain-containing protein 1A/B
ATP- adenosine triphosphate
BAF - BRG1/BRM-associated factor
BDNF - brain-derived neurotrophic factor
BER - base excision repair
BET - bromodomain and extraterminal
BMI-1 - B lymphoma mouse Moloney leukemia virus insertion region 1
BSA - bovine serum albumin
BWA - Burrows-Wheeler Aligner
CAFS - cancer associated fibroblasts
Camera - Correlation Adjusted Mean Rank
CDK4/6 - cyclin-dependent kinases 4/6
CDX - cell-line derived xenograft
ChIP - chromatin immunoprecipitation
CI - combination indices
COSMIC - Catalogue of Somatic Mutations in Cancer
cRNA - CRISPR guideRNA
CSCs - cancer stem cells
C_T - cycle threshold
CTCF - CCCTC-Binding Factor
DCC - difference cumulative change
DDEC - dedifferentiated endometrial carcinoma
ddPCR - droplet digital PCR
DDR – DNA damage response
DMSO - dimethyl sulfoxide
DNA - deoxyribonucleic acid
DNMTs - DNA methyltransferases
DSBs - double-stranded breaks
DTT - dithiothreitol
E2 - estradiols
EC - endometrial cancers
ECM – extracellular matrix
EGF - epidermal growth factor
EMA - epithelial membrane antigen
EMT – epithelial-to-mesenchymal transition
ENO1 – Enolase 1
ER-α - estrogen-receptor-α
ES – embryonic stem
EZH2 - enhancer of zeste homolog 2
FA - formic acid
FACS - fluorescence-activated cell sorting

FAM - 6-carboxyfluorescein
FBS - fetal bovine serum
FDR - false discovery rate
FFPE - formalin-fixed paraffin-embedded
FIGO - International Federation of Gynecology and Obstetrics
FGF - fibroblast growth factor
FMO - fluorescence minus one
FTO - fat mass and obesity-associated
gDNA - genomic DNA
GEMs - Gel Bead-In Emulsions
GLI1 - GLI Family Zinc Finger 1
GOF- gain of function
GSEA - gene set enrichment analysis
HATs - histone acetyltransferases
HDAC - histone deacetylases
HGF - hepatocyte growth factor
HR - homologous recombination
HRP - horseradish-peroxidase
IC_{50} - half maximal inhibitory concentration
ICL - cross-link repair
IDH - isocitrate dehydrogenases
IGV - Integrated Genome Viewer
iPSCs - induced pluripotent stem cells
IV - intravenous
JmjC – Jumonji C
KDMs - histone lysine demethylases
KLF17 - Kruppel-like factor 17
KO – knockout
LFQ - label free quantification
lncRNAs - long non-coding RNAs
MAPK- mitogen-activated protein kinase
MDS - Multidimensional scaling
MEM - minimal essential media
MFI – median fluorescence intensities
miRNAs – microRNAs
MLH1 - mutL protein homolog 1
MLL - mixed-lineage leukemia
MMR - mismatch repair
MNase - micrococcal nuclease
MSCs - mesenchymal stem cells
MSigDB - Molecular Signatures Database
MSH2 - mutS protein homolog 2
MSH6 - mutS protein homolog 6
MSI - microsatellite instability
MSI-H - microsatellite instable hypermutated
MWCO - molecular weight cutoff

NER - nucleotide excision repair
NES - normalized enrichment score
NHEJ - non-homologous end-joining
NHR - nuclear hormone receptors
NID1 - Nidogen 1
NSCLC - non-small-cell lung cancer
NSG - NOD.Cg-PrkdcscidIl2rg
NUPR1 - Nuclear Protein 1
OC - ovarian cancers
PARP - poly-(ADP-ribose) polymerase
PBAF - polybromo-associated BAF
PBS - phosphate buffered saline
PD-1 - programmed cell death-1
PD-L1 - PD-1 ligand 1
PDX - patient-derived xenograft
PFA - paraformaldehyde
PMS2 - postmeiotic segregation increased 2
PR - progesterone receptor
PRC2 - polycomb repressive complex 2
PTMs - post translational modifications
QQ - quantile-quantile
RANK - Receptor activator of nuclear factor-κB
Rb - retinoblastoma protein
REST - RE1 Silencing Transcription Factor
RIPA - radioimmunoprecipitation assay
RNA - ribonucleic acids
RNAi – RNA interference
RNP – ribonucleoprotein
RPKM - reads per kilobase per million mapped reads
ROS - reactive oxygen species
RT-qPCR - real-time quantitative PCR
SAHF - senescence-associated heterochromatin foci
SASP - senescence-associated secretory phenotype
SCCOHT - small cell cancer carcinoma of the ovary, hypercalcemic type
SCNA - somatic copy number alterations
SDS - sodium dodecyl sulfate
SET - Su(var)3-9, Enhancer of Zeste, Trithorax
scATAC - single-cell Assay of Transposase Accessible Chromatin
scRNA - single-cell RNA
sgRNA - single guide RNA
shRNA - short hairpin RNA
siRNA - small interfering RNA
SL - Synthetic lethality
SRA - steroid receptor activator
SWI/SNF - SWItch/Sucrose Non-Fermentable
TBST - TBS + 0.1% Tween

TCGA - The Cancer Genome Atlas Project
TET - Ten-Eleven Translocation
TF – transcription factor
TGF-β - transforming growth factor β
tracrRNA - trans-activating CRISPR RNA
TrkB - tyrosine kinase B
TUNEL - terminal deoxynucleotidyl transferase dUTP nick end labeling
UBE2C - ubiquitin-conjugating enzyme E2 C
UMAP - uniform manifold approximation and projection
UTRs - untranslated regions
WHO - World Health Organization
WT – wildtype

1 Introduction

1.1. Tumor Heterogeneity as a Driver of Therapy Resistance and Metastasis

Developments in cancer treatment strategies have improved outcomes for most cancer patients, and ongoing research continues to build on this foundation to create safer and more effective treatments. Currently, many patients still experience disease recurrence and progression, which are ultimately fatal. In patients that succumb to their disease, there is often an initial partial or apparent full response to treatment, but regrowth at the primary or secondary sites suggests the existence of heterogeneity, which results from tumors harboring numerous distinct populations of cells with divergent therapeutic vulnerabilities. With the advent of high-throughput sequencing, intertumoral heterogeneities, such as differences in mutational landscapes, have been documented between patients with the same types of tumor (1, 2). Intratumoral heterogeneity, on the other hand, is defined by the difference between cancer cells from a tumor that have undergone phenotypic or functional changes that are a result of genetic variation and alterations to gene expression (1, 3). Most cancer patients die from metastases that are a direct result of tumors acquiring protumorigenic phenotypes driven by genetic and phenotypic changes that prevent the complete eradication of cells (4). Moreover, growing evidence suggests that heterogeneity may also drive evasion of the immune system (4). Hence, more effective cancer treatment needs to factor in such intratumoral heterogeneity.

Many mechanisms have been proposed to explain the origin of tumor heterogeneity, such as epigenetic alterations, and deregulated signaling (5, 6). In the past, two theories emerged to explain intratumoral heterogeneity: clonal evolution and

cellular hierarchies sustained by cancer stem cells (CSCs) **[Figure 1.1]** (7). The model of clonal evolution arose from the observation that any single cancer cell can acquire genetic alterations stochastically over time **[Figure 1.1A]** (6). Those clones that acquire genetic mutations that provide a growth advantage persist within the tumor **[Figure 1.1A]** (1, 4, 8-12). The accumulated alterations result in both tumor progression and growing heterogeneity concomitant with metastatic potential and therapy resistance **[Figure 1.1A]** (2, 13, 14). This theory suggests that every cell within a tumor has an equal chance of generating a neoplasm and these transformed cells continue to possess the potential to keep changing their phenotype in response to stimuli by tolerating sustained genetic mutations **[Figure 1.1A]** (2, 15, 16).

In contrast, the hierarchical CSC-driven model was developed from the idea that only a subset of CSCs within a tumor are capable of long-term growth by altering their differentiation programs to foster self-renewal **[Figure 1.1B]** (1, 3, 13, 14). These subpopulations of tumor-initiating cancer cells possess the ability to both self-renew and differentiate into phenotypically distinct progeny, similar to the hierarchical organization of healthy tissues generated from normal stem cells **[Figure 1.1B]** (4, 16-19). Evidence suggests that CSCs are highly tumorigenic and responsible for giving rise to tumor heterogeneity, metastatic potential, and therapy resistance **[Figure 1.1B]** (2, 14).

While CSC-driven hierarchies are a source of phenotypic heterogeneity, one must also reconcile that tumors harbor numerous genetically distinct clones. To unite this hierarchical model one must also incorporate stochastic mutational events that enable dedifferentiation to a more CSC-like state that then generate new hierarchies (20). In this hybrid model **[Figure 1.1C]**, plasticity is acquired like any other phenotype is, through the

combination of mutational and signaling changes, however, phenotypic plasticity is unique in that it provides the means of altering the cellular-identity of the cell to adapt to new microenvironments or to therapy. This ability to 'switch fate' – moving between differentiated and undifferentiated phenotypes – and the concurrent alterations in the epigenome are referred to as cellular plasticity **[Figure 1.1C]**. While plasticity has been noted in neoplasia, it also occurs in healthy cells such as fibroblasts, driven by cues such as wound healing and hypoxia (1, 2, 14, 16, 21). However, unlike healthy systems, wherein normal phenotypes are reacquired upon a return to homeostasis, tumors appear to accumulate and maintain plasticity during progression. This unconstrained plasticity likely arises from persistent microenvironmental stresses and epigenome dysregulating mutations. Newer evidence shows the fluidity of phenotypes generated by acquired plasticity, having demonstrated that cancer cells can switch between non-CSC and CSC-like states in response to intrinsic and extrinsic signals **[Figure 1.1C]** (1, 2, 5, 16, 22). The acquisition of plasticity and the adaptability that it confers provides a means by which cancers cells can evade treatment or habituate to new environments during growth and metastasis.

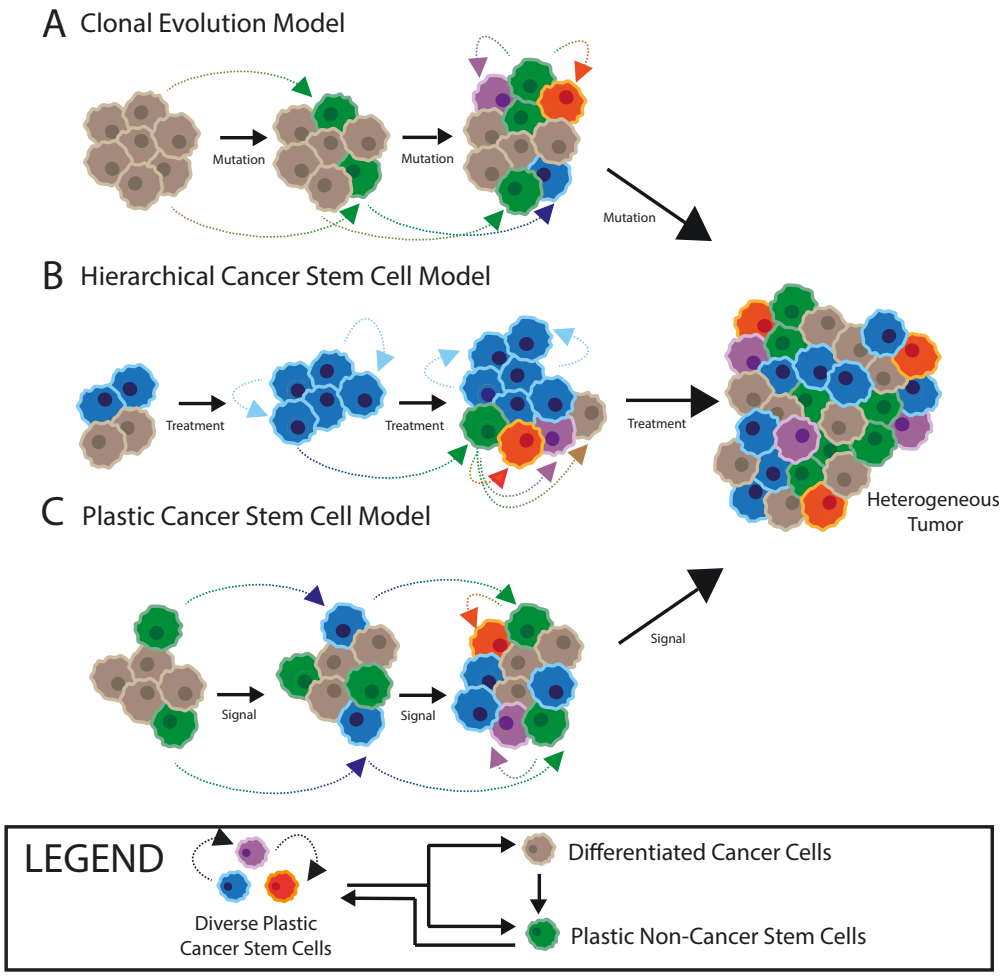


Figure 1.1. Three models that have been proposed to explain intra-tumoral heterogeneity. A) Clonal evolution **B)** Cellular hierarchies sustained by CSCs and **C)** A hybrid plasticity model incorporating CSC-driven hierarchies with stochastic mutational events.

1.2. Epigenetic Mechanisms of Gene Regulation

Through accumulated mutations and hierarchical development of phenotypic diversity, the derivation of cancer cells with distinct properties requires that unique and heritable gene expression patterns be established for each subset of cells. There is an intrinsic connection between the concepts of plasticity, cell fate, differentiation, dedifferentiation, and epigenetics. Alterations to the epigenome are how gene expression

signatures specify cell types, and thus, the epigenome is the mechanism by which plasticity is maintained, and differentiation coordinated. The epigenetic mechanisms that regulate plasticity and differentiation – activating or silencing genes through covalent modifications of deoxyribonucleic acid (DNA) and histones – drive different phenotypic results depending on cellular context **[Figure 1.2]**.

DNA methylation, the enzymatic addition of methyl groups to the cytosines of CpG dinucleotides, was first observed as an essential developmental phenomenon important in the silencing of the X chromosome which, soon after, was considered as a broader means of epigenetic silencing (23-26). DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which are recruited to protein complexes by transcription factors, chromatin modifiers, and non-coding ribonucleic acids (RNA) (reviewed in (27)). Likewise, demethylases such as Ten-Eleven Translocation (TET) enzymes reverse this reaction. Low methylation is associated with the accessible DNA of euchromatin.

The second major form of epigenetic regulation occurs at the level of histone modifications. Based on the X-ray structure, it was discovered that histones within the nucleosome complex possess basic histone amino (N)-terminal tails that could facilitate interactions between adjacent nucleosomes (28). Numerous histone modifications have been discovered, including methylation, acetylation, and phosphorylation, among others (a more extensive list is reviewed in (29)). Chromatin is a dynamic structure in which histones are constantly remodeling through processes of nucleosome dissociation, reassociation, movement, and histone dimer exchange (30).

Briefly, the process of histone acetylation is catalyzed by histone acetyltransferases (HATs), and the removal of these marks is governed by histone

deacetylases (HDAC). The acetylation of histones decreases the strength of the interactions between nucleosomes, and between nucleosomes and DNA by neutralizing the positive charge of lysine (31, 32). Acetylation is a significant factor in maintaining an open chromatin structure that is transcriptionally permissive. In stem cells, that are poised to undergo dramatic epigenetic changes, the coordination of HATs and HDACs is fundamental to either the maintenance of pluripotency and epigenetic plasticity or fate commitment (33-35). In one experiment, an epigenetic signature of fate commitment was discernible even before changes were detected at the RNA or protein level (35).

Unlike acetylation, methylation does not alter the charge of the histone; thus, it is unlikely to elicit effects through electrostatic interactions (36). Since the initial discovery of histone methylation, many proteins that can recognize and bind methylated histones have been identified, and it is the binding and activity of these proteins that determines the subsequent expression of genes by regulating chromatin structure and DNA accessibility (37-39). Methylation occurs at both lysine and arginine, with each lysine receiving up to three methyl groups, referred to as mono-, di- and trimethylation. The methylation of different residues also produces different effects on gene expression (40-50). Marks generally associated with activation include H3K4, H3K36, and H3K79, whereas H3K9, H3K27, and H4K20 show a higher association with silenced genes (40-49). Writers of activating marks include Su(var)3-9, Enhancer of Zeste, Trithorax (SET) and mixed-lineage leukemia (MLL) (51). Histone lysine demethylase (KDMs) family members remove these marks (51). Complexes like the polycomb repressive complex 2 (PRC2), methylate histones at lysine 27, and Jumonji C (JmjC) catalytic domain containing proteins like JMJD3/KDM6B, remove said marks (51).

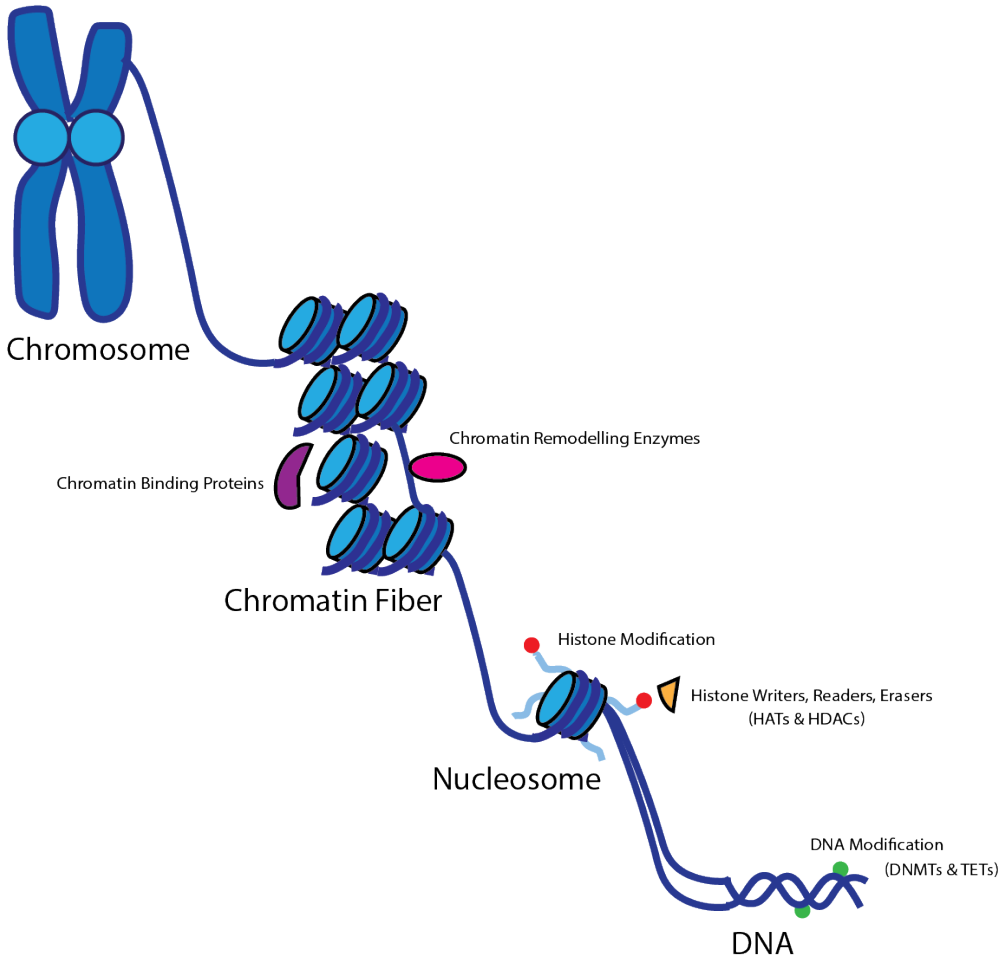


Figure 1.2. Broad overview of the epigenetic regulation of gene expression.

1.3. Acquisition of Phenotypic Plasticity through Mutations to Epigenomic Regulators

Since plasticity is associated with alterations to the epigenomic landscape, it is logical to posit that mutations in key epigenomic regulators may drive plasticity and cancer progression. To ensure tissue and cell-state-appropriate gene activity, the chromatin landscape must exist in a state of homeostasis that is tightly regulated by the combined action of PRC-family repressors, SET-family activators, and nucleosome remodelers (52, 53). In cancer, mutations observed in these genes disrupt this homeostasis. Accordingly, a growing number of studies have shown that mutations in genes governing epigenomic

phenomena such as DNA methylation, histone modifications, and nucleosome occupancy commonly occur in cancers (54). Moreover, mutations in epigenetic regulators commonly result in uncontrolled self-renewal and formation of CSCs (55, 56).

1.3.1. Mutations Affecting DNA Methylation Writer and Erasers

DNA methylation is catalyzed by DNMTs to promote the formation of heterochromatin. This family of proteins is indispensable in mammalian development (57, 58). As techniques for testing the effects of DNA methylation on gene expression have become more sophisticated, researchers have demonstrated that methylated DNA silences genes through reducing affinity of transcription factors for promoters, and by complexing with heterochromatin remodelers to alter chromatin structure, the effects of which are most potent in areas of dense CpG dinucleotides like CpG islands (59-64). Though evidence of these processes is accumulating rapidly, much of the mechanism remains unknown. It is also worth noting that there are some known exceptions to methylation being a negative regulator of gene expression (65-69). With the advent of bisulfate genome sequencing, it is possible to assess the epigenetic state of the whole genome in cells of different lineages. DNA methylation signatures for pluripotent stem cells, progenitor cells, and somatic stem cells have been identified (70). These signatures adhere to the paradigm that predicts stage-dependent hypomethylation of developmental and tissue-specific genes coordinating expression and hypermethylation and subsequent silencing of genes associated with precursors and other cell lineages (71).

DNA methylation contributes substantially to global chromatin restriction in cancer as methylation patterns are highly distorted from those found in healthy cells (53) **[Figure 1.3]**. Cytosines in CpG islands become hyper-methylated, while hypo-methylation exists

in GC-poor regions (53). Many tumors with diverse phenotypes have been demonstrated to possess CpG island hypermethylation, which leads to the silencing of both tumor suppressor and DNA repair genes (72, 73). Loss of the DNA methyltransferases DNMT3A, and DNMT3B have been shown, especially in leukemia, to contribute to CSC formation and maintenance (74, 75) **[Figure 1.3]**. Evidence from gastric, colon, and breast cancers has demonstrated that dysregulation of the DNA methylation patterns on tumor suppressors and oncogenes is a critical step in the formation of CSCs. The activation of stem cell and epithelial-to-mesenchymal transition (EMT)-related genes can be achieved through compromised DNA methylation patterning, as well (76). Disrupting DNA methylation at multiple levels seems to lead to the expansion of CSCs, a phenomenon witnessed in leukemia and solid tumors (77, 78). For example, isocitrate dehydrogenases (*IDH*) gain of function (GOF) mutations are incredibly common in glioma and leukemia. The mutated proteins produce metabolites that inhibit hydroxylases like TET that demethylate DNA (53, 79-81) **[Figure 1.3]**. Hypermethylation of tumors as a consequence of *IDH* mutations will also result in perturbed binding of CCCTC-Binding Factor (CTCF), a DNA-binding protein that is critical for higher-order chromatin structure and partitioning of the genome into functional domains or chromosomal loops that are regulated by specific enhancers (53) **[Figure 1.3]**. In *IDH* mutant tumors, large numbers of these chromosomal loops are perturbed, allowing for proximal gene expression and eventual loss of differentiation and emergence of CSCs (53) **[Figure 1.3]**. Dysregulation of DNA methylation coordinates the transcriptional repression of tumor suppressors and the activation of disease-promoting gene signatures like those associated with EMT and stem cells (53).

1.3.2. Mutations Affecting Histone Modifications

Histone modifications are the most complex means of regulating the epigenome. The number and variety of combinations makes histone modifications a source of versatile regulatory control, but also means that expression is the result of the sum of all post-translational modifications. Numerous positional variables also affect what role histone methylations have on gene expression. In embryonic stem cells, H3K4me3 found near a gene's promoter facilitates transcription; however, in the presence of H3K27me3, forming what is called a bivalent promoter, the combination forms a low expressing promoter that is poised for higher expression in response to environmental cues. Other post-translational modifications like H2B ubiquitylation act synergistically and facilitate increased writing of other marks like H3K4 methylation (82-85). Histone methylation comprises a complex code responsible for the regulation of many genes; however, the combinatorial effects of multiple modifications in addition to the requirement for the recruitment of protein complexes means that the mechanism by which any one gene is regulated in a specific cell will require precise investigation.

Enhancer of zeste homolog 2 (EZH2), the catalytic subunit of the PRC2 and writer of H3K27 methylation, is vital in a variety of malignancies to preserve the potential for self-renewal in a subset of cancer cells (86). *EZH2* GOF mutations are commonly observed in lymphoma and melanoma, where expansive H3K27 tri-methylation takes place, resulting in a cellular state with restricted expression of differentiation genes (53, 87) **[Figure 1.3]**. In breast cancer and B cell lymphomas, hyperactivation of EZH2 is sufficient for malignant transformation and depletion of active chromatin at loci encoding for terminal differentiation genes (88, 89). Loss of EZH2 in myeloid malignancies results

in stemness-related transcriptional programs (89, 90). Demethylases or modifying enzymes that oppose the activity of PRC2 – KDM6A/B, p300, MLL, and AT-rich interactive domain-containing protein 1A/B (ARID1A/B) – demethylate H3K27, acetylate H3K27 or methylate H3K4, respectively (52, 91) **[Figure 1.3]**. In numerous cancers, the abovementioned proteins are inactivated, leading to a shift in the balance of chromatin towards an overall more repressive state, impeding the appropriate engagement of promoters or enhancers needed for differentiation (92-95).

Malignant cells can also possess chromatin that is in a more plastic state, capable of switching between gene expression programs to facilitate adaptation (53). KMT2A/MLL is a histone methyltransferase that in several forms of leukemia such as acute myeloid leukemia and acute lymphoid leukemia, undergoes a translocation event forming an oncogenic fusion protein (96, 97). The ability of KMT2A/MLL to modify chromatin, allowing access to enhancer regions, is hijacked in MLL fusion proteins to allow already committed hematopoietic stem cells to reprogram themselves (98-100) **[Figure 1.3]**. The large number of KDMs within cells have been implicated in cancer as deregulation of their activity can bestow plasticity and facilitate reprogramming (101-103) **[Figure 1.3]**. High levels of KDM6 in glioblastoma stem cells aids in their survival post drug treatment through the acquisition of a more embryonic-like developmental state (104). The deregulation of histone methylation alters the expression patterns of cancer cell sufficiently to cause differentiation and the acquisition of plasticity. Mutations to regulators of the epigenome are not the only manner in which widespread changes at the epigenetic level can be induced, and differentiation programs in cancer cells can be altered. The linker histone H1.0 is not only expressed at different levels in numerous cancer types but

also within tumors, with differentiated cells possessing higher levels of histone H1.0 than cancer cells expressing stem cell markers (105). Histone H1.0 expression induces differentiation of non-tumorigenic cells through the silencing of self-renewal genes (105). In pediatric cases of glioblastoma, histone H3 is frequently mutated (106). Deep sequencing efforts revealed a K27M GOF substitution is most commonly observed (107). The presence of this mutation on Histone H3 impairs the action of the PRC2 from laying down the repressive histone H3 lysine 27 trimethylation mark across the genome (108). This blockade of repressive writing, in turn, leads to the establishment of an oncogenic self-renewal pathway reviving precursor cells from earlier in neural development (109). Whether the dysregulation of the epigenome is the result of the altered abundance of individual marks at specific loci or compositional changes to nucleosomes, the consequences are disruptions to differentiation and ultimately self-renewal.

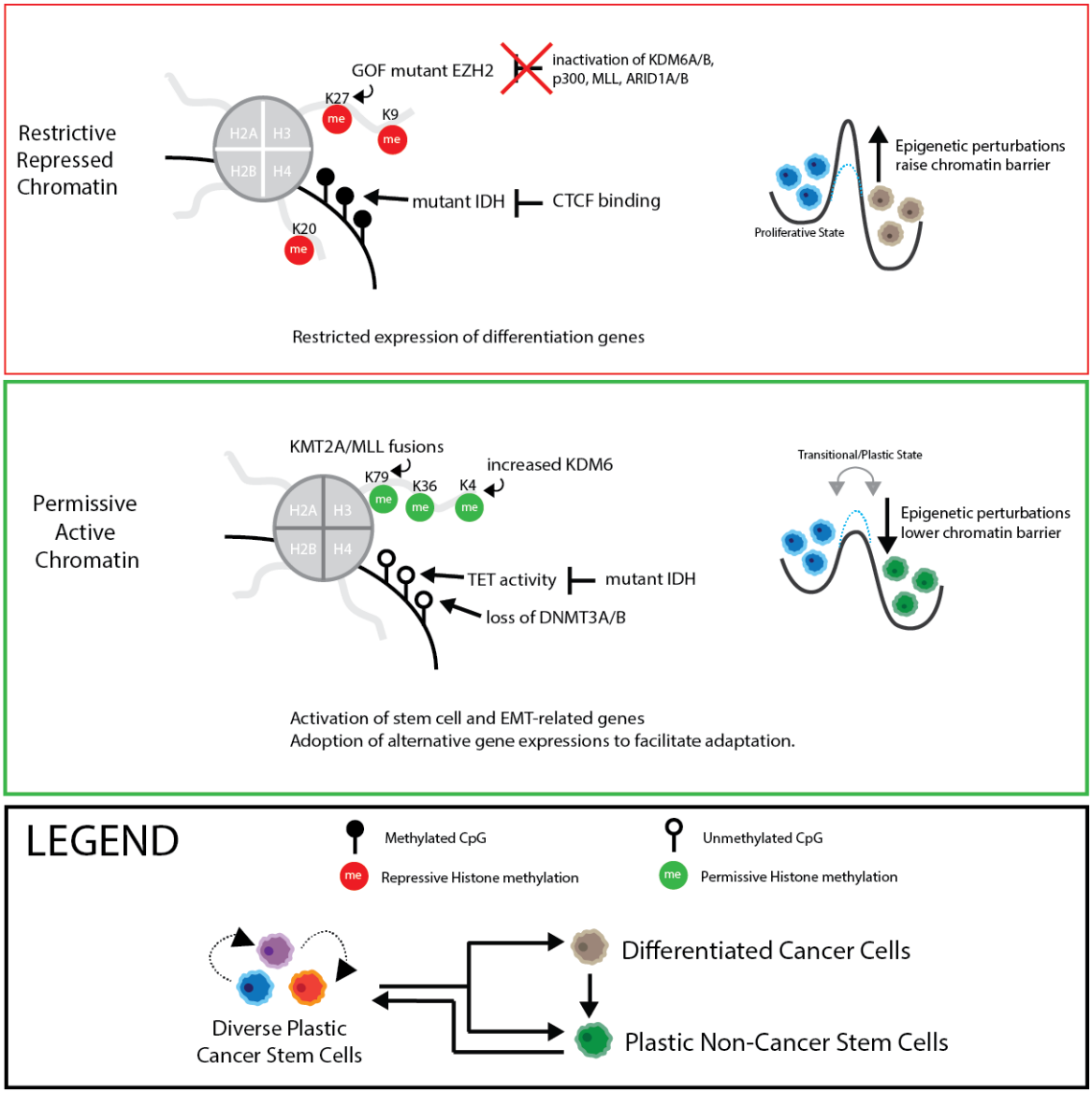


Figure 1.3. Mutations in genes governing epigenomic phenomena such as DNA methylation, histone modifications, and nucleosome occupancy commonly occur in cancers, resulting in disruptions to tissue and cell-state appropriate gene activity. Some factors associated with the acquisition of restrictive repressed chromatin wherein differentiation is suppressed include EZH2 GOF mutants which methylate H3K27, IDH mutants that impede CTCF binding and the loss of demethylases and chromatin modifying enzymes that oppose PRC2. Activation of stem cell and EMT-related gene expression through permissive active chromatin is achieved through loss of DNMT3A/B, GOF IDH mutants which influence TET activity and increased lysine demethylase activity.

1.3.3. Mutations in Chromatin Remodeling Genes

The SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complexes are vital to the differentiation process of many cell types (110). These complexes consist of approximately 9 to 12 proteins, with some subunits (SMARCA4/BRG1 and SMARCA2/BRM) possessing adenosine triphosphate (ATP)-dependent nucleosome remodelling activity (111-114). The complex normally exists in a few variations in mammalian cells including the BRG1/BRM-associated factor (BAF) and polybromo-associated BAF (PBAF) (113, 115, 116) **[Figure 1.4]**. The core subunits SMARCB1, SMARCC1, SMARCC2 and SMARCD proteins bind the more variable subunits to target assembly and define the regulatory roles of the complex (113, 114) **[Figure 1.4]**. The targets of SWI/SNF complexes are highly dependent on cell types, where they coordinate with transcription factors to activate lineage-specific genes (117, 118). The ATP-dependent chromatin remodeling complexes regulate chromatin packing by restructuring the nucleosome through sliding and ejecting actions, creating nucleosome depleted regions that are essential for the regulation of transcription (113, 119-121). Although SWI/SNF complexes were discovered based on their role in transcriptional activation, SWI/SNF complexes in mammalian cells also contribute substantially to transcriptional repression (122). Their ability to shift nucleosomes allows them to regulate transcription dynamically (123). In embryonic stem (ES) cells, the composition of SWI/SNF complexes is unique, allowing for the sustainment of properties of pluripotency (122). Inactivation of core subunits, SMARCA4 and ARID1A in ES cells results in their differentiation and substantial defects in self-renewal (124). Overexpression of SMARCA4 in fibroblasts results in their reprogramming into induced

pluripotent stem cells (iPSCs), most likely through enhanced binding of Oct4 to target genes (125). SMARCA4 has also been implicated in the repression of neuronal-specific genes in human neuronal stem cells due to its synergistic interaction and regulation of RE1 Silencing Transcription Factor (REST), a zinc finger transcription factor (126).

These studies in the context of developmental biology established the SWI/SNF complex as a vital regulator of self-renewal and plasticity and hinted at how mutations to SWI/SNF complex proteins may result in disruption of the balance between self-renewal and differentiation in cancer (122). Key targets of SWI/SNF complexes that have emerged include p16, which influences the phosphorylation status of Retinoblastoma protein (Rb), thereby affecting cell cycle progression and cellular differentiation (122). SWI/SNF complexes are also capable of interacting directly with Rb and repressing E2F and other genes downstream of Rb (127). The transcription factor c-Myc also associates with the SWI/SNF complex, heavily influencing gene expression programs, especially during differentiation (122, 128). Numerous nuclear hormone receptors (NHR) are also capable of interacting with SWI/SNF complexes (129). NHRs regulate gene expression that is induced in the presence of hormones and, in turn, affect cell proliferation and differentiation status (122). SWI/SNF complexes act as important facilitators of epigenetic reprogramming by inducing the tumorigenic signalling of plasticity factors, oncogenes, and cell cycle regulators.

Inactivating mutations in subunits of the SWI/SNF chromatin remodeling complex occur at a high frequency, approximately 20% in numerous cancers (122, 130). Across the numerous mammalian SWI/SNF genes, a wide range of mutations have been observed, most likely due to the fact that expression levels and even function of the

subunits changes across cancer types due to cell types (93). Presently, at least nine subunits of the SWI/SNF chromatin remodeling complex are recurrently mutated with specific subunits mutated in different malignancies across the body (131). In pediatric rhabdoid tumors, SMARCB1 loss has been implicated in the induction of CSCs through altered Hedgehog signalling (132). GLI Family Zinc Finger 1 (GLI1), a member of the Hedgehog pathway, is vital to differentiation, and many subunits within the SWI/SNF complex have been shown to interact with GLI1 and promotor regions to which it binds (132, 133). SMARCB1 loss in pediatric rhabdoid tumors reprograms cells at the transcriptional level and promotes pro-tumorigenic signalling, while blocking differentiation (133, 134). The SWI/SNF complex keeps c-Myc levels elevated through regulation of enhancer regions and, as a result, maintains the self-renewing CSC population in mixed lineage leukemia (135). Long non-coding RNAs (lncRNAs) capable of associating with SMARCA2 activate transcription and lead to tumorigenesis and CSC self-renewal in the liver (136). ARID1A/BAF250, an accessory subunit, plays a role in liver tumorigenesis but has also been identified as a driver of CSC generation in bladder cancer (137). Interestingly, mutations in SWI/SNF complexes have been discovered in numerous types of gynecologic cancers **[Figure 1.5]** (131). Not only do these mutations arise at various stages of tumor development and impact different subunits with the complex, it appears as if they possess distinct roles in each tumor type (131). In small cell cancer carcinoma of the ovary, hypercalcemic type (SCCOHT), inactivation of SMARCA4, is considered to be a driving event whereas inactivating mutations to SMARCA4, SMARCB1 and ARID1A in endometrial cancer appear to occur later and potentially serve to promote cancer progression (131). A more in-depth investigation is

still required to fully understand the variety of mechanisms employed by SWI/SNF chromatin remodelling complexes to facilitate the gene expression needed for oncogenic reprogramming. What is becoming clear, is that these complexes that exchange nucleosomes are dysregulated and act to direct CSC promoting gene expression.

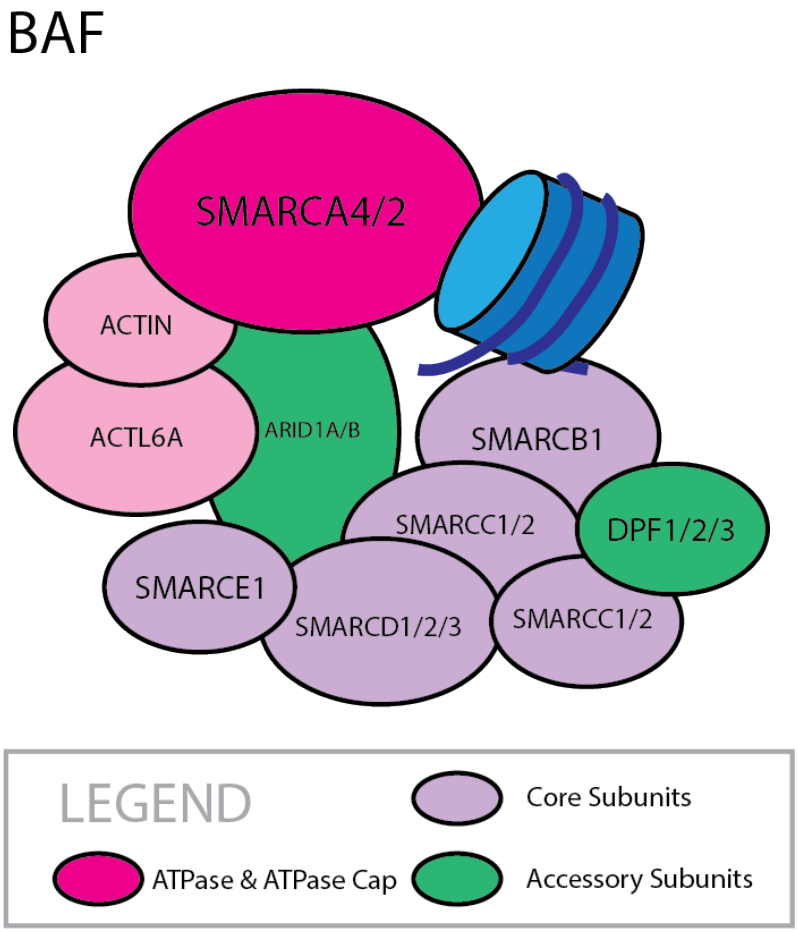


Figure 1.4. Cartoon representation of the BAF complex SWI/SNF chromatin remodeling complex. Based on the crystal structure published by He *et al.* (121) the nucleosome is sandwiched by SMARCA4/2 and SMARCB1. The canonical BAF complex also contains a mutually exclusive ARID1A or ARID1B which contrasts with the PBAF complex which incorporates ARID2, PBRM1 and BRD7 instead.

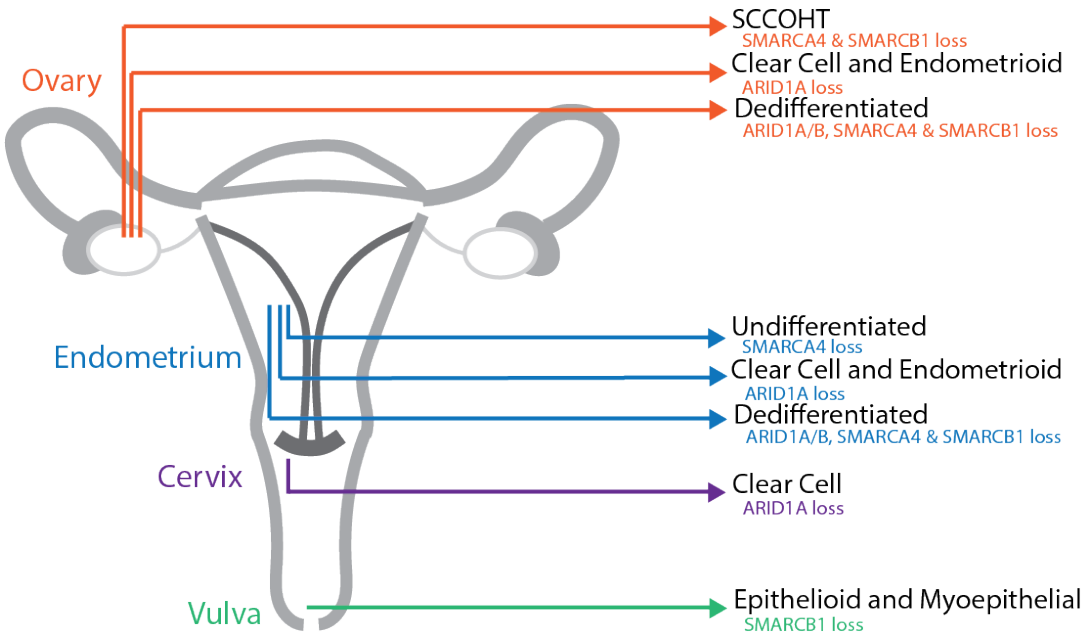


Figure 1.5. SWI/SNF mutations that lead to protein loss in gynecological cancers. Adapted from Wang *et al* (131).

1.4. Stress Induced Acquisition of Plasticity

Increasing evidence indicates that the presence of mutant clones alone may be insufficient for cancer development (7). For most cancers to progress, it is necessary for alterations to the epigenome to occur, many of which are mediated by the tumor microenvironment, leading to the acquisition of cellular plasticity. These factors include aspects of the microenvironment like hypoxia, secreted factors and extracellular matrix (ECM), as well as therapies like radiation and chemotherapy **[Figure 1.6]**. Each of these features has been shown to promote the acquisition of CSC-like phenotypes **[Figure 1.6]**. In this section, we shall review mechanisms of stress-induced plasticity with an emphasis on genotoxic and therapy-induced plasticity **[Figure 1.6]**. We will also discuss how the senescence-associated secretory phenotype (SASP) may mediate stress-induced plasticity **[Figure 1.6]**.

1.4.1. Plasticity Driven by the Tumor Microenvironment

Interactions with the tumor microenvironment are becoming increasingly important players in the acquisition of stemness by tumor cells. Carcinogenesis requires reciprocal interactions between cancer cells and microenvironmental components such as the extracellular matrix, fibroblasts, vasculature-associated endothelial cells, immune cells, and in specific contexts, adipose cells (138). While not fully understood, the epigenetic landscape of these cells may play a vital role in the maintenance of abnormal microenvironments suitable for cancer development. Tumors from the brain were the first to be documented to have their CSC stimulated by surrounding endothelial cells (139). The nitric oxide produced by the tumor endothelium can activate the Notch signalling pathway resulting in the emergence of stem-like characteristics (140). Many cancers exhibit CSC-like phenotypes in response to surrounding stromal cells secreting EMT or Nodal/Activin signalling factors (138, 141, 142). Cancer-associated fibroblasts can also induce CSCs in colorectal cancer through secretions, which, in turn, stimulate Wnt (143). In breast cancer cell lines, the bivalent marks at the promoter of EMT transcription factor ZEB1 primed *ZEB1* for expression (144). When the cells were exposed to TGFβ produced by cancer-associated fibroblasts the repressive H3K27me3 was lost (144). The increase in expression that resulted induced CSC formation and EMT (144). In breast cancer, *TNF*α and IL-6 from surrounding immune cells can also upregulate mesenchymal gene signatures (142, 145). The microenvironment has a profound capacity to enhance phenotypic plasticity through signalling cascades that culminate in epigenetic alterations to gene expression **[Figure 1.6]**.

1.4.2. The Senescence Associated Secretory Phenotype and Plasticity

The protein composition of the microenvironment including but not limited to ECM, growth factors, proteases, and inflammatory cytokines exert profound effects on cancer cells. A subset of cancer cells, senescent cells possess a profound ability to alter the contents of the tumour microenvironment. Senescence is defined as a cellular state in which irreversible cell cycle arrest and apoptosis resistance occur. Senescence results from a variety of external stimuli (146). In cancer, senescence can be induced by mutation-causing genotoxic insult and microenvironmental stresses such as hypoxia and therapy. The presence of SASP has been demonstrated to contribute to tumor recurrence and metastasis (146-149). At the epigenetic level, senescence requires substantial global genome reorganization, wherein Rb-E2F complexes recruit H3K39 methyltransferases to form repressive heterochromatin at E2F target genes to prevent entry into the S-phase [**Figure 1.6]** (150, 151). Since senescent cells undergo such extensive changes to their chromatin landscape, many of their cellular functions are reprogrammed epigenetically **[Figure 1.6]** (146). Maintaining a long-term senescent state can foster cancer development, particularly in the presence of chemotherapy. Cells that possess the SASP are capable of inducing cellular reprograming in adjacent cells through secreting high levels of IL-6 (148, 152-154). In mice, it has been demonstrated that transient exposure to the SASP causes the dedifferentiation of keratinocytes into stem cells (155). Sustained exposure to the SASP will eventually activate senescence in surrounding cells after increasing the expression of markers of stemness (146). It appears that senescence is most influential in establishing plasticity through eliciting functional changes in neighboring cells (146). Senescent cells can enhance reprogramming in neighboring cells

by secreting Yamanaka factors (Oct4, Sox2, c-Myc, KLF4) through their SASP (151). Wnt signalling is significantly enhanced through H3K4me3 and H3K27ac and remains constitutively on in CSCs after their exit from a senescent state, contributing to their role as tumor-reinitiating cells **[Figure 1.6]** (151, 153). Interestingly, in pluripotent stem cells, the senescent phenotype and its regulators block reprogramming from taking place (151, 156). It is hypothesized though that the stem-like nature achieved by senescent cancer cells could actually be a result of an epigenetically induced bivalent condition in which both terminal arrest and latent stemness are capable of existing simultaneously (151). The highly remodelled state of chromatin evident in senescent cancer cells is most likely capable of controlling both senescence and stemness, eventually reducing senescence-related gene expression until the barrier towards existence in a stem cell state is overcome (153, 157).

1.4.3. Genotoxic Stress and Plasticity

Genotoxic stress from reactive radicals, radiation, and toxins can inflict DNA damage (158). Cells have evolved conserved mechanisms of prevention and response to DNA damage such as checkpoints, cell cycle arrest and programmed cell death in order to protect cells from mutations and the eventual onset of malignancy (159). Cells possess multiple DNA repair mechanisms, including base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), and cross-link repair (ICL) (160). Double-stranded breaks (DSBs), depending on the phase of the cell cycle, are either repaired by homologous recombination (HR) or non-homologous end-joining (NHEJ) (160). If the damage to DNA is irreparable, then senescence is induced to prevent

cell division while allowing the cell to survive, or, if the damage is too severe, apoptosis is induced to kill the cell and prevent transformation (160).

CSCs are often resistant to chemo- and radiotherapies, and this avoidance of cytotoxicity can be attributed to various mechanisms (160). In breast cancer cells, the CSC populations are more radioresistant and have been shown to produce less reactive oxygen species (ROS) and upregulate DNA damage checkpoint responses **[Figure 1.6]** (161, 162). A distinct feature of CSCs is their highly effective DNA repair systems (163, 164). Gastrointestinal and breast carcinomas contain abundant CSCs that upregulate genes associated with ROS scavenging such as superoxide dismutase and catalase that lower their levels of ROS (160, 165). A link has also been made between markers of stemness and ROS metabolism, with CD44 in gastrointestinal stem-like cancer cells being capable of regulating glutathione levels through interactions with glutathione cysteine transporters (160). Whether the source of free radicals is intrinsic or extrinsic CSCs are uniquely poised to survive such insults.

Adaptations to DNA damage and replication stress can lead CSCs to be overly dependent on some DNA damage response (DDR) proteins such as ATR and RAD51, making them potential targets for therapy (166). Similarly, targeting CHK1 and CHK2 can increase the sensitivity of CSCs to radiation in glioblastoma and improve the efficacy of chemotherapy treatment in pancreatic and lung cancer (162, 167, 168). Leukemic CSCs appear to be sensitive to combination treatments of HDAC inhibitors and agents that target DDR (169). Combined inhibition of poly-(ADP-ribose) polymerase (PARP) and ATR also has been demonstrated to radiosensitize glioblastoma CSCs (170-172). CSCs possess multiple mechanisms to resist conventional therapy, therefore inhibiting their

ability to resist and recover from DNA damage may sensitize CSCs to conventional treatment modalities.

It should be noted that an improved DNA damage response is not universally observed across all CSCs (173). In breast cancer stem cells, EZH2 epigenetically represses DNA repair via H3K27 methylation, leading to genomic instability and increasing CSC frequency (170, 174). In mammary tumors, NHEJ activity is more prevalent in the CSC population relative to the non-CSC population (175). It will be valuable to understand how frequent these exceptions are and if they represent a targetable phenotype or will expression rebound in times of stress.

The DDR has been extensively studied in CSCs at the molecular level (159, 176). The interplay between chromatin, DNA damage signalling and DNA repair machinery is under-developed (177-180). The packaging of DNA into nucleosomes and higher-order chromatin structures limits the accessibility of DNA (181). Upon DNA damage, chromatin becomes disorganized and must be restored for the maintenance of cell identity (181). The specific remodeling events and epigenomic requirements in response to DNA damage have yet to be discovered in the context of cancer and CSCs (182). In human cells, it is known that all major families of chromatin remodelers participate in DNA repair (183, 184). Evidence exists indicating that epigenetic silencing occurs near DSBs, with the accumulation of silencing factors such as polycomb group proteins, HDACs, histone methyltransferases, and DNA methyltransferases (185-189). Formation of gamma H2A.X foci, as a result of phosphorylation of the histone variant H2A.X, not only recruits DNA repair proteins but also ensures that the larger chromatin domains impacted by the DNA damage are marked for repair and that cells arrest until the repair is completed (190). It

remains to be determined if parental histones are recycled post-DNA repair, and cellular identity is restored, or whether new information, in the form of post-translational modifications, is deposited as an indication of previous DNA damage to facilitate DNA repair in the future (181, 182). As DNA damage and epigenetic alterations are essential aspects transformation and loss of cellular identity, it will also be of interest to study whether chromatin scars, in the form of changes to DNA methylation, histone variants, histone post translational modifications (PTMs), and histone density upon genotoxic insults, contribute substantially to transcriptional reprogramming and loss of cell identity observed in some cancers (182).

1.4.4. Therapeutic Stress and Plasticity

Several studies have shown that therapies, such as radiation therapy and chemotherapy, can induce plasticity and stem cell-like phenotypes in cancer. For example, paclitaxel induces CSC populations in breast cancer, and radiotherapy increases CSC numbers in lung, prostate, colon, and breast cancer, amongst others. Mechanistically, it is unclear how therapy induces CSCs; however, NF-κB, NOTCH, NODAL, and EMT associated transcription factors have been implicated.

While therapies likely induce CSCs, alterations to the epigenetic landscape, such as aberrations to DNA methylation and histone modifications, also facilitate the development of resistance to chemotherapeutics **[Figure 1.6]** (191). Slow cycling cell populations can be generated by epigenetic factors and serve as reservoirs for the emergence of heterogeneous cells possessing even further epigenetic rearrangements that can lead to drug resistance (192). Chemoresistance in breast cancer is often a product of global hypomethylation, promoter hyper-methylation at specific locations, and

post-translational histone modifications (193-195). Aberrant HDAC, HAT, or p300 expression and overexpression of DNMTs lead to deregulation of histone modifications and DNA methylation, respectively, which in turn, gives rise to the expression of EMT and CSC-related genes **[Figure 1.6]** (194-196). These epigenetic mechanisms modulate the levels of the following genes, which also provide a survival advantage against cytotoxic threats: *ESR1, p16, p53, Survivin, Bcl2, MLH1,* and *MSH2* (195, 197-199). These genes among others have been found to provide drug tolerance to CSCs by acting through a variety of distinct mechanisms (196, 200).

Approximately half of the genome consists of repetitive elements which are tightly regulated to protect the host from the consequences of inappropriate activation. Recently, repetitive transposable elements have been discovered to be inappropriately activated in cancer cells due to global DNA demethylation (201). Chemoresistant CSCs are capable of maintaining repression at the repetitive elements within their genome by increasing H3K9 and H3K27 methylation during exposure to drugs (202). Targeted drugs are particularly potent activators of this repeat element misexpression response in cancer cells resulting in cell death (202). Derepression of these genomic repeat elements may increase the immunogenicity of cancer cells allowing for improved patient responses to immunotherapy and thus is being further investigated (201).

KDM 2/3/5/6/7 contribute to a drug-resistant state by generating slow-dividing stem-like cells (104, 202-209). Members of the KDM family of histone demethylases are overexpressed in several cancers and have been shown to confer resistance to tyrosine kinase inhibitors such as gefitinib, erlotinib, and imatinib (210-213). While KDMs are not fully understood, they do play a critical role in embryonic development and differentiation

(214, 215). In particular, they have been implicated in interacting with hormone receptors that modulate proliferation (210). Knockdown of KDM1A and KDM5B in non-small cell lung cancer prevents hypoxia-mediated resistance to gefitinib, as well as inhibiting EMT (216, 217). In ovarian cancer, resistance to platinum-based therapies has been linked to epigenetic modulators HDAC and DNMT1, and its association with regulator of G protein signalling 10 (RGS10) (218). The knockdown of either of these epigenetic proteins in chemoresistant ovarian cancer cells increases RGS10 levels permitting cell death (218). Targeting HDAC in murine xenograft models of ovarian cancer sensitizes them to cisplatin (219). Resistant cells have more HDAC1 bound at the promoter regions of the death receptor FAS, suppressing its expression, making these tumor cells better at evading immune cells (220). In breast cancer, KDM5B, bromodomain and extraterminal (BET) proteins, and EZH2 generate cells that are persistent following chemotherapeutic treatment through the modification of chromatin structure (205). While BET inhibitors in some contexts can revert the drug resistance phenotype, activation of PI3K signalling can lead to resistance to BET inhibitors over time (221). It remains to be tested whether long term antitumor effects can be achieved either with sequential or combination therapies. The combined use of both HDAC and BET inhibitors may be an effective strategy for overcoming chemoresistance (222). The upregulation of HDACs in pancreatic cancer supports CSCs and potentiates resistance to gemcitabine (223). HDAC inhibitors can reverse the expression of EMT and stem cell genes in pancreatic CSCs caused by HDAC overexpression, which resensitizes the cells to gemcitabine (223). It remains to be tested whether sustained antitumor effects can be achieved either with sequential or

combination therapies. The use of HDAC inhibitor in combination with and BET inhibitors or chemotherapy may be an effective strategy for overcoming chemoresistance (222).

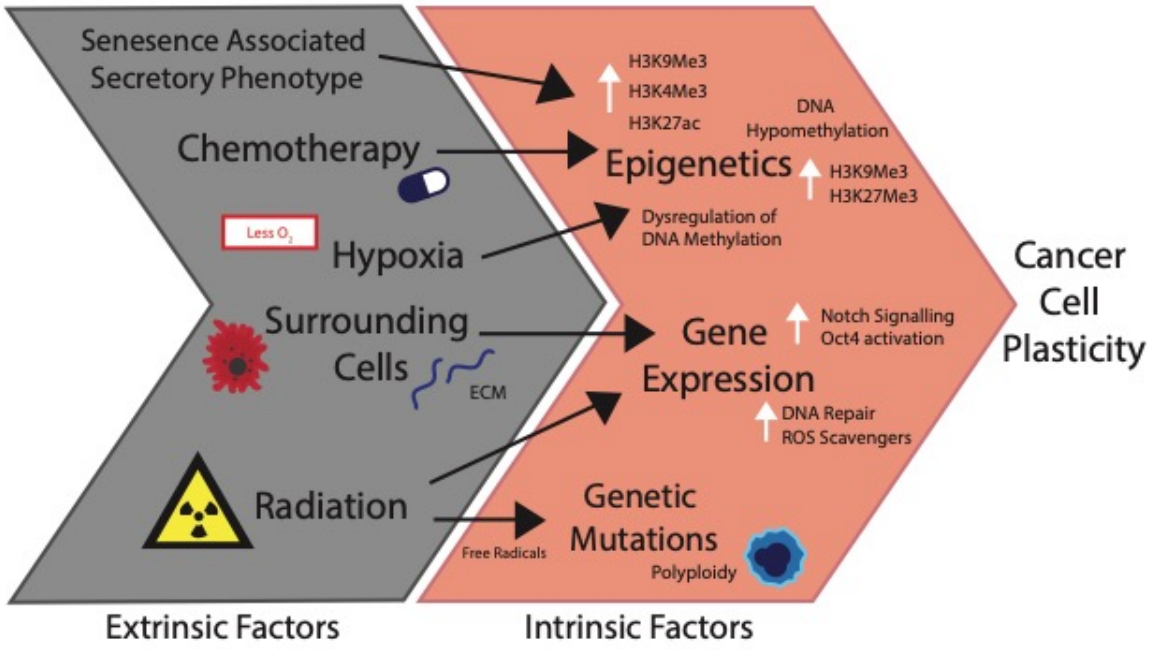


Figure 1.6. Aspects of the microenvironment like hypoxia, secreted factors and ECM, as well as therapies like radiation and chemotherapy have been shown to promote the acquisition of CSC-like phenotypes. For most cancers to progress, it is necessary for alterations to the epigenome to occur, many of which are mediated by the tumour microenvironment, leading to the acquisition of cellular plasticity. The SASP may also mediate stress-induced plasticity.

1.5. Evolution of Therapies Targeting Phenotypic Plasticity

Our adoption of the cancer cell plasticity model over the cancer stem cell model implies that CSCs can arise from more than just a subset of cells, but rather a variety of differentiated cell types, and this process of CSC generation can occur throughout tumorigenesis. The fact that non-CSCs can simply switch into CSCs and continually renew and expand is a challenge from a therapeutic perspective. It has been widely

demonstrated that CSCs heavily contribute to many of the pathological features that result in worse clinical prognosis for cancer patients. Any treatment aimed at targeting CSCs must take into consideration that a wide variety of distinct biological pathways may be endowing tumor cells with increased proliferation, survival and self-renewal properties.

The possibility of differentiation therapies being effective has diminished because pushing CSCs back towards a more non-stem-like state may not be possible when cells possess numerous genetic mutations that cannot be reversed **[Figure 1.7A]** (7). Targeting those oncogenic signals that have been shown to be vital to the acquisition and maintenance of stemness in cancer is not a long-term solution as many of these approaches are plagued by considerable side effects since adult stem cells are relying on the same molecules and biological pathways **[Figure 1.7A]** (89). The more successful approach to preventing progression and eventual metastasis of cancers with stem-like populations would be employing treatments that halt CSC proliferation **[Figure 1.7B]**. However, quiescent cells that are not actively dividing are emerging as major contributors to drug resistance in cancers whose cells are prone to undergoing switches in their cellular state. That is because non-CSC populations can simply recreate the initial CSC pool once it is initially eradicated **[Figure 1.7B]**. Non-CSCs are capable of leaving primary tumor sites and lying dormant at a secondary site before converting to a CSCs phenotype **[Figure 1.7B]** (7). One feasible approach perhaps for targeting quiescent CSCs may be through their metabolomic differences from cycling cancer cells (89). The impact the microenvironment plays in the transition of non-CSCs to CSCs in terms of initiating disease relapse at metastatic sites is substantial, thus patients may benefit from combination therapies that specifically alter the triggers of phenotypic switching (7).

Most importantly, further understanding of the drivers of cancer cell plasticity in the variety of different cancer types that exist is still urgently required to improve all patient outcomes. Inhibition of multiple epigenetic regulators may be promising, but again their efficacy when it comes to treating solid tumors is limited, and levels of toxicity are not ideal as normal stem cells again rely on the same epigenetic proteins **[Figure 1.7C]** (89). Combinatorial approaches using both targeted therapies against kinases and epigenetic drugs appear to be the most valuable therapeutic strategy moving forward **[Figure 1.7C]** (89). The use of both simultaneously may prevent drug tolerance from developing as chromatin remodelling enzymes are unable to develop adaptive transcriptional responses to targeted therapies **[Figure 1.7C]** (89). Ultimately understanding the interplay between the progressive acquisition of plasticity, the subsequent phenotypic heterogeneity and the underlying epigenetic changes that produce these changes will allow us to understand the mechanisms of therapeutic escape and develop new and more comprehensive strategies to treat patients.

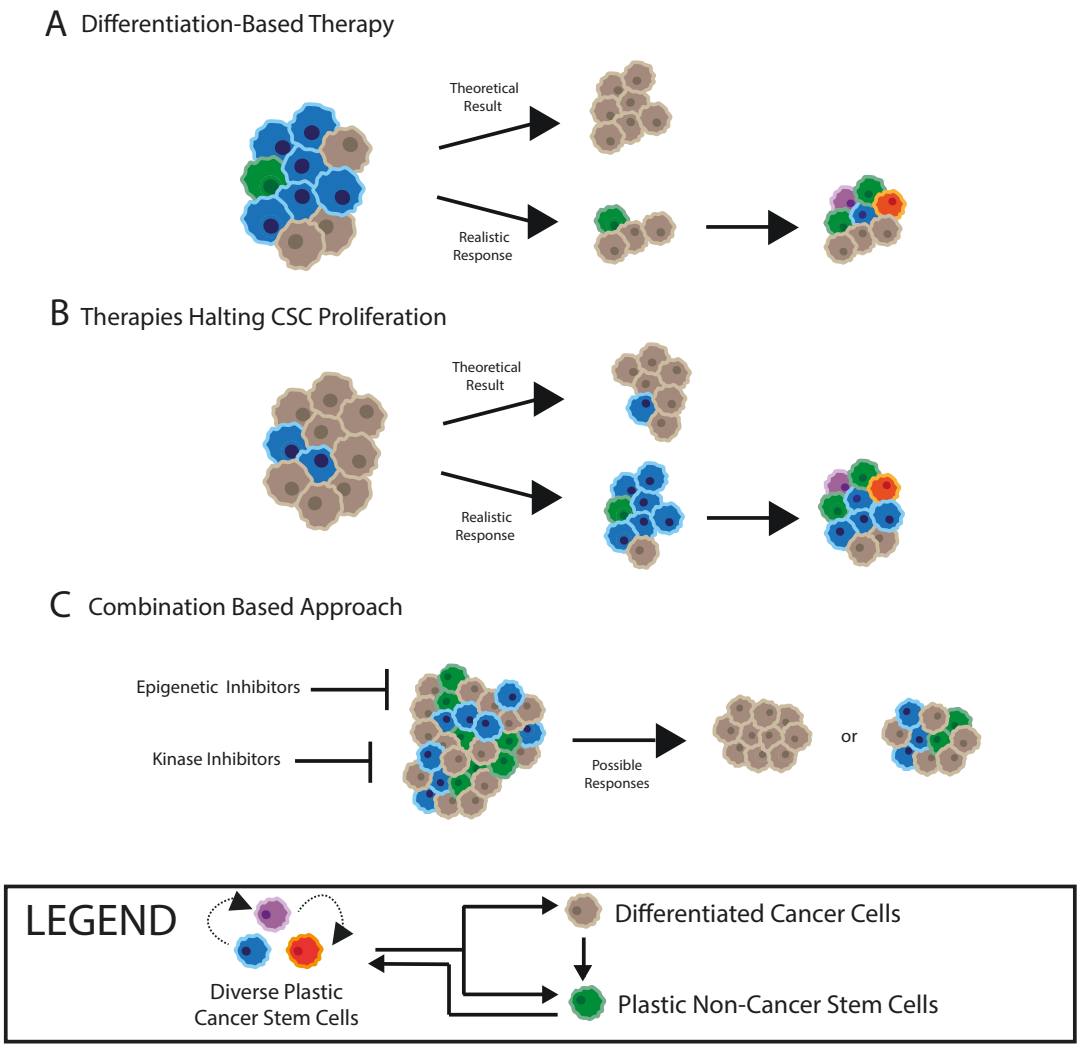


Figure 1.7. Several approaches that have been or are currently being considered to target CSCs. A) Differentiation based therapies aimed at pushing CSCs back towards a more non-stem-like state but is plagued by side effect since adult stem cells rely on the same biological pathways. **B)** Treatments that halt CSC proliferation initially seemed promising however CSCs are often quiescent and can simply repopulate the initial CSC pool after the initial eradication event. **C)** Combinatorial approaches using both targeted therapies against kinases and epigenetic drugs appear to be the most valuable therapeutic strategy moving forward. Drug tolerance could be prevented from developing through the lack of adaptive transcriptional responses to targeted therapies in the absence of function chromatin remodeling enzymes.

1.6. Endometrial Cancer: A Common Gynecological Malignancy

In Western countries, the majority of malignancies affecting the female reproductive tract are comprised of ovarian (OCs) and endometrial cancers (ECs). EC is considered to be the most commonly diagnosed cancer of the female genital tract with approximately 382,069 new cases worldwide (224-226). Ovarian cancer, on the other hand, with an estimated 184,799 deaths worldwide, is reported to be the most lethal gynecological carcinoma (227). Both malignancies affect both premenopausal and postmenopausal women. In 10% of all women with OC and 5% of women with EC, the cancers of the endometrium and ovary coexist (228). Pathological changes in the endometrium can sometimes occur as a consequence of OC. Ovarian and endometrial tumors in general do share common etiology related to reproductive factors such as number of ovulatory cycles, as well as hormone replacement therapy (229).

Despite primarily afflicting women over the age of 45 and after the onset of menopause, EC is the most frequently diagnosed gynecological malignancy in Western countries. In Canada, in 2020, it was estimated that 1,300 of the 7400 women diagnosed with EC, will die from this disease (230). Increased life expectancy and the rising incidence of obesity have both contributed to an increase in the prevalence of EC. Although the 5-year survival rate is high at 90% for International Federation of Gynecology and Obstetrics (FIGO) Stage I and II EC, approximately 10–15% of patients will experience recurrent metastatic disease (231). Taken together with FIGO Stage III and IV EC, these recurrent non-uterine confined and advanced-stage cases of EC have median survival that has been reported to barely exceed 1 year (232).

Endometrial carcinogenesis has been proposed to follow a dualistic model and ECs can be grouped into two types based on immunohistochemical and molecular features (233). Linked to obesity, estrogen excess and hormone receptor positivity, Type I endometriod ECs have more favorable outcomes than Type II serous tumors that are found mostly in older women (233). Treatment of early stages of Type I ECs has primarily been adjuvant radiotherapy whereas advanced stages of Type I and serous Type II tumors are frequently targeted by chemotherapy (234). In order to apply appropriate treatment to EC patients, proper subtype classification has been further supported by the characterization of commonly mutated genes within each histological subtype. Type I ECs frequently contain PTEN mutations coexisting with mutations to other genes in the P13K-Akt pathway (235, 236). Mutations to FGFR2, ARID1A, CTNNB1, PIK3CA, PIK3R1, and KRAS are also common in Type I tumors whereas TP53, PIK3CA, and PP2R1A mutations are most frequent in Type II ECs (237-241).

Further characterization at the molecular level using multiple platforms has provided an even more refined subdivision of ECs into different subtypes. Examination of somatic copy number alterations (SCNA) and microsatellite instability (MSI) resulted in EC clustering into four groups (234). One group consisted of mostly serous EC with *TP53* mutation and extensive SCNA (p53-mutated EC by WHO 2020 classification) (234). The remaining endometriod tumors could be divided into three subtypes: (1) ultramutated EC characterized by *POLE* exonuclease domain mutation with very high rates of mutations (*POLE*-ultramutated EC); (2) a group of hypermutated MSI EC (MMR-deficient EC); and (3) microsatellite stable EC with low frequencies of mutations (no specific molecular profile/NSMP EC) (234). Only the *POLE*-ultramutated subtype has progression-free

survival near 100%, which strengthens the notion that a better understanding of the other subtypes is required to improve therapeutic application to patients who present with EC tumors genomically classified in this manner.

As the genomic contribution to aggressive forms of EC is being elucidated, a growing understanding of the other molecular and microenvironmental contributions to these tumors is also coming to light. Certain cases of EC display a great degree of heterogeneity at the phenotypic level. For example, dedifferentiated endometrial carcinoma (DDEC) exhibits a solid growth pattern lacking appreciable features of differentiation in the undifferentiated component that is juxtaposed to well differentiated carcinoma component (242). Evidence in the literature is building a strong case that the interplay between genetic mutations, aberrations to signaling factor activity and cues from the tumor microenvironment can drive EMT, or changes to the extracellular matrix of EC cells. The reported aggressive clinical behavior of DDEC could be explained by EC having undergone EMT to become undifferentiated, motile stem-like cells that in turn do not respond to conventional chemotherapy.

1.7. Epithelial to Mesenchymal Transition in Endometrial Cancer

EMT is a highly regulated physiological process in the early embryonic development and ontogenesis of the female reproductive tract (243). Dysfunction of EMT in the normal epithelial cells of the reproductive organs, the ovary and the uterus, results in pathological processes such as adenomyosis, endometriosis, cancer development and metastasis (244). During EMT, epithelial cells not only lose their polarity but also their adhesion to adjacent cells and the basement membrane and acquire properties that promote

migration and invasion. These phenotypic changes are marked by the acquisition of a fibroblast-like mesenchymal appearance and cellular plasticity (244). In cancer cells, this developmental process is hijacked, allowing the tumor cells to dissociate, migrate, and metastasize (245, 246). Furthermore, EMT induces the emergence of CSC traits, prevents apoptosis and senescence, induces resistance to chemotherapy, and contributes to immunosuppression (247). EMT is regulated epigenetically, transcriptionally, and post-transcriptionally. Downregulation of epithelial cell-specific tight and adherens junction proteins like E-cadherin in conjunction with the novel expression of mesenchymal proteins Vimentin and N-cadherin are trademark responses to the EMT program (248, 249). Numerous signaling pathways including PI3K/Akt, transforming growth factor β (TGF-β), epidermal growth factor (EGF), hepatocyte growth factor (HGF), mitogen-activated protein kinase (MAPK)/ERK, NF-kβ, WNT, Notch, estrogen-receptor-α (ER-α), and HIF-1α cross talk to participate in EMT upregulation. These signaling pathways act to mobilize embryonic transcription factors as well as epigenetic modifiers to reprogram epithelial cells toward a more mesenchymal-like fate (250). It can logically be hypothesized that the extreme invasiveness and poorer patient prognosis associated with high grade EC and DDEC is a direct result of EC cells having undergone the process of EMT. Although EMT can lead EC cells to lose their cell–cell adhesions and acquire the ability to migrate and proliferate, studies have not yet proven that EMT in EC cells will eventually result in metastases. More experimentation is required to gain a clearer picture of the role of EMT in EC cancer progression.

1.7.1. Role Transcription Factors Play in EMT in Endometrial Cancer

Transcription factors are among the best-characterized mediators of EMT (248). In EC tissue specifically, overexpression of Twist, Slug, ZEB1, ZEB2, and Snail is linked to reduced expression of E-cadherin (251) **[Figure 1.8]**. Nuclear β-catenin has also been shown to promote EMT by upregulating Slug expression (250, 252-255). SALL4, an essential transcription factor with a well-described role in the maintenance of pluripotent embryonic stem cells is aberrantly expressed in EC, promoting invasiveness through the up regulation of mesenchymal cell markers such as N-cadherin (256). SALL4 induces EMT through c-Myc, another transcription factor and oncogene (256). Kruppel-like factor 17 (KLF17) was initially thought to be an inhibitor of EMT and a tumor suppressor in several cancers including breast cancer (257-259). In the context of EC, KLF17 functions as a driver of EMT. EC tissue has elevated levels of KLF17 and expression of KLF17 in EC cell lines, leads to an upregulation of EMT-inducing transcription factors (260). Hence, some transcriptional programs may regulate EMT in a tissue specific manner.

Another molecular player that has been shown to drive EMT is the neurotrophic receptor tyrosine kinase B (TrkB). Long recognized as an important oncogenic factor in a neurogenic context, when the TrkB signaling pathway involving the brain-derived neurotrophic factor (BDNF) is activated in other tumor types, tumor cell proliferation, invasion, and metastatic potential are all stimulated (261). This pathway has also been linked to anoikis resistance in multiple cancers by inhibiting cell death and, therefore, leading to metastatic spread of cancer (262). TrkB and its high affinity ligand, BNDF are detected at high levels in EC and OC (261, 263). TrkB levels determine the fate of EC cell lines, causing the Cadherin switch most commonly associated with an EMT event

(261). The Akt and MAPK pathways are downstream of the TrkB signaling pathway, which could provide an explanation as to how the actions of several transcription factors can converge on the single yet complex cellular process of EMT.

1.7.2. Role Metabolism Plays in EMT in Endometrial Cancer

EMT may also be regulated by metabolic processes. Enolase 1 (ENO1), an enzyme functioning in the glycolytic pathway was hypothesized to have some role in tumor development. This idea was based on the logical observation that increased glucose uptake and aerobic glycolysis are characteristic features of rapidly growing cells (264). In EC, silencing ENO1 decreases Snail and N-cadherin expression while upregulating E-cadherin levels (265). At the same time, silencing ENO1 downregulates levels of proteins in the PI3K/Akt pathway, also resulting in Snail being expressed at lower levels (265). It is hypothesized then that ENO1 could be a potential oncogene, activating the PI3K/Akt pathway and eventually initiating downstream EMT signaling cascades in EC **[Figure 1.8]**. For endometrial cancer patients also suffering with diabetes, high glucose levels may induce mitochondrial dysfunction through the upregulation of Drp1 (266). The mitochondrial damage and induced by Drp1 disrupts mitochondrial homeostasis and can promote EMT and EC progression (266). Endometrial cancer cells capable of prospering under high glucose levels also express high levels of GLUT4, a glucose transport protein in addition to upregulating VEGF/VEGFR, which altogether with increasing ER, promotes EMT and accelerates EC development (267). Insulin and insulin-like growth factors also play vital roles in maintaining balanced metabolism with their dysregulation being linked to diabetes and cancer (268). The insulin receptors, IR and IGF-1R are overexpressed and have been implicated in EC development (268).

Reduction of IR and IGF-1R alone or together sufficiently reduces the ability of EC cells to migrate and invade and inhibits EMT through inactivation of PI3K/AKT and ERK signalling pathways (268).

1.7.3. Role Non-coding RNAs Play in EMT in Endometrial Cancer

Most recently, the effects of non-coding RNA have been shown to be critical to the development of cancer (269). MicroRNAs (miRNAs) and lncRNAs in particular have been found to be upregulated in many cancers, including endometrial cancer, acting as oncogenes or tumor suppressor genes (270, 271) **[Figure 1.8]**. miRNAs act as regulators, binding the 3′ untranslated regions (UTRs) of coding RNAs triggering either the repression of mRNA translation or the degradation of the RNA completely (272). lncRNAs usually associate with protein complexes and have been discovered to be both effectors and repressors that are involved in regulating gene expression at both the transcriptional and translational level (273, 274). In general, high levels of miRNAs are associated with a variety of cancers but an understanding of how specific miRNAs regulate the expression of different oncogenes in EC is gradually being uncovered. While some studies have demonstrated that differential expression of lncRNAs and abnormal functioning can be associated with endometrial tumor development much more examination of the molecular mechanisms linking lncRNAs to EMT remain to be discovered.

Presently, EMT is suppressed in EC through the action of a growing number of microRNAs. miR-194 has been linked to B lymphoma mouse Moloney leukemia virus insertion region 1 (BMI-1), a protein associated with self-renewal and malignant transformation. In EC cell lines, BMI-1 can be linked to enhanced invasiveness, and miR-

194 levels in highly invasive EC *in vitro* are inversely correlated with BMI-1 expression (275, 276). miR-194 transfection decreases cell invasion in the HEC50B cell line while simultaneously inducing a loss of the EC cell line's mesenchymal phenotype (275). miR-101 is downregulated in both endometriod and serous EC and has been found to inhibit proliferation of EC cells in the aggressive serous type. Notably, increasing miR-101 levels in EC cells reverses EMT (277). Specific to EC, miR-101 suppression of EMT can partly be linked to enhanced expression of EZH2, a histone-lysine *N*-methyltransferase enzyme that participates in histone methylation and, ultimately, transcriptional repression. EZH2 downregulates mesenchymal markers and Wnt/β-catenin signaling, leading to MET (277). miR-23a has also been found at significantly reduced levels in EC tissue (278). Overexpression of miR-23a *in vitro,* led to inhibition of EMT in HEC 1A cells through the targeting of SMAD3 (278). Downregulated in EC, miR-124 expression is partially attenuated by DNA methylation (279). Much like miR-23a, miR-124 when expressed at higher levels reverses the EMT-like phenotype, exhibiting reduced migration, invasion and proliferation through the upregulation of the scaffolding protein IQGAP1 (279). Overexpression of miR-20a-5p in EC cells inhibits EMT by targeting STAT3 directly and thereby has been posited to inhibit invasion of EC cells as well (280). miR-320a and miR-340-5p binding the 3'UTR of eIF4E have also been demonstrated to supress EMT in EC that is induced by TGF-β (281). It has also been demonstrated using EC cell lines, that miR-195 and miR-214-3p upon upregulation inhibit EMT through at least partly targeting either SOX4 or TWIST1, respectively (282, 283). miR-202 inhibits EMT yet has been shown in EC to be downregulated. The target of this microRNA has been shown to be FGF2 as well as the Wnt/β -Catenin signalling pathway which explains its' ability to inhibit

EC cell migration and invasion and how down-regulation of miR-202 can be associated with poorer prognosis in patients (284). An axis of microRNAs (miR-302b-3p/302c-3p/302d-3p) are also normally found to be downregulated in EC and upon overexpression in EC cells are capable of inhibiting the progression of EMT as a result of regulating ZEB1 expression levels and promotion of apoptosis (285). Another microRNA, miR-34a also acts as a tumor suppressor, capable of suppressing tumor growth upon its overexpression and inhibiting EMT through decreasing Notch1 levels (286).

Recently, several microRNAs have been found to promote EMT in the context of endometrial cancer. miR-21-5p upon overexpression promotes EMT in endometrial cancer cell lines through inhibition of SOX17 protein expression via its 3'UTR (287). There is also evidence that overexpression of miR-135a can also promote EMT *in vitro* most likely through modulation of PTEN and p-AKT levels (288). Acting upon the Wnt/β -Catenin pathway, is miR-373 which is upregulated in EC and whose overexpression in EC cells promotes EMT (289). Overexpression of miR-215 can downregulate LEFTY2, in turn affecting EC cell proliferation and fostering EMT in the presence of low levels of the tumor suppressor protein (290). Interestingly, there are also interactions between transcription factors such as BHLHE40 and BHLHE41 and a family of microRNAs, the miR130 family, that have been found to mutually suppress each other and thus regulate EMT in EC (291).

Undifferentiated endometrial carcinoma frequently possesses a reduction in E-cadherin expression (292). MiRNAs have been implicated in the modulation of the epithelial differentiation status by repressing the action of ZEB1 and ZEB2, which are

transcriptional repressors of E-cadherin (292). In particular, members of the miR-200 family inhibit the expression of ZEB1 and ZEB2. These transcription factors inhibit E-cadherin expression and thus drive EMT. Hence, miRNA-200 family members lead to a reduction and/or reversal of these processes (293). Of note, ZEB1 and ZEB2 can also bind to promoter regions of miR-200, leading to reduced expression. DICER1, a cytoplasmic RNase III enzyme responsible for cleaving miRNA into active 22 nucleotide species is also downregulated in undifferentiated EC (294). By preventing miR-200 processing, dysregulation of DICER1 leads to reduced E-cadherin levels concomitant with the upregulation of Vimentin, N-cadherin, Twist1, Snail and ZEB2. Therefore, in EC, miRNAs' regulation of oncogene expression can influence the induction of EMT and the ability of endometrial cells to acquire phenotypes with the potential to metastasize.

In terms of lncRNA being implicated in EC progression through EMT, steroid receptor activator (SRA) has been found at higher levels in many gynecological cancer tissues including EC (295). When knocked down in EC cells, EMT is not promoted through eIF4E-BP1 or Wnt/β-catenin signalling pathways (295). HOTAIRM1 is another lncRNA whose downregulation in EC leads to reduced acquisition of a mesenchymal phenotype through EMT (296). This non-coding RNA has been connected to HOXA1, regulating the ability of this oncogene to stimulate cell proliferation and other hallmarks of aggressive disease (296). lncRNAs can also module miRNA signalling pathways as is the case with H19 and its ability to regulate miR-20b-5p levels (297). In both EC cells and tissue, H19 is expressed at higher levels, whereas miR-20b-5p is downregulated (297). As miR-20b-5p controls HIF-1α expression, elevated H19 leads to higher HIF-1α expression and therefore stimulation of EMT (297).

1.7.4. Role Hormones Play in EMT in Endometrial Cancer

Estrogen and progestins can also upregulate ZEB1 in the stroma and myometrium of the uterus and in human cells *in vitro* (298, 299). Interestingly though, there are no hormone response elements upstream of the translational start site of ZEB1. In aggressive cases of EC, such as grade 3 endometrioid and type II serous carcinomas, ZEB1 overexpression is not limited to the stroma and myometrium. Indeed, the ZEB1 protein is aberrantly expressed in epithelial-derived carcinoma cells as well (298). Loss of E-cadherin expression paired with ZEB1 expression in a high percentage of epithelial cells is characteristic of EMT and suggests hormonal regulation of the entire process.

During the normal menstrual cycle, the steroid hormone, progesterone can induce differentiation in EC cells. Progesterone induces the expression of inhibitors of Wnt signaling which in turn downregulate EMT and slow down cancer progression (300). Loss of progesterone receptors has been found in patient tissues with progressive EC and also witnessed in these cases, is a significant upregulation of pathways involved in the progression of cells to a mesenchymal phenotype (300). Taken together with the fact that application of medroxyprogesterone acetate, a synthetic variant of progesterone, to EC cells *in vitro* inhibits migration and downregulates Vimentin; a strong case for progesterone mediated inhibition of EMT can be presented (300). Progesterone also downregulates TGF-β, a signaling pathway which is a major driving force behind EMT **[Figure 1.8]**.

Much like in OC, elevated levels of estradiols (E2) contribute to the enhanced proliferation and invasive capacity of EC cells through the activation of the PI3K/Akt and

MAPK signaling pathways and fat mass and obesity-associated (FTO) gene expression (301). A study by Wik and colleagues not only provided new insight into the mechanisms of E2-induced proliferation and invasion of EC but also provided a link to obesity (301). Lack of ER-α in EC correlates with activation of Wnt-, Sonic Hedgehog- and TGF-β signaling pathways and induction of EMT suggesting ER-α independent mechanisms of EMT regulation (301) **[Figure 1.8]**. Most recently, conditioned media from normal endometrial stromal factors has been found to inhibit estrogen induced EMT through regulation of Slug and E-cadherin expression levels (302). Metformin, a commonly used drug used to treat type 2 diabetes can also reduce E2-induced cell proliferation and EMT in EC cells through suppression of ERK1/2 signaling and activation of AMPKα signaling (302) **[Figure 1.8]**. Ubiquitin-conjugating enzyme E2 C (UBE2C) also mediates EMT in EC, with E2 being capable of stimulating UBE2C gene expression (303).

1.7.5. Role Cytokines Play in EMT in Endometrial Cancer

Most aggressive forms of EC have tumor cells that have migrated to nearby lymph nodes and have invaded through the myometrium of the uterus. Gene-expression microarrays followed by bioinformatic analysis revealed a potentially prominent role for the cytokine, TGF-β in promoting invasion through the induction of EMT (304) **[Figure 1.8]**. Other directors of the embryo implantation process such as FOS, MMP-9, MapK1, and RHOA were also associated with aggressive EC cases, which hints at a possible parallel between the molecular events associated with controlled trophoblast implantation and uncontrolled endometrial tumor invasion (305). In HEC1A and RL95-2 EC cell lines, EMT can be induced by TGF-β as tested at the morphological and molecular levels (304, 306). TGF-β can also act as a chemo attractant for these *in vitro* cell lines, increasing

their invasive capacity (304). Co-treatment of Ishikawa cells with the cytokine, IL-6 and TGF-β resulted in messenchymal-like morphological changes that coincided with increased expression levels of the genes, Snail, N-cadherin, and Twist (307). Thus, TGF-β has been hypothesized to play a critical role in early invasion of EC through initiating the process associated with EMT. ERM/ETV5 (Ets family of transcription factors), is also upregulated in association with myometrial invasion (308). Overexpression of this particular transcription factor promotes cell migration and invasion and induces EMT by upregulating ZEB1 expression (309). In HEC1A EC cells, gene-expression microarray assays revealed Nidogen 1 (NID1) and Nuclear Protein 1 (NUPR1) to be direct targets of the ETV5 transcription factor when it was stably expressed *in vitro* (310). At the invasive front, both NUPR1 and NID1 had similar expression levels to ETV5 (310) . Knocking down NID1, a glycoprotein secreted by mesenchymal cells, in cells overexpressing ETV5 led to a significant decrease in cell invasion (310). Inhibiting NID1 in orthotopic EC models results in smaller tumors, an effect that is probably further enhanced by the microenvironment of the tumor (310). Additionally, inhibition of both NID1 and NUPR1 decreased the number of metastases (309). In HEC1A cells, ETV5 was shown to directly influence EMT by performing its function as a transcription factor and activating ZEB1. LPP, a protein implicated in cell-cell adhesion and cell motility is a transcriptional coactivator for other members of the transcription factor family of ETV5 (311). EMT induced by ETV5, led to localization of LPP from cell-cell contacts to focal adhesions (309). This accumulation of LPP at focal adhesions could lead to an amplification of extracellular signals and in turn its trans- location to the nucleus, where in it could further

activate ETV5, propagating its transcriptional activity and promoting persistent EC invasion through further EMT events.

Receptor activator of nuclear factor-κB (RANK) and its associated ligand RANKL, have been implicated in numerous physiological processes such as immune responses but also have been shown to be critical for the formation of lymph nodes (312). In EC tissue, RANK/RANKL expression is significantly higher and overexpression of RANK in EC cell lines, results in induction of EMT (313). CCL20 was found at increased levels in RANKL-treated RANK overexpressing cells. Furthermore, a neutralizing antibody targeting CCL20 could suppress EMT (313).

Autocrine motility factor (AMF) has also been implicated in EMT and, therefore, in promoting invasiveness and metastasis of endometrial carcinoma. Immunohistochemical analysis revealed high levels of AMF in EC tissue compared to normal endometrial tissue that showed a positive correlation with EMT markers (314). Silencing of AMF followed by gene-expression profiling showed altered expression of EMT mediators such as Snail (314). Additionally, treatment of EC cell lines with MAPK specific inhibitors downregulated EMT marker expression, suggesting that AMF promotes EMT in EC through the MAPK signaling pathway (314) **[Figure 1.8]**.

Cancer associated fibroblasts (CAFS) have also been found to serve an important role in EC through stimulating EMT (315). Conditioned media from CAFs is sufficient to induce EMT in EC cells, decreasing E-cadherin and increasing N-cadherin and vimentin expression (315). This phenomenon is attributed to the concentration of growth factors (EGF, TGF-β, HGF and FGF) present in the conditioned media of CAFs (315).

1.7.6. Role Hypoxia and Oxidative Stress Play in EMT in Endometrial Cancer

A hypoxic microenvironment is also a substantial inducer of EMT in EC, as it tends to accompany rapidly growing solid malignancies and results from poor blood supply to surrounding healthy tissue. Directly or indirectly, HIF-1 has been shown to control Snail, ZEB and other regulators of EMT (316). In primary EC samples, HIF-1 was overexpressed in over 65% of cases (316). This elevated expression of HIF-1 coincided with increased expression of Twist and decreased levels of E-cadherin (316). It can be speculated that under low oxygen conditions HIF-1 regulates Twist expression by direct binding to its promoter and, therefore, promotes EMT and aggressiveness of EC.

Cancer cells, through activation of proteasome pathways, are capable of tolerating oxidative stress. REGY-associated proteasomes can degrade specific proteins like cell-cycle inhibitors in an ubiquitin and ATP-independent manner. In EC, mutant p53- R248Q can bind to the promoter of and upregulate the expression of REGY (317). Depletion of REGY in EC lines reduces cell proliferation, migration, and invasion whereas expression of mutant p53-R248Q promotes EMT (317). Overexpression of p53-R248Q cannot restore REGY protein levels in REGY-depleted EC cells, hinting at an alternative mode in which the restoration of these cells' malignant properties occurs (317). Insight from EC cells that are resistant to inhibitors of proteasomes, has led to the hypothesis that EMT in this environmental context is brought on by miR-200-ZEB1/ZEB2 protein regulation (318). p53-R248Q can also bind to the promoter of miR-130b, inhibiting its transcription and subsequently allowing ZEB1 to bring about the EMT phenotype (319). Interestingly, p53-R248Q has also been found to promote EMT in EC through the disruption of the p68-Drosha complex, which is responsible for the processing of miR-26a (320). Reduced miR-

26a levels leads to overexpression of its downstream target EZH2, a promoter of EC tumor progression through EMT (320).

Although further research is required to fully understand the extent to which hypoxia influences the onset of EMT in the context of the endometrium, HIF-1 has been shown to significantly induce EMT in both EC and OC. Whether oxidative stress can also upregulate HIF-1 and bring about EMT in the endometrium also remains to be fully explored.

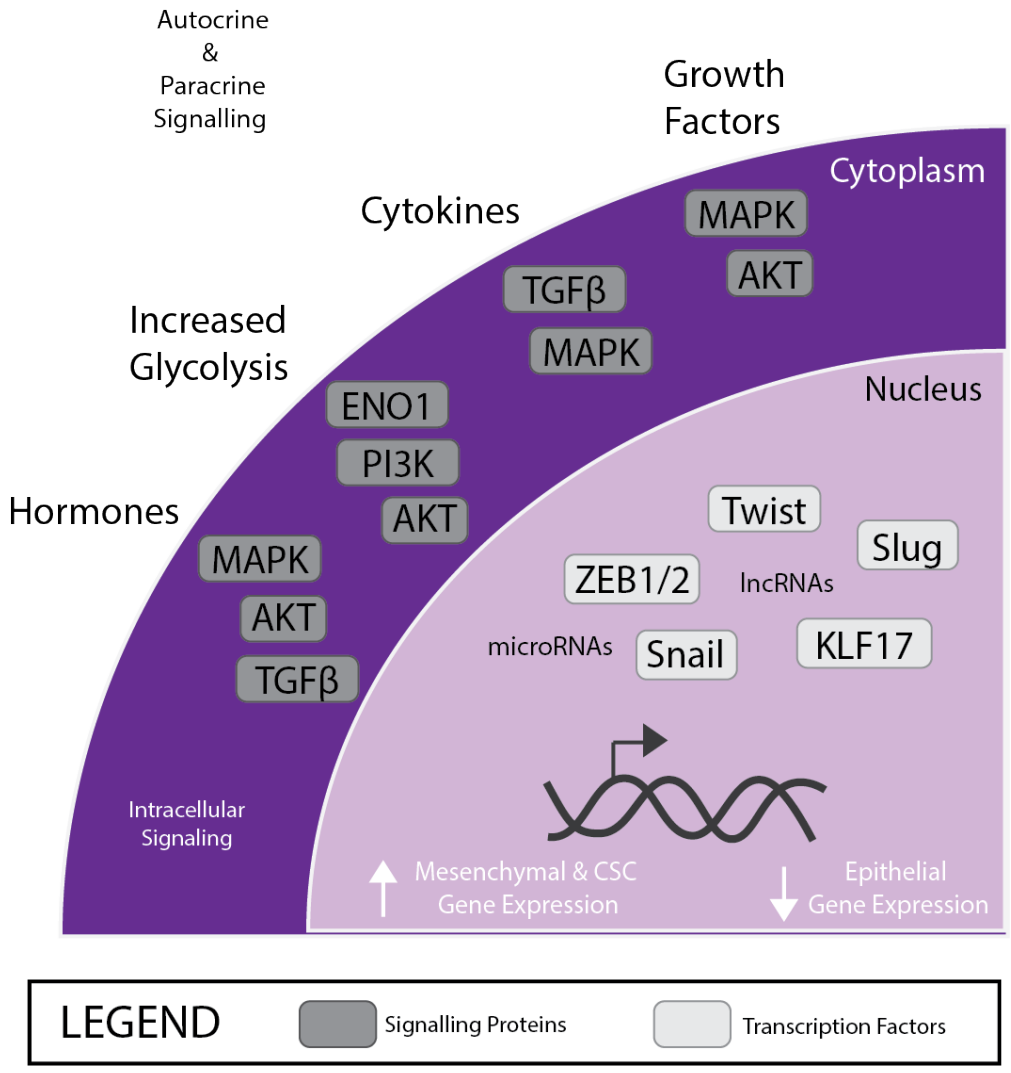


Figure 1.8. EMT in endometrial cancer. Regulation of EMT and CSC-inducing signalling pathways through extra and intra-cellular signaling and the action of transcription and epigenetic factors.

1.8. Dedifferentiated Endometrial Cancer

DDEC is a rare neoplasm, comprising less than 5% of endometrial cancer cases yet is more aggressive than high grade endometrioid carcinoma (321-324). Silva et al, in 2006 defined DDEC as cases of endometrial carcinoma in which low-grade-endometrioid carcinoma was combined with undifferentiated carcinoma (323). The transition between

the tumor components is abrupt with a sharp border (325). Nearly half of patients with DDEC already possess advanced stage disease with nearly 40% of those patients dying anywhere from half a month to less than two years from initial diagnosis (323, 326). This unfavorable prognosis is even evident when the undifferentiated component constitutes less than 20% of the whole tumor (323, 325). The diagnosis of DDEC can be made on biopsy (i.e., endometrial biopsy or biopsy of metastatic disease) but the diagnosis may only become apparent when the tumor can be more thoroughly examined in hysterectomy. The differentiated components are often found on the surface of the tumor whereas the undifferentiated portions which is the more actively growing portion typically invades deeper into the myometrium (326). As such, endometrial biopsy which typically samples from the more superficial portion of the tumor may only sample the well differentiated component of the tumor, which limits the recognition of the more aggressive nature of the disease pre-operatively. Conversely, in a biopsy of suspected metastatic mass, pathologists may not recognize an undifferentiated tumor as the undifferentiated component of a DDEC. Consequently, DDEC may very well be underrecognized and its incidence much higher. The majority of DDEC patients receive adjuvant therapy whether that is only chemotherapy, only radiotherapy or both chemotherapy and radiation therapy (327).

A recent systematic review and meta-analysis revealed that 44% of DDEC or undifferentiated endometrial carcinomas fall into the MSI TCGA classification (MMR-deficient EC) with p53 wildtype/copy number low (NSMP EC) being the next most common molecular group, while only rarely did DDEC belong to the p53 abnormal/copy number high (p53-mutated EC) groups (327). As has been witnessed with the numerous

clinical pathology-based studies is that all four TCGA groups are represented in DDEC cases but classification as MSI-H group appears to be most represented (327). The percentage of mismatch repair deficiency and *POLE* mutations in DDEC and UEC is higher than that observed for all endometrial carcinomas (327). The prevalence of the *POLE*-ultramutated group is also two-fold higher than that observed overall for endometrial carcinomas (327). Given the over-represented MSI-H and *POLE*-mutated subgroups, the majority of undifferentiated and dedifferentiated endometrial carcinomas have high mutational loads which contrasts with what is seen with other histologies of endometrial cancer (327). It remains to be seen though if those DDEC/UEC cases with abnormal p53, result in a prognosis that is worse than hypermutated/ultramutated DDEC as this phenomenon among endometrial carcinomas is generally associated with poor prognosis (327). Limitations to reviews of this type though and particularly in the context of these neoplasm lie in the few studies on DDEC that exist and the frequent patient overlap between studies due to the rarity of the disease (327).

The TCGA's stratification of endometrial carcinomas based on alterations at the level of molecular genetic included mixed endometrial carcinomas but little information on dedifferentiated endometrial carcinomas was evident (328). The small number of cases that were of mixed histology were either clustered into the copy number high/serous-like group of the copy number low group (234, 328). An investigation of a small number of DDEC cases revealed POLE exonuclease domain mutations to either P286R or V411L or mutations in the exonuclease domains (S297F, P436L, A456P) (328). POLE is a catalytic subunit within the POLE DNA polymerase enzyme complex that uses its exonuclease activity to locate and replace erroneous bases in daughter strands (328,

329). Within the POLE ultramutated group described by the TCGA, endometrial cancers are characterized by *POLE* exonuclease domain mutations, have a high rate of somatic mutations and favorable prognosis (234). DDEC patients possessing mutations to *POLE* were more likely to be alive without disease than those patients with DDEC tumors without POLE mutations (328). It appears therefore that *POLE* mutation could be a marker of good prognosis in endometrial cancer regardless of histological appearance (328). This improved prognosis is mostly likely a result of more tumor-infiltrating lymphocytes which exhibit an enhanced T-cell anti-tumor response in the context of tumors with high mutational burden (330-332). An increased immune response should decrease the metastatic potential of tumors leading to a less aggressive neoplasm (330). Future studies will be necessary to investigate in a larger cohort of DDEC cases whether *POLE* exonuclease domain mutations consistently show a high rate of tumor-infiltrating lymphocytes.

In close to 60% of DDEC cases, somatic mutations are observed to mismatch repair proteins: mutL protein homolog 1 (MLH1), postmeiotic segregation increased 2 (PMS2), mutS protein homolog 2 (MSH2) and mutS protein homolog 6 (MSH6) (333). The frequency of MMR deficiency in DDEC is much higher, almost double than what is seen in common endometrial cancer cases (326, 333). Findings in other cancer types have demonstrated that those with a higher mutational burden due to defective DNA repair mechanisms may be more immunogenic and possess immune escape mechanisms such as upregulated programmed cell death-1 (PD-1) and PD-1 ligand 1 (PD-L1) pathways (333, 334). The interaction of these two proteins ensures over activation of the immune system does not take place and T-cell proliferation is inhibited.

Tumor cells though, can aberrantly express PD-L1, halting normal immune surveillance (335). When there is high infiltration of CD8+ T cells, immune checkpoint inhibitors have been shown to more effective as they can restore activation of the immune system (336). The degree of CD8+ T cell infiltration into tumors and expression of PD-L1 have not been studied extensively in the context of MMR deficient DDEC tumors (337). DDEC being a cancer subtype that is in urgent need of therapeutic advances, prognosis could be improved by administration of immune checkpoint inhibitors. In 2019, Ono *et al*., discovered PD-L1 expression in 65% of the 17 DDEC cases in their study group and found that expression of PD-L1 expression was only evident in the undifferentiated component (333). As hypothesized, those DDEC cases that were MMR-deficient, possessed more CD8 positive T cell infiltration (333). Similar results were also independently observed by Hacking *et al.* (338). The results from this examination suggested that DDEC could be a target for immune checkpoint inhibitors especially as the underlying molecular mechanisms associated with the disease otherwise are poorly understood (339). Immune escape mechanisms may be potentially occurring during the process of dedifferentiation but more cases will need to be examined and further experimentation required to examine the actual efficacy of immune checkpoint inhibitors alone or in combination with other treatment strategies.

Only in 2014, did the World Health Organization (WHO) add DDEC to the classification of tumors of the female reproductive organs (327). As the pathology of DDEC has only recently been recognized much more remains to be revealed in terms of clinical features, incidence and treatment. From a pathological standpoint, diagnosis requires differentiating the undifferentiated component from other higher-grade

endometrial cancer. Identification of the interspersed foci of glandular formation within the tumor would favor a poorly differentiated carcinoma over an undifferentiated carcinoma. However, accurate distinction can be difficult in clinical practice, particularly in biopsy sample but sometimes in hysterectomy specimen as well. Diagnostic biomarkers are therefore sought after to aid in proper identification of undifferentiated carcinoma. Candidate immunohistochemical markers include ER and PR due to the fact that they are strongly positive in differentiated regions and are almost to completely lost in undifferentiated areas in addition to keratins, epithelial membrane antigen (EMA) which exhibit an opposite expression tendency (326, 340, 341).

Claudin-4, a protein critical for intercellular junction formation and thereby important in cell adhesion and differentiation too has been discovered to be lowly expressed in low grade (FIGO grade 1 or 2) endometrioid carcinomas (342, 343). Recently a large-scale examination of claudin-4 expression in DDEC was undertaken based on findings in other SWI/SNF deficient tumors that were shown to lack claudin-4 expression (344). This study revealed that the undifferentiated component of DDEC tumors consistently exhibits loss of claudin-4 while the differentiated low-grade endometrioid component possess claudin-4 (345). This suggests that absence of claudin-4 is a frequent observation during cellular dedifferentiation in the context of the endometrium (345). The mechanisms behind acquisition of claudin-4 loss remain to be defined. Currently, loss of or reduced claudin-4 levels appears to only indicate the primitive, undifferentiated state of portions of DDEC tumors. Intriguingly, other endometrial cancer subtypes also exhibited claudin-4 loss in a significant proportion of cases, hinting that absence of claudin-4 is not specific to DDEC and highlights the

importance of considering claudin-4 loss in conjunction with other histological and immunophenotypical features during diagnosis.

Focal expression of neuroendocrine markers such as chromogranin, synaptophysin or CD56 is evident within the undifferentiated components (346). Carcinomas originating from various organs, with neuroendocrine differentiation have typically been associated with a poorer prognosis (346-348). A series of 4 DDEC tumors were closely inspected for neuroendocrine features in Espinosa et al. with at least 70% of cells within the undifferentiated portions of DDEC tumors staining positive for neuroendocrine expression (346). This work demonstrates again how heterogenous at the molecular level DDEC happens to be and how complex the genomic landscape and interplay of mutations is that leads to acquisition of dedifferentiation in endometrial cancer.

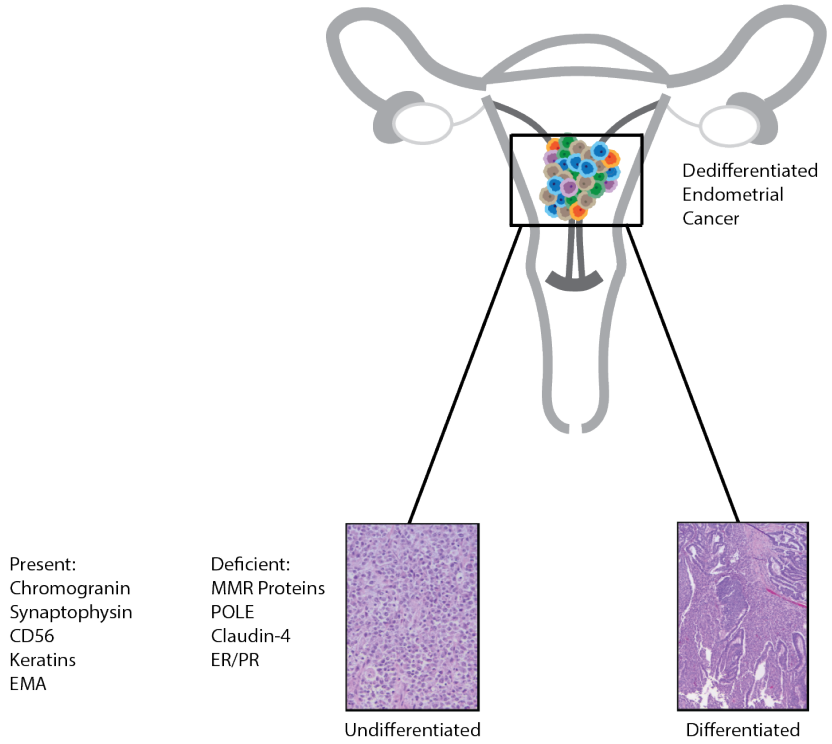


Figure 1.9. Proteins found to either be expressed at high levels or mutated and/or absent within the undifferentiated component of DDEC tumors. Representative H&E images from the undifferentiated and differentiated regions of a DDEC tumor.

1.9. Thesis Rationale, Hypothesis and Aims:

Although there are a growing number of human cancers that exhibit dedifferentiated, embryonic-like phenotypes, there still exists a lack of insight as to how such features are developed and sustained over time. Next-generation sequencing has revealed loss of chromatin remodeling proteins in many different cancer types. I hypothesize that loss of the chromatin remodelling proteins such as SMARCA4, SMARCB1 and ARID1B is critical to attainment of dedifferentiated endometrial cancer. These may be adopted as biomarkers to improve diagnostic accuracy (Chapter 2). Through the development of cell line models of DDEC, I examined how SMARCA4 loss contributes to cellular dedifferentiation and the role chromatin remodeling proteins may play in the acquisition of stemness and aggressiveness by endometrial cancer cells

(Chapter 3). To address the lack of treatment options for DDEC patients, I also explored whether taking a synthetic lethality-based approach may be the best strategy to overcoming the chemotherapy resistance and metastasis exhibited by SMARCA4 deficient dedifferentiated regions of DDEC tumors (Chapter 4). Taken together, extensive characterization of DDEC will serve to deepen our understanding of cellular dedifferentiation at the molecular level and will only further our understanding of the role epigenetics play in the ability of cancers to adapt through hijacking of developmental pathways. Furthermore, DDEC is an ideal tumor type to assess the suitability of combination therapies to combat heterogenous cancers with propensities towards exhibiting cancer cell plasticity.

1.10. References

1. da Silva-Diz V, Lorenzo-Sanz L, Bernat-Peguera A, Lopez-Cerda M, Munoz P. Cancer cell plasticity: Impact on tumor progression and therapy response. Semin Cancer Biol. 2018;53:48-58.
2. Rich JN. Cancer stem cells: understanding tumor hierarchy and heterogeneity. Medicine (Baltimore). 2016;95(1 Suppl 1):S2-7.
3. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013;501(7467):328-37.
4. Schwitalla S. Tumor cell plasticity: the challenge to catch a moving target. J Gastroenterol. 2014;49(4):618-27.
5. Das PK, Pillai S, Rakib MA, Khanam JA, Gopalan V, Lam AKY, et al. Plasticity of Cancer Stem Cell: Origin and Role in Disease Progression and Therapy Resistance. Stem Cell Rev Rep. 2020.
6. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976;194(4260):23-8.
7. Marjanovic ND, Weinberg RA, Chaffer CL. Cell plasticity and heterogeneity in cancer. Clin Chem. 2013;59(1):168-79.
8. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer. 2012;12(5):323-34.
9. Gerlinger M, Swanton C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. Brit J Cancer. 2010;103(8):1139-43.
10. Swanton C. Intratumor Heterogeneity: Evolution through Space and Time. Cancer Res. 2012;72(19):4875-82.

11. Waclaw B, Bozic I, Pittman ME, Hruban RH, Vogelstein B, Nowak MA. A spatial model predicts that dispersal and cell turnover limit intratumour heterogeneity. Nature. 2015;525(7568):261-+.
12. Anderson ARA, Weaver AM, Cummings PT, Quaranta V. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. Cell. 2006;127(5):905-15.
13. Gerdes MJ, Sood A, Sevinsky C, Pris AD, Zavodszky MI, Ginty F. Emerging understanding of multiscale tumor heterogeneity. Front Oncol. 2014;4:366.
14. Michor F, Polyak K. The origins and implications of intratumor heterogeneity. Cancer Prev Res (Phila). 2010;3(11):1361-4.
15. Dick JE. Looking ahead in cancer stem cell research. Nat Biotechnol. 2009;27(1):44-6.
16. Hernandez-Camarero P, Jimenez G, Lopez-Ruiz E, Barungi S, Marchal JA, Peran M. Revisiting the dynamic cancer stem cell model: Importance of tumour edges. Crit Rev Oncol Hematol. 2018;131:35-45.
17. Clevers H. The cancer stem cell: premises, promises and challenges. Nat Med. 2011;17(3):313-9.
18. Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. Cancer Cell. 2012;21(3):283-96.
19. Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cell concept. J Clin Invest. 2010;120(1):41-50.
20. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell. 2014;14(3):275-91.
21. Cabrera MC, Hollingsworth RE, Hurt EM. Cancer stem cell plasticity and tumor hierarchy. World J Stem Cells. 2015;7(1):27-36.
22. Greaves M. Evolutionary determinants of cancer. Cancer Discov. 2015;5(8):806-20.
23. Ohno S, Kaplan WD, Kinosita R. Formation of the sex chromatin by a single X-chromosome in liver cells of Rattus norvegicus. Exp Cell Res. 1959;18:415-8.
24. Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature. 1961;190:372-3.
25. Riggs AD. X inactivation, differentiation, and DNA methylation. Cytogenet Cell Genet. 1975;14(1):9-25.
26. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. Science. 1975;187(4173):226-32.
27. Laisne M, Gupta N, Kirsh O, Pradhan S, Defossez PA. Mechanisms of DNA Methyltransferase Recruitment in Mammals. Genes (Basel). 2018;9(12).
28. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature. 1997;389(6648):251-60.
29. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21(3):381-95.
30. Fierz B, Poirier MG. Biophysics of Chromatin Dynamics. Annu Rev Biophys. 2019;48:321-45.
31. Zhang R, Erler J, Langowski J. Histone Acetylation Regulates Chromatin Accessibility: Role of H4K16 in Inter-nucleosome Interaction. Biophys J. 2017;112(3):450-9.

32. Chang L, Takada S. Histone acetylation dependent energy landscapes in tri-nucleosome revealed by residue-resolved molecular simulations. Sci Rep. 2016;6:34441.
33. Hezroni H, Tzchori I, Davidi A, Mattout A, Biran A, Nissim-Rafinia M, et al. H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells. Nucleus. 2011;2(4):300-9.
34. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389(6649):349-52.
35. Bhanu NV, Sidoli S, Garcia BA. Histone modification profiling reveals differential signatures associated with human embryonic stem cell self-renewal and differentiation. Proteomics. 2016;16(3):448-58.
36. Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol. 2005;6(11):838-49.
37. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature. 2001;410(6824):120-4.
38. Huyen Y, Zgheib O, Ditullio RA, Jr., Gorgoulis VG, Zacharatos P, Petty TJ, et al. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature. 2004;432(7015):406-11.
39. Wysocka J, Swigut T, Milne TA, Dou Y, Zhang X, Burlingame AL, et al. WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. Cell. 2005;121(6):859-72.
40. Li B, Gogol M, Carey M, Lee D, Seidel C, Workman JL. Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science. 2007;316(5827):1050-4.
41. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, et al. Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci U S A. 2002;99(13):8695-700.
42. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, et al. Active genes are tri-methylated at K4 of histone H3. Nature. 2002;419(6905):407-11.
43. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet. 2007;39(3):311-8.
44. Soldi M, Mari T, Nicosia L, Musiani D, Sigismondo G, Cuomo A, et al. Chromatin proteomics reveals novel combinatorial histone modification signatures that mark distinct subpopulations of macrophage enhancers. Nucleic Acids Res. 2017;45(21):12195-213.
45. Steger DJ, Lefterova MI, Ying L, Stonestrom AJ, Schupp M, Zhuo D, et al. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. Mol Cell Biol. 2008;28(8):2825-39.
46. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature. 2006;441(7091):349-53.
47. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells. Cell. 2006;125(2):301-13.

48. Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, et al. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. Mol Cell. 2008;30(6):755-66.
49. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, et al. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev. 2004;18(11):1251-62.
50. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012;13(5):343-57.
51. Zhang T, Cooper, S., Brockdorff, N. The interplay of histone modifications – writers that read. Embo Rep. 2015;15(11):1467-81.
52. Piunti A, Shilatifard A. Epigenetic balance of gene expression by Polycomb and COMPASS families. Science. 2016;352(6290):aad9780.
53. Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. Science. 2017;357(6348).
54. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012;22(1):9-20.
55. Suva ML, Riggi N, Bernstein BE. Epigenetic reprogramming in cancer. Science. 2013;339(6127):1567-70.
56. Laugesen A, Helin K. Chromatin repressive complexes in stem cells, development, and cancer. Cell Stem Cell. 2014;14(6):735-51.
57. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell. 1992;69(6):915-26.
58. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247-57.
59. Yin Y, Morgunova E, Jolma A, Kaasinen E, Sahu B, Khund-Sayeed S, et al. Impact of cytosine methylation on DNA binding specificities of human transcription factors. Science. 2017;356(6337).
60. Dennis K, Fan T, Geiman T, Yan Q, Muegge K. Lsh, a member of the SNF2 family, is required for genome-wide methylation. Genes Dev. 2001;15(22):2940-4.
61. Myant K, Termanis A, Sundaram AY, Boe T, Li C, Merusi C, et al. LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. Genome Res. 2011;21(1):83-94.
62. Esteve PO, Chin HG, Smallwood A, Feehery GR, Gangisetty O, Karpf AR, et al. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. Genes Dev. 2006;20(22):3089-103.
63. Mancini DN, Singh SM, Archer TK, Rodenhiser DI. Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors. Oncogene. 1999;18(28):4108-19.
64. Kitazawa S, Kitazawa R, Maeda S. Transcriptional regulation of rat cyclin D1 gene by CpG methylation status in promoter region. J Biol Chem. 1999;274(40):28787-93.
65. Bell AC, Felsenfeld G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature. 2000;405(6785):482-5.
66. Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature. 2000;405(6785):486-9.

67. Kemme CA, Marquez R, Luu RH, Iwahara J. Potential role of DNA methylation as a facilitator of target search processes for transcription factors through interplay with methyl-CpG-binding proteins. Nucleic Acids Res. 2017;45(13):7751-9.
68. Kaffer CR, Srivastava M, Park KY, Ives E, Hsieh S, Batlle J, et al. A transcriptional insulator at the imprinted H19/Igf2 locus. Genes Dev. 2000;14(15):1908-19.
69. Clark SJ, Harrison J, Molloy PL. Sp1 binding is inhibited by (m)Cp(m)CpG methylation. Gene. 1997;195(1):67-71.
70. Noguchi H, Miyagi-Shiohira C, Nakashima Y. Induced Tissue-Specific Stem Cells and Epigenetic Memory in Induced Pluripotent Stem Cells. Int J Mol Sci. 2018;19(4).
71. Bibikova M, Chudin E, Wu B, Zhou L, Garcia EW, Liu Y, et al. Human embryonic stem cells have a unique epigenetic signature. Genome Res. 2006;16(9):1075-83.
72. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358(11):1148-59.
73. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer. 2011;11(10):726-34.
74. Brunetti L, Gundry MC, Goodell MA. DNMT3A in Leukemia. Cold Spring Harb Perspect Med. 2017;7(2).
75. Zheng Y, Zhang H, Wang Y, Li X, Lu P, Dong F, et al. Loss of Dnmt3b accelerates MLL-AF9 leukemia progression. Leukemia. 2016;30(12):2373-84.
76. Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, et al. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43(8):768-75.
77. Lu R, Wang P, Parton T, Zhou Y, Chrysovergis K, Rockowitz S, et al. Epigenetic Perturbations by Arg882-Mutated DNMT3A Potentiate Aberrant Stem Cell Gene-Expression Program and Acute Leukemia Development. Cancer Cell. 2016;30(1):92-107.
78. Yang L, Rodriguez B, Mayle A, Park HJ, Lin X, Luo M, et al. DNMT3A Loss Drives Enhancer Hypomethylation in FLT3-ITD-Associated Leukemias. Cancer Cell. 2016;30(2):363-5.
79. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328-33.
80. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321(5897):1807-12.
81. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360(8):765-73.
82. Young MD, Willson TA, Wakefield MJ, Trounson E, Hilton DJ, Blewitt ME, et al. ChIP-seq analysis reveals distinct H3K27me3 profiles that correlate with transcriptional activity. Nucleic Acids Res. 2011;39(17):7415-27.
83. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature. 2007;448(7153):553-60.
84. Zhu B, Zheng Y, Pham AD, Mandal SS, Erdjument-Bromage H, Tempst P, et al. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol Cell. 2005;20(4):601-11.

85. Kim J, Guermah M, McGinty RK, Lee JS, Tang Z, Milne TA, et al. RAD6-Mediated Transcription-Coupled H2B Ubiquitylation Directly Stimulates H3K4 Methylation in Human Cells. Cell. 2009;137(3):459-71.
86. Wen Y, Cai J, Hou Y, Huang Z, Wang Z. Role of EZH2 in cancer stem cells: from biological insight to a therapeutic target. Oncotarget. 2017;8(23):37974-90.
87. Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med. 2016;22(2):128-34.
88. Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A. 2010;107(49):20980-5.
89. Poli VF, L.; Zippo A. . Tumorigenic Cell Reprogramming and Cancer Plasticity: Interplay between Signaling, Microenvironment, and Epigenetics. Stem Cells International. 2018.
90. Simon JA, Kingston RE. Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. Mol Cell. 2013;49(5):808-24.
91. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487-500.
92. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell. 2012;150(1):12-27.
93. Hodges C, Kirkland JG, Crabtree GR. The Many Roles of BAF (mSWI/SNF) and PBAF Complexes in Cancer. Cold Spring Harb Perspect Med. 2016;6(8).
94. Zhang J, Dominguez-Sola D, Hussein S, Lee JE, Holmes AB, Bansal M, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. Nat Med. 2015;21(10):1190-8.
95. Ryan RJ, Drier Y, Whitton H, Cotton MJ, Kaur J, Issner R, et al. Detection of Enhancer-Associated Rearrangements Reveals Mechanisms of Oncogene Dysregulation in B-cell Lymphoma. Cancer Discov. 2015;5(10):1058-71.
96. Shilatifard A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu Rev Biochem. 2012;81:65-95.
97. Sanjuan-Pla A, Bueno C, Prieto C, Acha P, Stam RW, Marschalek R, et al. Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia. Blood. 2015;126(25):2676-85.
98. Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes Dev. 2003;17(24):3029-35.
99. Somervaille TCP, Matheny CJ, Spencer GJ, Iwasaki M, Rinn JL, Witten DM, et al. Hierarchical Maintenance of MLL Myeloid Leukemia Stem Cells Employs a Transcriptional Program Shared with Embryonic Rather Than Adult Stem Cells. Cell Stem Cell. 2009;4(2):129-40.
100. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature. 2006;442(7104):818-22.
101. Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. Mol Cell. 2012;48(4):491-507.

102. Audergon PN, Catania S, Kagansky A, Tong P, Shukla M, Pidoux AL, et al. Epigenetics. Restricted epigenetic inheritance of H3K9 methylation. Science. 2015;348(6230):132-5.
103. Ragunathan K, Jih G, Moazed D. Epigenetics. Epigenetic inheritance uncoupled from sequence-specific recruitment. Science. 2015;348(6230):1258699.
104. Liau BB, Sievers C, Donohue LK, Gillespie SM, Flavahan WA, Miller TE, et al. Adaptive Chromatin Remodeling Drives Glioblastoma Stem Cell Plasticity and Drug Tolerance. Cell Stem Cell. 2017;20(2):233-46 e7.
105. Torres CM, Biran A, Burney MJ, Patel H, Henser-Brownhill T, Cohen AS, et al. The linker histone H1.0 generates epigenetic and functional intratumor heterogeneity. Science. 2016;353(6307).
106. Wainwright EN, Scaffidi P. Epigenetics and Cancer Stem Cells: Unleashing, Hijacking, and Restricting Cellular Plasticity. Trends Cancer. 2017;3(5):372-86.
107. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet. 2012;44(3):251-3.
108. Lewis PW, Muller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, et al. Inhibition of PRC2 Activity by a Gain-of-Function H3 Mutation Found in Pediatric Glioblastoma. Science. 2013;340(6134):857-61.
109. Funato K, Major T, Lewis PW, Allis CD, Tabar V. Use of human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation. Science. 2014;346(6216):1529-33.
110. Romero OA, Sanchez-Cespedes M. The SWI/SNF genetic blockade: effects in cell differentiation, cancer and developmental diseases. Oncogene. 2014;33(21):2681-9.
111. Cairns BR, Kim YJ, Sayre MH, Laurent BC, Kornberg RD. A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc Natl Acad Sci U S A. 1994;91(5):1950-4.
112. Cote J, Quinn J, Workman JL, Peterson CL. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science. 1994;265(5168):53-60.
113. Wang W, Cote J, Xue Y, Zhou S, Khavari PA, Biggar SR, et al. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. Embo J. 1996;15(19):5370-82.
114. Phelan ML, Sif S, Narlikar GJ, Kingston RE. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell. 1999;3(2):247-53.
115. Wu JI, Lessard J, Crabtree GR. Understanding the words of chromatin regulation. Cell. 2009;136(2):200-6.
116. Mashtalir N, D'Avino, AR., Michel, BC., Luo, J., Pan, J., Otto, JE., Zullow, HJ., McKenzie, ZM., Kubiak, RL., St Pierre, R., Valencia, AM., Poynter, SJ., Cassel, SH., Ranish, JA., Kadoch, C. Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. Cell. 2018;175(5):1272-88.
117. Flowers S, Nagl NG, Jr., Beck GR, Jr., Moran E. Antagonistic roles for BRM and BRG1 SWI/SNF complexes in differentiation. J Biol Chem. 2009;284(15):10067-75.
118. de la Serna IL, Ohkawa Y, Imbalzano AN. Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers. Nat Rev Genet. 2006;7(6):461-73.

119. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv. 2015;1(5):e1500447.
120. Dechassa M, Sabri, A., Pondugula, S., Kassabov, Sr., Chatterjee, N., Kladde, MP., Bartholomew, B. SWI/SNF has intrinsic nucleosome dissambly activity that is dependent on adjacent nucleosomes. Mol Cell. 2010;38(4):590-602.
121. He S, Wu Z, Tian Y, Yu Z, Yu J, Wang X, et al. Structure of nucleosome-bound human BAF complex. Science. 2020;367(6480):875-81.
122. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer. 2011;11(7):481-92.
123. Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: the industrial revolution of DNA around histones. Nat Rev Mol Cell Biol. 2006;7(6):437-47.
124. Gao X, Tate P, Hu P, Tjian R, Skarnes WC, Wang Z. ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a. Proc Natl Acad Sci U S A. 2008;105(18):6656-61.
125. Singhal N, Graumann J, Wu G, Arauzo-Bravo MJ, Han DW, Greber B, et al. Chromatin-Remodeling Components of the BAF Complex Facilitate Reprogramming. Cell. 2010;141(6):943-55.
126. Gunes C, Paszkowski-Rogacz, M., Rahmig, S., Camgoz, A., Wermke, M., Dahl, A., Bornhauser, M., Waskow, C., Bucholz, F. Comparative RNAi Screens in Isogenic Human Stem Cells Reveal SMARCA4 as a Differential Regulator. Stem Cell Reports. 2019;12(5):1084-98.
127. Trouche D, Le Chalony C, Muchardt C, Yaniv M, Kouzarides T. RB and hbrm cooperate to repress the activation functions of E2F1. Proc Natl Acad Sci U S A. 1997;94(21):11268-73.
128. Cheng SW, Davies KP, Yung E, Beltran RJ, Yu J, Kalpana GV. c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. Nat Genet. 1999;22(1):102-5.
129. Trotter KW, Archer TK. The BRG1 transcriptional coregulator. Nucl Recept Signal. 2008;6:e004.
130. St Pierre R, Kadoch C. Mammalian SWI/SNF complexes in cancer: emerging therapeutic opportunities. Curr Opin Genet Dev. 2017;42:56-67.
131. Wang Y, Hoang L, Ji JX, Huntsman DG. SWI/SNF Complex Mutations in Gynecologic Cancers: Molecular Mechanisms and Models. Annu Rev Pathol. 2020;15:467-92.
132. Jagani Z, Mora-Blanco EL, Sansam CG, McKenna ES, Wilson B, Chen D, et al. Loss of the tumor suppressor Snf5 leads to aberrant activation of the Hedgehog-Gli pathway. Nat Med. 2010;16(12):1429-33.
133. Nakayama RTP, J.L.; Valencia A.M et al.,. SMARCB1 is required for widespread BAF complex–mediated activation of enhancers and bivalent promoters. Nature Genetics. 2017;49(11):1613-23.
134. Wang XL, R.S.; Alver, B.H.; et al., . SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. Nature Genetics. 2017;49(2):289-95.
135. Shi J, Whyte WA, Zepeda-Mendoza CJ, Milazzo JP, Shen C, Roe JS, et al. Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. Genes Dev. 2013;27(24):2648-62.

136. Zhu P, Wang Y, Wu J, Huang G, Liu B, Ye B, et al. LncBRM initiates YAP1 signalling activation to drive self-renewal of liver cancer stem cells. Nat Commun. 2016;7:13608.
137. Yang Z, Li C, Fan Z, Liu H, Zhang X, Cai Z, et al. Single-cell Sequencing Reveals Variants in ARID1A, GPRC5A and MLL2 Driving Self-renewal of Human Bladder Cancer Stem Cells. Eur Urol. 2017;71(1):8-12.
138. Eun K, Ham SW, Kim H. Cancer stem cell heterogeneity: origin and new perspectives on CSC targeting. BMB Rep. 2017;50(3):117-25.
139. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell. 2007;11(1):69-82.
140. Charles N, Ozawa T, Squatrito M, Bleau AM, Brennan CW, Hambardzumyan D, et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell. 2010;6(2):141-52.
141. Hamada S, Masamune A, Takikawa T, Suzuki N, Kikuta K, Hirota M, et al. Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells. Biochem Biophys Res Commun. 2012;421(2):349-54.
142. Poltavets V, Kochetkova M, Pitson SM, Samuel MS. The Role of the Extracellular Matrix and Its Molecular and Cellular Regulators in Cancer Cell Plasticity. Front Oncol. 2018;8:431.
143. Vermeulen L, de Sousa e Melo F, Richel DJ, Medema JP. The developing cancer stem-cell model: clinical challenges and opportunities. Lancet Oncol. 2012;13(2):e83-9.
144. Chaffer CL, Marjanovic N.D., Lee, T., Bell, G., Kleer, C.G., Reinhardt, F., D'alessio, A.C., Young, R.A., Weinberg, R.A. Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. Cell. 2013;154(1):61-74.
145. Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, et al. Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. Oncogene. 2009;28(33):2940-7.
146. Rhinn M, Ritschka B, Keyes WM. Cellular senescence in development, regeneration and disease. Development. 2019;146(20).
147. Gonzalez-Meljem JM, Apps JR, Fraser HC, Martinez-Barbera JP. Paracrine roles of cellular senescence in promoting tumourigenesis. Br J Cancer. 2018;118(10):1283-8.
148. Zacarias-Fluck MF, Morancho B, Vicario R, Luque Garcia A, Escorihuela M, Villanueva J, et al. Effect of cellular senescence on the growth of HER2-positive breast cancers. J Natl Cancer Inst. 2015;107(5).
149. Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov. 2017;7(2):165-76.
150. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell. 2003;113(6):703-16.
151. Lee S, Schmitt CA. The dynamic nature of senescence in cancer. Nat Cell Biol. 2019;21(1):94-101.
152. Achuthan S, Santhoshkumar TR, Prabhakar J, Nair SA, Pillai MR. Drug-induced senescence generates chemoresistant stemlike cells with low reactive oxygen species. J Biol Chem. 2011;286(43):37813-29.

153. Milanovic M, Fan DNY, Belenki D, Dabritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature. 2018;553(7686):96-100.
154. Guillon J, Petit C, Moreau M, Toutain B, Henry C, Roche H, et al. Regulation of senescence escape by TSP1 and CD47 following chemotherapy treatment. Cell Death Dis. 2019;10(3):199.
155. Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP, et al. The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. Genes Dev. 2017;31(2):172-83.
156. Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, et al. Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. Nature. 2009;460(7259):1132-5.
157. Chandra T, Ewels PA, Schoenfelder S, Furlan-Magaril M, Wingett SW, Kirschner K, et al. Global reorganization of the nuclear landscape in senescent cells. Cell Rep. 2015;10(4):471-83.
158. Velegzhaninov IO, Ievlev VA, Pylina YI, Shadrin DM, Vakhrusheva OM. Programming of Cell Resistance to Genotoxic and Oxidative Stress. Biomedicines. 2018;6(1).
159. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell. 2010;40(2):179-204.
160. Louka M, Boutou E, Bakou V, Pappa V, Georgoulis A, Sturzbecher HW, et al. DNA Damage Response/Repair in Cancer Stem Cells - Potential vs. Controversies. Advances in DNA Repair. 2015:415-44.
161. Singer E, Judkins J, Salomonis N, Matlaf L, Soteropoulos P, McAllister S, et al. Reactive oxygen species-mediated therapeutic response and resistance in glioblastoma. Cell death & disease. 2015;6.
162. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756-60.
163. Skvortsov S, Debbage P, Lukas P, Skvortsova I. Crosstalk between DNA repair and cancer stem cell (CSC) associated intracellular pathways. Seminars in Cancer Biology. 2015;31:36-42.
164. Mathews LA, Cabarcas SM, Hurt EM, Zhang X, Jaffee EM, Farrar WL. Increased expression of DNA repair genes in invasive human pancreatic cancer cells. Pancreas. 2011;40(5):730-9.
165. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009;458(7239):780-3.
166. Gallmeier E, Hermann PC, Mueller MT, Machado JG, Ziesch A, De Toni EN, et al. Inhibition of ataxia telangiectasia- and Rad3-related function abrogates the in vitro and in vivo tumorigenicity of human colon cancer cells through depletion of the CD133(+) tumor-initiating cell fraction. Stem Cells. 2011;29(3):418-29.
167. Bartucci M, Svensson S, Romania P, Dattilo R, Patrizii M, Signore M, et al. Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy. Cell death and differentiation. 2012;19(5):768-78.

168. Al-Ejeh F, Pajic M, Shi W, Kalimutho M, Miranda M, Nagrial AM, et al. Gemcitabine and CHK1 inhibition potentiate EGFR-directed radioimmunotherapy against pancreatic ductal adenocarcinoma. Clin Cancer Res. 2014;20(12):3187-97.
169. Zhou L, Zhang Y, Chen S, Kmieciak M, Leng Y, Lin H, et al. A regimen combining the Wee1 inhibitor AZD1775 with HDAC inhibitors targets human acute myeloid leukemia cells harboring various genetic mutations. Leukemia. 2015;29(4):807-18.
170. Vitale I, Manic G, De Maria R, Kroemer G, Galluzzi L. DNA Damage in Stem Cells. Mol Cell. 2017;66(3):306-19.
171. Ahmed SU, Carruthers R, Gilmour L, Yildirim S, Watts C, Chalmers AJ. Selective Inhibition of Parallel DNA Damage Response Pathways Optimizes Radiosensitization of Glioblastoma Stem-like Cells. Cancer Res. 2015;75(20):4416-28.
172. Venere M, Hamerlik P, Wu Q, Rasmussen RD, Song LA, Vasanji A, et al. Therapeutic targeting of constitutive PARP activation compromises stem cell phenotype and survival of glioblastoma-initiating cells. Cell death and differentiation. 2014;21(2):258-69.
173. Schulz A, Meyer F, Dubrovska A, Borgmann K. Cancer Stem Cells and Radioresistance: DNA Repair and Beyond. Cancers (Basel). 2019;11(6).
174. Chang CJ, Yang, J.Y., Xia, W., Chen, C.T., Xie, X., Chao, C.H., Woodward, W.A., Hsu, J.M., Hortobagyi, G.N., Hung, M.C. EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-β-catenin signaling. Cancer Cell. 2011;19(1):86-100.
175. Chang CH, Zhang M, Rajapakshe K, Coarfa C, Edwards D, Huang S, et al. Mammary Stem Cells and Tumor-Initiating Cells Are More Resistant to Apoptosis and Exhibit Increased DNA Repair Activity in Response to DNA Damage. Stem Cell Reports. 2015;5(3):378-91.
176. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071-8.
177. Smeenk G, van Attikum H. The chromatin response to DNA breaks: leaving a mark on genome integrity. Annu Rev Biochem. 2013;82:55-80.
178. Papamichos-Chronakis M, Peterson CL. Chromatin and the genome integrity network. Nat Rev Genet. 2013;14(1):62-75.
179. Peterson CL, Almouzni G. Nucleosome Dynamics as Modular Systems that Integrate DNA Damage and Repair. Cold Spring Harbor perspectives in biology. 2013;5(9).
180. Price BD, D'Andrea AD. Chromatin Remodeling at DNA Double-Strand Breaks. Cell. 2013;152(6):1344-54.
181. Adam S, Dabin J, Polo SE. Chromatin plasticity in response to DNA damage: The shape of things to come. DNA Repair (Amst). 2015;32:120-6.
182. Dabin J, Fortuny A, Polo SE. Epigenome Maintenance in Response to DNA Damage. Mol Cell. 2016;62(5):712-27.
183. Seeber A, Hauer M, Gasser SM. Nucleosome remodelers in double-strand break repair. Curr Opin Genet Dev. 2013;23(2):174-84.
184. Lans H, Marteijn JA, Vermeulen W. ATP-dependent chromatin remodeling in the DNA-damage response. Epigenetics Chromatin. 2012;5:4.

185. Schick S, Fournier D, Thakurela S, Sahu SK, Garding A, Tiwari VK. Dynamics of chromatin accessibility and epigenetic state in response to UV damage. Journal of cell science. 2015;128(23):4380-94.
186. Alagoz M, Katsuki Y, Ogiwara H, Ogi T, Shibata A, Kakarougkas A, et al. SETDB1, HP1 and SUV39 promote repositioning of 53BP1 to extend resection during homologous recombination in G2 cells. Nucleic Acids Res. 2015;43(16):7931-44.
187. Gong FD, Miller KM. Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation. Mutat Res-Fund Mol M. 2013;750(1-2):23-30.
188. O'Hagan HM. Chromatin modifications during repair of environmental exposure-induced DNA damage: a potential mechanism for stable epigenetic alterations. Environ Mol Mutagen. 2014;55(3):278-91.
189. Soria G, Polo SE, Almouzni G. Prime, Repair, Restore: The Active Role of Chromatin in the DNA Damage Response. Molecular Cell. 2012;46(6):722-34.
190. Scully R, Xie A. Double strand break repair functions of histone H2AX. Mutat Res. 2013;750(1-2):5-14.
191. Emran AA, Marzese DM, Menon DR, Hammerlindl H, Ahmed F, Richtig E, et al. Commonly integrated epigenetic modifications of differentially expressed genes lead to adaptive resistance in cancer. Epigenomics. 2019;11(7):732-7.
192. De Angelis ML, Francescangeli F, La Torre F, Zeuner A. Stem Cell Plasticity and Dormancy in the Development of Cancer Therapy Resistance. Front Oncol. 2019;9:626.
193. Wilting RH, Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. Drug Resist Updat. 2012;15(1-2):21-38.
194. Chekhun VF, Lukyanova NY, Kovalchuk O, Tryndyak VP, Pogribny IP. Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarcinoma cells reveals novel hyper- and hypomethylated targets. Mol Cancer Ther. 2007;6(3):1089-98.
195. Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, Tze S, et al. Global levels of histone modifications predict prognosis in different cancers. Am J Pathol. 2009;174(5):1619-28.
196. Ponnusamy LM, P.K.S.; Chang, Y-W.; Singh K.P. Role of cellular reprogramming and epigenetic dysregulation in acquired chemoresistance in breast cancer. Cancer Drug Resistance 2019 2:297-312.
197. Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, Davidson NE, et al. Aberrant methylation of the estrogen receptor and E-cadherin 5' CpG islands increases with malignant progression in human breast cancer. Cancer Res. 2000;60(16):4346-8.
198. Aubele M, Schmitt M, Napieralski R, Paepke S, Ettl J, Absmaier M, et al. The Predictive Value of PITX2 DNA Methylation for High-Risk Breast Cancer Therapy: Current Guidelines, Medical Needs, and Challenges. Dis Markers. 2017;2017:4934608.
199. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer. 2002;2(1):48-58.
200. Calcagno AM, Salcido CD, Gillet JP, Wu CP, Fostel JM, Mumau MD, et al. Prolonged drug selection of breast cancer cells and enrichment of cancer stem cell characteristics. J Natl Cancer Inst. 2010;102(21):1637-52.
201. Ishak CA, Classon M, De Carvalho DD. Deregulation of Retroelements as an Emerging Therapeutic Opportunity in Cancer. Trends in Cancer. 2018;4(8):583-97.

202. Guler GD, Tindell CA, Pitti R, Wilson C, Nichols K, KaiWai Cheung T, et al. Repression of Stress-Induced LINE-1 Expression Protects Cancer Cell Subpopulations from Lethal Drug Exposure. Cancer Cell. 2017;32(2):221-37 e13.
203. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 2010;141(1):69-80.
204. Dalvi MP, Wang L, Zhong R, Kollipara RK, Park H, Bayo J, et al. Taxane-Platin-Resistant Lung Cancers Co-develop Hypersensitivity to JumonjiC Demethylase Inhibitors. Cell Rep. 2017;19(8):1669-84.
205. Risom T, Langer EM, Chapman MP, Rantala J, Fields AJ, Boniface C, et al. Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer. Nature communications. 2018;9.
206. Staberg M, Rasmussen RD, Michaelsen SR, Pedersen H, Jensen KE, Villingshoj M, et al. Targeting glioma stem-like cell survival and chemoresistance through inhibition of lysine-specific histone demethylase KDM2B. Molecular Oncology. 2018;12(3):406-20.
207. Vinogradova M, Gehling VS, Gustafson A, Arora S, Tindell CA, Wilson C, et al. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. Nat Chem Biol. 2016;12(7):531-8.
208. Hinohara K, Wu HJ, Vigneau S, McDonald TO, Igarashi KJ, Yamamoto KN, et al. KDM5 Histone Demethylase Activity Links Cellular Transcriptomic Heterogeneity to Therapeutic Resistance. Cancer Cell. 2018;34(6):939-53 e9.
209. Leadem BR, Kagiampakis I, Wilson C, Cheung TK, Arnott D, Trojer P, et al. A KDM5 Inhibitor Increases Global H3K4 Trimethylation Occupancy and Enhances the Biological Efficacy of 5-Aza-2'-Deoxycytidine. Cancer Res. 2018;78(5):1127-39.
210. White JCP, P.; Crea, F. The role of histone lysine demethylases in cancer cells' resistance to tyrosine kinase inhibitors. Cancer Drug Resistance. 2019;2:326-34.
211. Tian X, Zhang S, Liu HM, Zhang YB, Blair CA, Mercola D, et al. Histone lysine-specific methyltransferases and demethylases in carcinogenesis: new targets for cancer therapy and prevention. Curr Cancer Drug Targets. 2013;13(5):558-79.
212. Suzuki T, Terashima M, Tange S, Ishimura A. Roles of histone methyl-modifying enzymes in development and progression of cancer. Cancer science. 2013;104(7):795-800.
213. Rotili D, Mai A. Targeting Histone Demethylases: A New Avenue for the Fight against Cancer. Genes Cancer. 2011;2(6):663-79.
214. Kang MK, Mehrazarin S, Park NH, Wang CY. Epigenetic gene regulation by histone demethylases: emerging role in oncogenesis and inflammation. Oral Dis. 2017;23(6):709-20.
215. Fu X, Zhang P, Yu B. Advances toward LSD1 inhibitors for cancer therapy. Future Med Chem. 2017;9(11):1227-42.
216. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. 2017;14(10):611-29.
217. Lu YH, Liu YF, Oeck S, Glazer PM. Hypoxia Promotes Resistance to EGFR Inhibition in NSCLC Cells via the Histone Demethylases, LSD1 and PLU-1. Molecular Cancer Research. 2018;16(10):1458-69.

218. Cacan E, Ali MW, Boyd NH, Hooks SB, Greer SF. Inhibition of HDAC1 and DNMT1 modulate RGS10 expression and decrease ovarian cancer chemoresistance. PloS one. 2014;9(1):e87455.
219. Liu X, Yu Y, Zhang J, Lu C, Wang L, Liu P, et al. HDAC1 Silencing in Ovarian Cancer Enhances the Chemotherapy Response. Cell Physiol Biochem. 2018;48(4):1505-18.
220. Cacan E. Histone Deacetylase-1-mediated Suppression of FAS in Chemoresistant Ovarian Cancer Cells. Anticancer research. 2016;36(6):2819-26.
221. Iniguez AB, Alexe G, Wang EJ, Roti G, Patel S, Chen L, et al. Resistance to Epigenetic-Targeted Therapy Engenders Tumor Cell Vulnerabilities Associated with Enhancer Remodeling. Cancer Cell. 2018;34(6):922-38 e7.
222. Manzotti G, Ciarrocchi A, Sancisi V. Inhibition of BET Proteins and Histone Deacetylase (HDACs): Crossing Roads in Cancer Therapy. Cancers (Basel). 2019;11(3).
223. Cai MH, Xu XG, Yan SL, Sun Z, Ying Y, Wang BK, et al. Depletion of HDAC1, 7 and 8 by Histone Deacetylase Inhibition Confers Elimination of Pancreatic Cancer Stem Cells in Combination with Gemcitabine. Sci Rep. 2018;8.
224. Evans T, Sany O, Pearmain P, Ganesan R, Blann A, Sundar S. Differential trends in the rising incidence of endometrial cancer by type: data from a UK population-based registry from 1994 to 2006. Br J Cancer. 2011;104(9):1505-10.
225. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a cancer journal for clinicians. 2011;61(2):69-90.
226. Zhang S, Gong TT, Liu FH, Jiang YT, Sun H, Ma XX, et al. Global, Regional, and National Burden of Endometrial Cancer, 1990-2017: Results From the Global Burden of Disease Study, 2017. Front Oncol. 2019;9:1440.
227. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
228. Sozen H, Vatansever D, Iyibozkurt AC, Topuz S, Ozsurmeli M, Salihoglu Y, et al. Clinicopathologic and survival analyses of synchronous primary endometrial and epithelial ovarian cancers. J Obstet Gynaecol Res. 2015;41(11):1813-9.
229. Merritt MA, Cramer DW. Molecular pathogenesis of endometrial and ovarian cancer. Cancer Biomark. 2010;9(1-6):287-305.
230. Brenner DR, Weir HK, Demers AA, Ellison LF, Louzado C, Shaw A, et al. Projected estimates of cancer in Canada in 2020. CMAJ. 2020;192(9):E199-E205.
231. Creasman WT, Odicino F, Maisonneuve P, Beller U, Benedet JL, Heintz AP, et al. Carcinoma of the corpus uteri. J Epidemiol Biostat. 2001;6(1):47-86.
232. Obel JC, Friberg G, Fleming GF. Chemotherapy in endometrial cancer. Clin Adv Hematol Oncol. 2006;4(6):459-68.
233. Lax SF, Kurman RJ. A dualistic model for endometrial carcinogenesis based on immunohistochemical and molecular genetic analyses. Verh Dtsch Ges Pathol. 1997;81:228-32.
234. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73.

235. Cheung LW, Hennessy BT, Li J, Yu S, Myers AP, Djordjevic B, et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. Cancer Discov. 2011;1(2):170-85.
236. Levine RL, Cargile CB, Blazes MS, van Rees B, Kurman RJ, Ellenson LH. PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. Cancer Res. 1998;58(15):3254-8.
237. Byron SA, Gartside M, Powell MA, Wellens CL, Gao F, Mutch DG, et al. FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features. PloS one. 2012;7(2):e30801.
238. McConechy M, Ding, JR., Cheang, MC., Wiegand, K., Senz, J., Tone, A., Yang, W., Prentice, L., Tse, K., Zeng, T., McDonald, H., Schmidt, AP., Mutch, DG., McAlpine, JN., Hirst, M., Shah, SP., Lee, CH., Goodfellow, PJ., Gilks, CB. Huntsman, D.G. Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol. 2012;228(1):20-30.
239. Urick M, Rudd, ML., Godwin, AK., Sgroi, D., Merino, M., Bell, DW. PIK3R1 (p85α) Is Somatically Mutated at High Frequency in Primary Endometrial Cancer. Cancer Res. 2011;71(12):4061-7.
240. Kuhn E, Wu RC, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. J Natl Cancer Inst. 2012;104(19):1503-13.
241. Le Gallo M, O'Hara AJ, Rudd ML, Urick ME, Hansen NF, O'Neil NJ, et al. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. Nat Genet. 2012;44(12):1310-5.
242. Kuhn E, Ayhan A, Bahadirli-Talbott A, Zhao C, Shih Ie M. Molecular characterization of undifferentiated carcinoma associated with endometrioid carcinoma. The American journal of surgical pathology. 2014;38(5):660-5.
243. Kim YS, Yi BR, Kim NH, Choi KC. Role of the epithelial-mesenchymal transition and its effects on embryonic stem cells. Exp Mol Med. 2014;46:e108.
244. Lamouille S, Xu, J., Derynck, R. Molecular mechamisms of epithelial-mesenchymal transition. Nature Reviews Molecular Cell Biology. 2014;15:178-96.
245. Kudo-Saito C, Shirako, H., Takeuchi, T., Kawakami, Y. Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell. 2009;15(3):195-206.
246. Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, et al. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer science. 2010;101(2):293-9.
247. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene. 2010;29(34):4741-51.
248. Thiery JP. Epithelial-mesenchymal transitions in tumor progression. Nat Rev Cancer. 2002;2(6):442-54.
249. Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X. Epithelial-to-mesenchymal transition and stem cells in endometrial cancer. Hum Pathol. 2013;44(10):1973-81.

250. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature. 2004;432(7015):332-7.
251. Montserrat N, Mozos A, Llobet D, Dolcet X, Pons C, de Herreros AG, et al. Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma. Hum Pathol. 2012;43(5):632-43.
252. Saegusa M, Hashimura M, Kuwata T, Okayasu I. Requirement of the Akt/beta-catenin pathway for uterine carcinosarcoma genesis, modulating E-cadherin expression through the transactivation of slug. Am J Pathol. 2009;174(6):2107-15.
253. Lau MT, Klausen C, Leung PC. E-cadherin inhibits tumor cell growth by suppressing PI3K/Akt signaling via beta-catenin-Egr1-mediated PTEN expression. Oncogene. 2011;30(24):2753-66.
254. Inoue H, Takahashi H, Hashimura M, Eshima K, Akiya M, Matsumoto T, et al. Cooperation of Sox4 with beta-catenin/p300 complex in transcriptional regulation of the Slug gene during divergent sarcomatous differentiation in uterine carcinosarcoma. BMC cancer. 2016;16:53.
255. Bian Y, Chang X, Liao Y, Wang J, Li Y, Wang K, et al. Promotion of epithelial-mesenchymal transition by Frizzled2 is involved in the metastasis of endometrial cancer. Oncol Rep. 2016;36(2):803-10.
256. Liu L, Zhang, J., Yang, X., Fang, C., Xu, H., Xi, X. SALL4 as an Epithelial-Mesenchymal Transition and Drug Resistance Inducer Through the Regulation of c-Myc in Endometrial Cancer. PloS one. 2015;10(9).
257. Gumireddy K, Li, A., Gimotty, P.A., Klein-Szanto, A.J., Showe, L.C., Katsaros, D., Coukos, G., Zhang, L., Huang, Q. KLF17 is a negative regulator of epithelial–mesenchymal transition and metastasis in breast cancer. Nature Cell Biology. 2009:1297–304.
258. Cai XD, Zhou YB, Huang LX, Zeng QL, Zhang LJ, Wang QQ, et al. Reduced expression of Kruppel-like factor 17 is related to tumor growth and poor prognosis in lung adenocarcinoma. Biochem Biophys Res Commun. 2012;418(1):67-73.
259. Liu FY, Deng YL, Li Y, Zeng D, Zhou ZZ, Tian DA, et al. Down-regulated KLF17 expression is associated with tumor invasion and poor prognosis in hepatocellular carcinoma. Medical oncology (Northwood, London, England). 2013;30(1):425.
260. Dong P, Kaneuchi, M., Xiong, Y., Cao, L., Cai, M., Liu, X., Guo, S-W., Ju, J., Jia, N., Konno, Y., Watari, H., Hosaka, M., Sudo, S., Sakuragi, N. Identification of KLF17 as a novel epithelial to mesenchymal transition inducer via direct activation of TWIST1 in endometrioid endometrial cancer. Carcinogenesis. 2014;35(4):760-8.
261. Bao W, Qiu H, Yang T, Luo X, Zhang H, Wan X. Upregulation of TrkB promotes epithelial-mesenchymal transition and anoikis resistance in endometrial carcinoma. PloS one. 2013;8(7):e70616.
262. Douma S, Van Laar T, Zevenhoven J, Meuwissen R, Van Garderen E, Peeper DS. Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature. 2004;430(7003):1034-9.
263. Zheng W, Dai Q, Tao P, Sun A, Wang Y, Bao L, et al. Overexpression of tyrosine kinase receptor B promotes metastasis of ovarian serous adenocarcinoma by lymphangiogenesis. Tumori. 2011;97(6):756-61.
264. Altenberg B, Greulich, KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics. 2004;84(6):1014-20.

265. Zhao M, Fang, W., Wang, Y., Guo, S., Shu, L., Wang, L., Chen, Y., Fu, Q., Liu, Y., Hua, S., Fan, Y., Liu, Y., Deng, X., Luo, R., Mei, Z., Jiang, Q., Liu. Enolase-1 Is a Therapeutic Target in Endometrial Carcinoma. Oncotarget. 2015;6(17):15610-27.
266. Guo J, Ye F, Jiang X, Guo H, Xie W, Zhang Y, et al. Drp1 mediates high glucose-induced mitochondrial dysfunction and epithelial-mesenchymal transition in endometrial cancer cells. Exp Cell Res. 2020;389(1):111880.
267. Gu CJ, Xie F, Zhang B, Yang HL, Cheng J, He YY, et al. High Glucose Promotes Epithelial-Mesenchymal Transition of Uterus Endometrial Cancer Cells by Increasing ER/GLUT4-Mediated VEGF Secretion. Cell Physiol Biochem. 2018;50(2):706-20.
268. Wang C, Su K, Zhang Y, Zhang W, Zhao Q, Chu D, et al. IR-A/IGF-1R-mediated signals promote epithelial-mesenchymal transition of endometrial carcinoma cells by activating PI3K/AKT and ERK pathways. Cancer Biol Ther. 2019;20(3):295-306.
269. Tsai HC, Baylin SB. Cancer epigenetics: linking basic biology to clinical medicine. Cell Res. 2011;21(3):502-17.
270. Castilla M, Moreno-Bueno, G., Romero-Perez, L., Van De Vijver, K., Biscuola, M., Lopez-Garcia, MA., Prat, J., Matias-Guiu, X., Cano, A., Oliva, E., Palacios, J. Micro-RNA Signature of the Epithelial-Mesenchymal Transition in Endometrial Carcinosarcoma. J Pathol. 2011;223(1):72-80.
271. Lin X, Qui, J., Hua, K. Long non-coding RNAs as emerging regulators of epithelial to mesenchymal transition in gynecologic cancers. BioScience Trends. 2018;12(4):342-53.
272. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet. 2008;9(2):102-14.
273. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature. 2011;472(7341):120-4.
274. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA. 2010;16(8):1478-87.
275. Dong P, Kaneuchi M, Watari H, Hamada J, Sudo S, Ju J, et al. MicroRNA-194 inhibits epithelial to mesenchymal transition of endometrial cancer cells by targeting oncogene BMI-1. Mol Cancer. 2011;10:99.
276. Yu J, Chen, L., Bao, Z., Liu, Y., Liu, G., Liu, G., Li, F., Li, L. BMI-1 Promotes Invasion and Metastasis in Endometrial Adenocarcinoma and Is a Poor Prognostic Factor. Oncol Rep. 2020;43(5):1630-40.
277. Konno Y, Dong, P., Xiong, Y., Suzuki, F., Lu, J., Cai, M., Watari, H., Mitamura, T., Hosaka, M., Hanley, SJB., Kudo, M., Sakuragi, N. MicroRNA-101 Targets EZH2, MCL-1 and FOS to Suppress Proliferation, Invasion and Stem Cell-Like Phenotype of Aggressive Endometrial Cancer Cells. Oncotarget. 2014;5(15):6049-62.
278. Liu P, Wang C, Ma C, Wu Q, Zhang W, Lao G. MicroRNA-23a regulates epithelial-to-mesenchymal transition in endometrial endometrioid adenocarcinoma by targeting SMAD3. Cancer Cell Int. 2016;16(1):67.
279. Dong P, Ihira K, Xiong Y, Watari H, Hanley SJ, Yamada T, et al. Reactivation of epigenetically silenced miR-124 reverses the epithelial-to-mesenchymal transition and

inhibits invasion in endometrial cancer cells via the direct repression of IQGAP1 expression. Oncotarget. 2016;7(15):20260-70.
280. Huang Y, Yang N. MicroRNA-20a-5p inhibits epithelial to mesenchymal transition and invasion of endometrial cancer cells by targeting STAT3. Int J Clin Exp Pathol. 2018;11(12):5715-24.
281. Zhang HH, Li R, Li YJ, Yu XX, Sun QN, Li AY, et al. eIF4Erelated miR320a and miR3405p inhibit endometrial carcinoma cell metastatic capability by preventing TGFbeta1induced epithelialmesenchymal transition. Oncol Rep. 2020;43(2):447-60.
282. Zhao X, Dai L, Yue Q, Wang H, Wang XU, Li Y, et al. MiR-195 inhibits migration, invasion and epithelial-mesenchymal transition (EMT) of endometrial carcinoma cells by targeting SOX4. J Biosci. 2019;44(6).
283. Fang YY, Tan MR, Zhou J, Liang L, Liu XY, Zhao K, et al. miR-214-3p inhibits epithelial-to-mesenchymal transition and metastasis of endometrial cancer cells by targeting TWIST1. Onco Targets Ther. 2019;12:9449-58.
284. Chen P, Xing, T., Want, Q., Liu, A., Liu, H., Hu, Y., Ji, Y. Song, Y. Wang, D. . MicroRNA-202 Inhibits Cell Migration and Invasion Through Targeting FGF2 and Inactivating Wnt/β-catenin Signaling in Endometrial Carcinoma. Biosci Rep. 2019;39(10).
285. Li Y, Huo J, Pan X, Wang C, Ma X. MicroRNA 302b-3p/302c-3p/302d-3p inhibits epithelial-mesenchymal transition and promotes apoptosis in human endometrial carcinoma cells. Onco Targets Ther. 2018;11:1275-84.
286. Wang Z, Wang W, Huang K, Wang Y, Li J, Yang X. MicroRNA-34a inhibits cells proliferation and invasion by downregulating Notch1 in endometrial cancer. Oncotarget. 2017;8(67):111258-70.
287. Wang C, Li Q, He Y. MicroRNA215p promotes epithelial to mesenchymal transition by targeting SRYbox 17 in endometrial cancer. Oncol Rep. 2020;43(6):1897-905.
288. Wang J, Zhang L, Jiang W, Zhang R, Zhang B, Silayiding A, et al. MicroRNA-135a promotes proliferation, migration, invasion and induces chemoresistance of endometrial cancer cells. Eur J Obstet Gynecol Reprod Biol X. 2020;5:100103.
289. Li Y, Sun D, Gao J, Shi Z, Chi P, Meng Y, et al. MicroRNA-373 promotes the development of endometrial cancer by targeting LATS2 and activating the Wnt/beta-Catenin pathway. J Cell Biochem. 2018.
290. Gao X, Cai Y, An R. miR215 promotes epithelial to mesenchymal transition and proliferation by regulating LEFTY2 in endometrial cancer. Int J Mol Med. 2018;42(3):1229-36.
291. Asanoma K, Hori, E., Yoshida, S., Yagi, H., Onoyama, I., Kodama, K., Yasunaga, M., Ohgami, T., Kaneki, E., Okugawa, K. Mutual suppression between BHLHE40/BHLHE41 and the MIR301B-MIR130B cluster is involved in epithelial-to-mesenchymal transition of endometrial cancer cells. Oncotarget. 2019;10(45):4640-54.
292. Yoshida S, Furukawa N, Haruta S, Tanase Y, Kanayama S, Noguchi T, et al. Expression profiles of genes involved in poor prognosis of epithelial ovarian carcinoma: a review. Int J Gynecol Cancer. 2009;19(6):992-7.
293. Burk U, Schubert, J., Wellner, U., Schmalhofer, O., Vincan, E., Spaderna, S., Brabletz, T. A Reciprocal Repression Between ZEB1 and Members of the miR-200 Family Promotes EMT and Invasion in Cancer Cells. Embo Rep. 2008;9(6):582-9.

294. Bracken C, Gregory, PA., Kolesnikoff, N., Bert, AG., Wang, J., Shannon, MF., Goodall, GJ. A Double-Negative Feedback Loop between ZEB1-SIP1 and the microRNA-200 Family Regulates Epithelial-Mesenchymal Transition. 2008;68(19).
295. Park S-A, Kim, LK., Kim, YT., Heo, T-H., Kim, HJ. Long non-coding RNA steroid receptor activator promotes the progression of endometrial cancer via Wnt/ β-catenin signaling pathway. Int J Biol Sci. 2020;16(1):99-115.
296. Li X, Pang L, Yang Z, Liu J, Li W, Wang D. LncRNA HOTAIRM1/HOXA1 Axis Promotes Cell Proliferation, Migration And Invasion In Endometrial Cancer. Onco Targets Ther. 2019;12:10997-1015.
297. Zhu H, Jin, Y-M., Lyu, X-M., Fan, L-M., Wu, F. Long noncoding RNA H19 regulates HIF-1α/AXL signaling through inhibiting miR-20b-5p in endometrial cancer. Cell Cycle. 2019;18(19):2454-64.
298. Spoelstra N, Manning, NG., Higashi, Y., Darling, D., Singh, M., Shroyer, KR., Broaddus, RR., Horwitz, KB., Richer JK. The Transcription Factor ZEB1 Is Aberrantly Expressed in Aggressive Uterine Cancers. 2006;66(7):3893-902.
299. Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem. 2002;277(7):5209-18.
300. van der Horst P, Wang, W., Vandenput, I., Kuhne, LC., Ewing, PC., van IJcken, WFJ., van der Zee, M., Amant, F., Burger, CW., Blok, LJ. Progesterone Inhibits Epithelial-to-Mesenchymal Transition in Endometrial Cancer. PloS one. 2012;7(1).
301. Wik E, Raeder MB, Krakstad C, Trovik J, Birkeland E, Hoivik EA, et al. Lack of estrogen receptor-alpha is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma. Clin Cancer Res. 2013;19(5):1094-105.
302. Zhang H, Li H, Qi S, Liu Z, Fu Y, Li M, et al. Normal endometrial stromal cells regulate 17beta-estradiol-induced epithelial-mesenchymal transition via slug and E-cadherin in endometrial adenocarcinoma cells in vitro. Gynecol Endocrinol. 2017;33(1):82-6.
303. Liu Y, Zhao, R., Chi, S., Zhang, W., Xiao, C., Zhou, X., Zhao, Y., Wang, H. . UBE2C Is Upregulated by Estrogen and Promotes Epithelial–Mesenchymal Transition via p53 in Endometrial Cancer. Mol Cancer Res. 2020;18(2).
304. Muinelo-Romay L, Colas E, Barbazan J, Alonso-Alconada L, Alonso-Nocelo M, Bouso M, et al. High-risk endometrial carcinoma profiling identifies TGF-beta1 as a key factor in the initiation of tumor invasion. Mol Cancer Ther. 2011;10(8):1357-66.
305. Bischof P, Campana A. A putative role for oncogenes in trophoblast invasion? Hum Reprod. 2000;15 Suppl 6:51-8.
306. Lei X, Wang L, Yang J, Sun LZ. TGFbeta signaling supports survival and metastasis of endometrial cancer cells. Cancer Manag Res. 2009;2009(1):15-24.
307. So K, Min, KJ., Hong, JH., Lee, J-K. Interleukin-6 Expression by Interactions Between Gynecologic Cancer Cells and Human Mesenchymal Stem Cells Promotes Epithelial-Mesenchymal Transition. Int J Oncol. 2015;47(4):1451-9.
308. Planaguma J, Abal M, Gil-Moreno A, Diaz-Fuertes M, Monge M, Garcia A, et al. Up-regulation of ERM/ETV5 correlates with the degree of myometrial infiltration in endometrioid endometrial carcinoma. J Pathol. 2005;207(4):422-9.

309. Colas E, Muinelo-Romay L, Alonso-Alconada L, Llaurado M, Monge M, Barbazan J, et al. ETV5 cooperates with LPP as a sensor of extracellular signals and promotes EMT in endometrial carcinomas. Oncogene. 2012;31(45):4778-88.
310. Pedrola N, Devis L, Llaurado M, Campoy I, Martinez-Garcia E, Garcia M, et al. Nidogen 1 and Nuclear Protein 1: novel targets of ETV5 transcription factor involved in endometrial cancer invasion. Clinical & experimental metastasis. 2015;32(5):467-78.
311. Guo B, Sallis RE, Greenall A, Petit MM, Jansen E, Young L, et al. The LIM domain protein LPP is a coactivator for the ETS domain transcription factor PEA3. Mol Cell Biol. 2006;26(12):4529-38.
312. Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y, et al. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proc Natl Acad Sci U S A. 2010;107(7):2989-94.
313. Liu Y, Wang J, Ni T, Wang L, Wang Y, Sun X. CCL20 mediates RANK/RANKL-induced epithelial-mesenchymal transition in endometrial cancer cells. Oncotarget. 2016;7(18):25328-39.
314. Li Y, Che Q, Bian Y, Zhou Q, Jiang F, Tong H, et al. Autocrine motility factor promotes epithelial-mesenchymal transition in endometrial cancer via MAPK signaling pathway. Int J Oncol. 2015;47(3):1017-24.
315. Wang X, Zhang, W., Sun, X., Lin, Y., Chen, W. Cancer-associated Fibroblasts Induce Epithelial-Mesenchymal Transition Through Secreted Cytokines in Endometrial Cancer Cells. Oncol Lett. 2018;15(4):5694-702.
316. Feng Z, Gan H, Cai Z, Li N, Yang Z, Lu G, et al. Aberrant expression of hypoxia-inducible factor 1alpha, TWIST and E-cadherin is associated with aggressive tumor phenotypes in endometrioid endometrial carcinoma. Jpn J Clin Oncol. 2013;43(4):396-403.
317. Wang H, Bao W, Jiang F, Che Q, Chen Z, Wang F, et al. Mutant p53 (p53-R248Q) functions as an oncogene in promoting endometrial cancer by up-regulating REGgamma. Cancer letters. 2015;360(2):269-79.
318. Asakura T, Yamaguchi N, Ohkawa K, Yoshida K. Proteasome inhibitor-resistant cells cause EMT-induction via suppression of E-cadherin by miR-200 and ZEB1. Int J Oncol. 2015;46(5):2251-60.
319. Dong P, Karaayvaz M, Jia N, Kaneuchi M, Hamada J, Watari H, et al. Mutant p53 gain-of-function induces epithelial-mesenchymal transition through modulation of the miR-130b-ZEB1 axis. Oncogene. 2013;32(27):3286-95.
320. Jiang FZ, He YY, Wang HH, Zhang HL, Zhang J, Yan XF, et al. Mutant p53 induces EZH2 expression and promotes epithelial-mesenchymal transition by disrupting p68-Drosha complex assembly and attenuating miR-26a processing. Oncotarget. 2015;6(42):44660-74.
321. Wu ES, Shih Ie M, Diaz-Montes TP. Dedifferentiated endometrioid adenocarcinoma: An under-recognized but aggressive tumor? Gynecol Oncol Case Rep. 2013;5:25-7.
322. Silva EG, Deavers MT, Malpica A. Undifferentiated carcinoma of the endometrium: a review. Pathology. 2007;39(1):134-8.
323. Silva EG, Deavers MT, Bodurka DC, Malpica A. Association of low-grade endometrioid carcinoma of the uterus and ovary with undifferentiated carcinoma: a new type of dedifferentiated carcinoma? Int J Gynecol Pathol. 2006;25(1):52-8.

324. Yokomizo R, Yamada K, Iida Y, Kiyokawa T, Ueda K, Saito M, et al. Dedifferentiated endometrial carcinoma: A report of three cases and review of the literature. Mol Clin Oncol. 2017;7(6):1008-12.
325. Morioka S, Tanase Y, Kawaguchi R, Uchiyama T, Kobayash H. Two Cases of Dedifferentiated Endometrioid Carcinoma: Case Presentation and Brief Review of the Literature. Case Rep Obstet Gynecol. 2018;2018:7624785.
326. Tafe LJ, Garg K, Chew I, Tornos C, Soslow RA. Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms. Mod Pathol. 2010;23(6):781-9.
327. Travaglino A, Raffone A, Mascolo M, Guida M, Insabato L, Zannoni GF, et al. TCGA Molecular Subgroups in Endometrial Undifferentiated/Dedifferentiated Carcinoma. Pathol Oncol Res. 2020;26(3):1411-6.
328. Espinosa I, Lee, CH., D'Angelo, E., Palacios, J., Prat, J. Undifferentiated and Dedifferentiated EndometrialCarcinomas With POLE Exonuclease Domain MutationsHave a Favorable Prognosis. The American journal of surgical pathology. 2017;41:1121-8.
329. Nick McElhinny S, Gordenin, DA., Stith, CM., Burgers, PMJ., Kunkel, TA. Division of labor at the eukaryotic replication fork. Mol Cell. 2008;30(2):137-44.
330. Bakhsh S, Kinloch, M., Hoang, LN., Soslow, RA., Kobel, M., Lee, CH., McAlpine, JN., McConechy, MK., Blake Gilks, C. . Histopathological features of endometrial carcinomas associated with POLE mutations: implications for decisions about adjuvant therapy. Histopathology. 2016;68:916-24.
331. van Gool IC, Eggink FA, Freeman-Mills L, Stelloo E, Marchi E, de Bruyn M, et al. POLE Proofreading Mutations Elicit an Antitumor Immune Response in Endometrial Cancer. Clin Cancer Res. 2015;21(14):3347-55.
332. Nelson B, McAlpine, JN. The more tumors change, the more theystay tame: do T cells keep POLE ultramutated endometrialcarcinomas in check? Gynecol Oncol. 2015;138:1-2.
333. Ono R, Nakayama, K., Nakamura, K., Yamashita, H., Ishibashi, T., Ishikawa, M., Minamoto, T., Razia, S., Ishikawa, N., Otsuki, Y., Nakayama, S., Onuma, H., Kurioka, H., Kyo, S. Dedifferentiated Endometrial Carcinoma Could be A Target for Immune Checkpoint Inhibitors
(Anti PD-1/PD-L1 Antibodies). Int J Mol Sci 2019;20(3744).
334. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509-20.
335. Alsaab H, Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S., Iyer, AK. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Front Pharmacol. 2017;8(8).
336. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-cell Infiltration and PD-L1. Cancer Res. 2015;75(11):2139-45.
337. Fader A, Diaz, LA., Armstrong, DK., Tanner. EJ., Uram, J., Eyring, A., Wang, H., Fisher, G., Grenten, T., Le, D. . Preliminary results of a phase II study: PD-1 blockade in mismatch repair–deficient, recurrent or
persistent endometrial cancer. Gynecol Oncol. 2016;141:206-7.

338. Abcouwer SF, Marjon PL, Loper RK, Vander Jagt DL. Response of VEGF expression to amino acid deprivation and inducers of endoplasmic reticulum stress. Invest Ophthalmol Vis Sci. 2002;43(8):2791-8.
339. Hacking S, Jin C, Komforti M, Liang S, Nasim M. MMR deficient undifferentiated/dedifferentiated endometrial carcinomas showing significant programmed death ligand-1 expression (sp 142) with potential therapeutic implications. Pathol Res Pract. 2019;215(10):152552.
340. Malpica A. How to approach the many faces of endometrioid carcinoma. Modern Pathology. 2016;29:doi.org/10.1038/modpathol.2015.142.
341. Altrabulsi B, Malpica A, Deavers MT, Bodurka DC, Broaddus R, Silva EG. Undifferentiated carcinoma of the endometrium. The American journal of surgical pathology. 2005;29(10):1316-21.
342. Van Itallie C, Rahner, C., Anderson, JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. J Clin Invest. 2001;107:1319-27.
343. Pan XY, Wang B, Che YC, Weng ZP, Dai HY, Peng W. Expression of claudin-3 and claudin-4 in normal, hyperplastic, and malignant endometrial tissue. Int J Gynecol Cancer. 2007;17(1):233-41.
344. Schaefer IM, Agaimy A, Fletcher CD, Hornick JL. Claudin-4 expression distinguishes SWI/SNF complex-deficient undifferentiated carcinomas from sarcomas. Mod Pathol. 2017;30(4):539-48.
345. Tessier-Cloutier B, Soslow RA, Stewart CJR, Kobel M, Lee CH. Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas. Histopathology. 2018;73(2):299-305.
346. Espinosa I, De Leo A, D'Angelo E, Rosa-Rosa JM, Corominas M, Gonzalez A, et al. Dedifferentiated endometrial carcinomas with neuroendocrine features: a clinicopathologic, immunohistochemical, and molecular genetic study. Hum Pathol. 2018;72:100-6.
347. Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. Cancer. 2001;91(11):1992-2000.
348. Aytac E, Ozdemir Y, Ozuner G. Long term outcomes of neuroendocrine carcinomas (high-grade neuroendocrine tumors) of the colon, rectum, and anal canal. J Visc Surg. 2014;151(1):3-7.

2

Examination of clinical cases of dedifferentiated endometrial cancer reveals absence of chromatin remodelling proteins and loss of gynecological differentiation markers

2.1. Introduction

Since Silva *et al.* initially reported dedifferentiated endometrial cancer in 2006-2007, it has steadily become more recognized by pathologists as a subtype of endometrial cancer possessing both differentiated (D) and undifferentiated components (UC) (1-4). In 2014, the work of Kuhn *et al.,* demonstrated somatic mutations were shared across both components hinting at a clonal origin between the differentiated endometrioid and undifferentiated regions of DDEC (2). However, little research has been conducted aiming to understand the molecular mechanisms potentially underlying the hypothesized progression of the low-grade endometrioid carcinoma to undifferentiated carcinoma. Better understanding the oncogenic mechanisms responsible for the development of dedifferentiated endometrial cancer, may allow for earlier detection strategies and improved treatment modalities to be created and adopted to combat such a highly aggressive disease. By targeted sequencing and immunohistochemical analysis, we compared the endometrioid and undifferentiated component of a series of DDEC cases to discover likely molecular events underlying dedifferentiation in most dedifferentiated carcinomas. As inactivation of the SWI/SNF chromatin remodeling proteins, SMARCA4 and SMARCB1, were only observed in half of the histologically prototypical DDEC cases we performed further targeted sequencing and immunohistochemistry studies on not only a different series of endometrial dedifferentiated carcinomas but we also analyzed a larger number of SWI/SNF complex proteins to unearth the mechanism(s) underlying dedifferentiation in the remaining undefined DDEC neoplasms. As the function of the

SWI/SNF complex is associated with transcriptional regulation, tumor cells lacking SWI/SNF subunits appear to possess an inability to activate genes that promote differentiation and cell-cell adhesion. Currently, the presence of the undifferentiated carcinoma component is what mainly separates DDEC from endometrial carcinoma. However, PAX8 and ER expression have been shown to be lost in up to 85-92% and 69-95% of DDEC tumors, respectively (5, 6). Furthermore, aberrant p53 immunostaining has been exhibited in 26-33% of DDEC cases examined (2, 6). As much of the immunophenotypic surveying of DDEC cases took place prior to discoveries that SMARCA4 and SMARCB1 inactivation are a frequent molecular event in DDEC, we also aimed to evaluate the expression of PAX8, ER and p53 in SMARCA4/SMARCB1 deficient and intact DDEC to not only gain further biological insight into these neoplasms but hopefully to also gain diagnostic insights as well. Finally having successfully interrogated DNA and protein expression patterns in the same DDEC samples we sought to further profile our limited formalin-fixed paraffin-embedded (FFPE) DDEC tissue by simultaneously examining RNA and protein levels. The NanoString nCounter Gene Expression Assay fills a niche between gene expression profiling methods that are genome-wide such as microarrays and RNA-sequencing and those assays that are targeted (real-time quantitative PCR (RT-qPCR)) (7). Through the hybridization of sequence-specific fluorescent barcodes, it is now possible to digitally detect and quantify mRNA molecules directly (8). NanoString technology is particularly useful in generating accurate genomic information from small amounts of fixed patient tissue (9). There is also substantial evidence indicating the successful utilization of nCounter systems in both prognostic and predictive settings (9, 10). We used targeted NanoString gene expression

profiling to preliminarily investigate gene expression patterns in DDEC neoplasms, which had lost SMARCA4 protein expression. The purpose of all these studies was to better understand the biology and heterogeneity underlaying DDEC and more specifically illuminate major molecular players pivotal in cellular dedifferentiation in the context of endometrial cancer.

2.2. Results

2.2.1. *Frameshift and nonsense mutations to SWI/SNF complex proteins, SMARCA4 AND SMARCB1, results in their loss in the undifferentiated regions of DDEC neoplasms.*

Targeted next generation sequencing focused on 26 recurrently mutated genes in gynecological carcinomas: *AKT1, ARID1A, FBXW7, FGR2, JAK1, KRAS, MLH1, MSH2, MSH6, NRAS, PIK3CA, PIK3R1, PIK3R2, PMS2, POLE, PPP2R1A, PTEN, RNF43, RPL22, SMARCA4, STK11, SPOP, TPF2, FOXL2, CTNNB1* and *BRAF* was performed on an index series of 8 DDEC cases with both the endometrioid and undifferentiated components analyzed in half of the cases and the undifferentiated component analyzed in the remaining 4 cases. All the DDEC carcinomas displayed a mix of low-grade (FIGO grade 1 or 2) endometroid carcinoma mixed with regions of undifferentiated carcinoma. The undifferentiated component constituted anywhere from 10-95% of the overall tumor and displayed a sheet-like proliferation of monotonous oval to round cells. Tumor necrosis, cellular discohesion and prominent mitotic activity were also evident within the undifferentiated component. Six of the eight tumors were MMR-deficient in both the corresponding endometrioid and undifferentiated components of the same DDEC lesions. We identified recurrent mutations to the following genes: *PTEN* (8/8), *ARID1A* (6/8),

PIK3CA (5/8), *SMARCA4* (5/8), *JAK1* (4/8), *KRAS* (4/8), *PIK3R1* (4/8), *POLE* (4/8), *RPL22* (3/8), *FBXW7* (3/8), *SPOP* (3/8) and *CTNNB1* (2/8) **[Figure 2.1]**. When both the undifferentiated and endometrioid components were both analyzed approximately 67% of the mutations were found in both histologic sections. The overall mutation profile is consistent with the endometrioid histotype, except for the discovery of frequent frameshift and/or nonsense SMARCA4 mutations found solely in the undifferentiated component **[Figure 2.1]** (11). Four tumors possessed inactivating *SMARCA4* mutations and Sanger sequencing confirmed the somatic nature of the frameshift/nonsense *SMARCA4* mutations in these 4 MMR deficient tumors **[Figure 2.1]**.

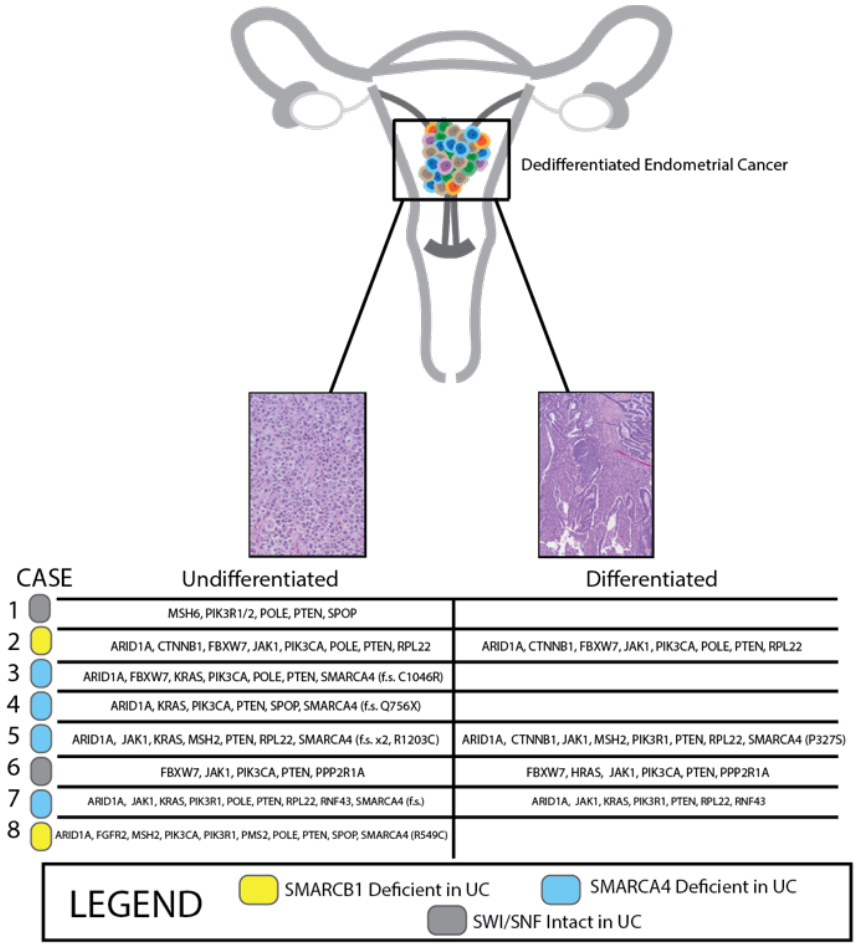


Figure 2.1. Summary of the molecular features or mutation profiles of an index series of eight dedifferentiated endometrial carcinomas. f.s. indicates frameshift mutation.

To validate the findings at the mutational level, we examined the expression of SMARCA4 by immunohistochemistry. The four MMR deficient tumors that harboured *SMARCA4* inactivating mutations showed a complete loss of SMARCA4 protein expression in the undifferentiated region but intact SMARCA4 expression in the corresponding endometrioid component **[Figure 2.2]**. SMARCB1, a member of the SWI/SNF complex was expressed in all 4 of the SMARCA4-deficient tumors **[Figure 2.2]**.

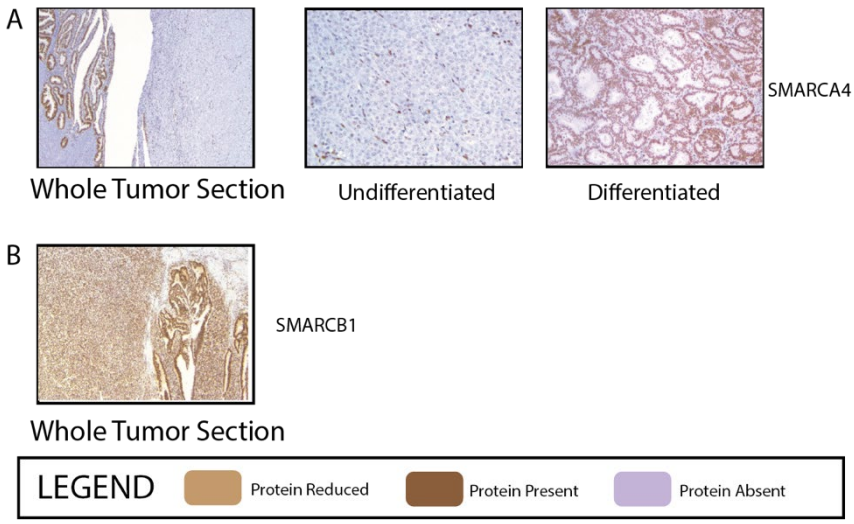


Figure 2.2. SMARCA4 and SMARCB1 immunostaining in SMARCA4-deficient dedifferentiated endometrial carcinomas. A) Case 3 showing a complete loss of nuclear SMARCA4 in the undifferentiated component and intact SMARCA4 nuclear staining in the differentiated component. **B)** SMARCA4 deficient Case 7 showing intact SMARCB1 in both the differentiated and undifferentiated component.

Three tumors with wildtype or missense mutations in *SMARCA4* showed intact SMARCA4 expression in both components of the tumor. Half of the SMARCA4 intact tumors had lost expression of SMARCB1 only in the undifferentiated component but had retained expression of SMARCB1 in the differentiated endometroid component **[Figure 2.3]**. The remaining two SMARCA4 intact tumors had intact SMARCB1 expression through the tumor.

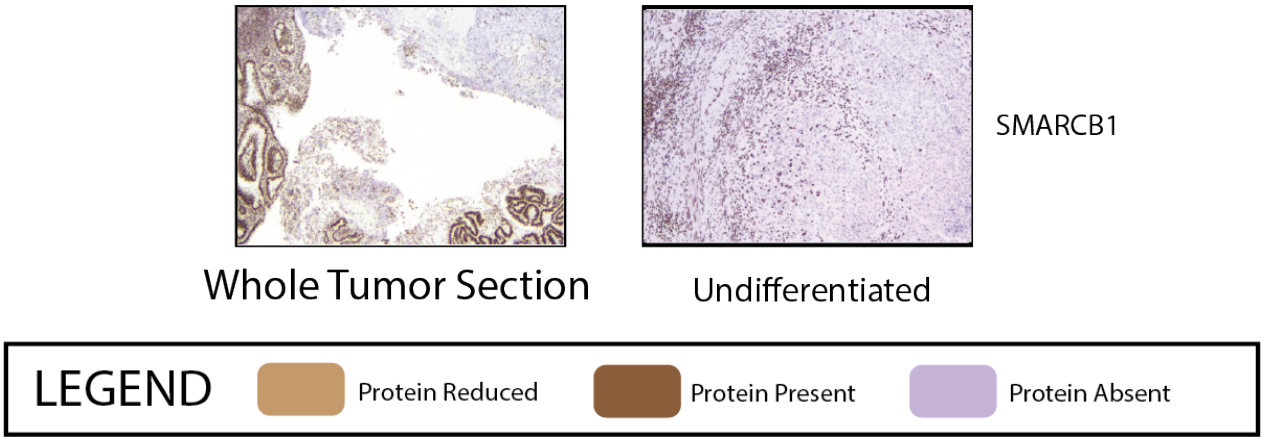


Figure 2.3. SMARCB1 immunostaining in SMARCB1-deficient dedifferentiated endometrial carcinomas. Case 1 showing a complete loss of nuclear SMARCB1 staining in the undifferentiated component.

To ascertain the frequency of SMARCA4/SMARCB1 loss, an examination of their expression levels in a series of 22 centrally reviewed DDEC cases was carried out. As with the index series the undifferentiated component ranged in size from 5 to 95% of the overall tumor but on average constituted 60%. From the tumors examined, 70% were MMR deficient, with the same MMR proteins lost in in both components regardless of their differentiation status. Within this validation cohort, 7 of the 22 tumors (~30%) showed complete absence of SMARCA4 immunostaining only in the undifferentiated region. Of the remaining tumors in which SMARCA4 expression was present in the undifferentiated component, 2 out of 13 displayed complete loss of SMARCB1. A series of 32 FIGO grade 3 endometrioid carcinomas were included for comparison and none of those cases showered either SMARCA4 and/or SMARCB1 loss.

Table 2.1. Summary of SMARCA4, SMARCB1 and MMR protein IHC findings for the entire study cohort. Frequency of MMR deficiency was determined out of 30 cases with interpretable staining results.

	SMARCA4/SMARCB1 Status	MMR Deficiency
Dedifferentiated Endometrial Carcinoma (n=30)		21/30 (70%)
SMARCA4 Deficient/SMARCB1 Intact	11/30 (37%)	8/11 (73%)
SMARCA4 Intact/SMARCB1 Deficient	4/30 (13%)	2/4 (50%)
SMARCA4 Intact/SMARCB1 Intact	15/30 (50%)	11/15 (73%)
FIGO Grade 3 Endometrioid Carcinoma (n=31)		19/30 (63%)
SMARCA4 Deficient/SMARCB1 Intact	0/31 (0%)	
SMARCA4 Intact/SMARCB1 Deficient	0/31 (0%)	
SMARCA4 Intact/SMARCB1 Intact	31/31 (100%)	19/30 (63%)

Taken altogether, our index and validation series revealed 37% (11/30) of DDEC cases possessed SMARCA4 deficiency while 12% (4/30) of DDEC tumors examined harbored SMARCB1 deficiency in the undifferentiated components. As SMARCA4 or SMARCB1 loss was mutually exclusive, loss of either SWI/SNF chromatin remodelling subunit was present in 50% (15/30) of the DDEC tumors we studied. Interestingly, MMR deficiency was equally prevalent regardless of SMARCA4 status with 73% (8/11) of SMARCA4 deficient tumors and 73% (11/15) SMARCA4/SMARCB1 intact tumors lacking complete MMR machinery. From a histological perspective, all undifferentiated components whether SMARCA4 or SMARCB1 deficient were predominant within the tumor and the differentiated portions all exhibited typical FIGO grade 1 or 2 endometrioid morphology. The undifferentiated regions all showed tumor necrosis as well as similar

morphology of sheet-like proliferation of monomorphoric round cells with patches that have underwent cellular discohesion and are rhabdoid-like. Mitotic activity was also high in the undifferentiated component with an average mitotic rate of 49 MF/10 HPF.

The average age at diagnosis was 61 years for patients with SMARCA4/SMARCB1-deficient DDEC only slightly younger or similar to patients with SMARCA4/SMARCB1-intact DDEC or grade 3 endometrioid carcinomas, respectively. Notably, 47% of DDEC patients presenting with SMARCA4/SMARCB1 deficient DDEC possessed stage 2-4 disease which is much higher than the 33% of SMARCA4/SMARCB1 intact DDEC patients and 23% of patients diagnosed with grade 3 endometrioid carcinoma. 70% of DDEC patients received adjuvant therapy (radiation therapy and/or chemotherapy) compared to 83% of patients with grade 3 endometrioid carcinoma. DDEC has worse disease-specific survival when compared to FIGO grade 3 endometrioid carcinoma **[Figure 2.4]**. No significant disease specific survival was found between DDEC cases based on SMARCA4/SMARCB1 presence or absence in the undifferentiated regions of these tumors **[Figure 2.4]**.

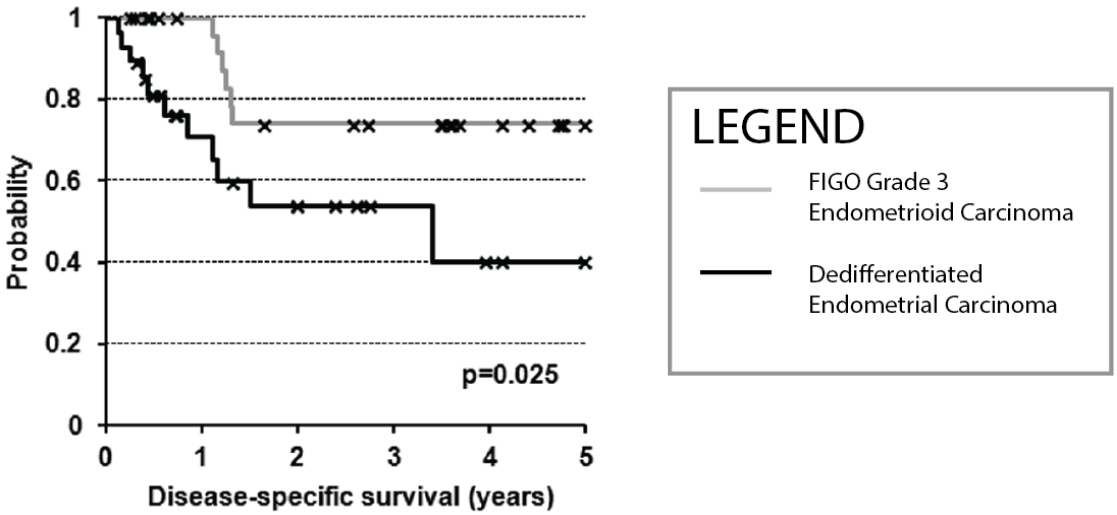


Figure 2.4. Kaplan-Meier survival analysis showing decreased disease-specific survival in review-confirmed DDEC tumors (n=30) compared to FIGO grade 3 endometrioid carcinomas (n=31).

2.2.2. *Consistent absence of PAX8 and ER expression in molecularly defined DDEC.*

Early investigations of the undifferentiated regions of DDEC neoplasms, revealed loss of markers of gynecological differentiation such as PAX8 and ER but was not carried out in the context of SWI/SNF inactivation (6). Therefore, twenty previously described SMARCA4 or SMARCB1 deficient dedifferentiated endometrial carcinomas were examined. Of those 20, 15 had absent SMARCA4 nuclear immunostaining in the undifferentiated component but had retained SMARCA4 expression in the corresponding endometrioid component. The other 5 DDEC tumors had absent SMARCB1 nuclear expression in the undifferentiated component compared to the intact expression of SMARCB1 in the differentiated regions. SMARCA4 and SMARCB1 loss was mutually exclusive. 30% of patients presented with extrauterine spread. There were no distinctive histological differences between SMARCA4/SMARCB1 intact and SMARCA4/SMARCB1 deficient DDEC tumors. 65% of the DDEC tumors in this study were MMR deficient with

the majority 9 cases possessing concurrent MLH1 and PMS2 loss, 3 with PMS2 deficiency alone and 1 with simultaneous MSH2 and MSH6 deficiencies.

Immunostaining for PAX8, ER and p53 was performed. In 95% (19/20) of DDEC cases profiled, PAX8 was expressed in the differentiated endometrioid component **[Table 2.2 & Figure 2.5]**. Similarly, ER was determined to be expressed in the endometrioid component of 89% (16/18) of DDEC tumors **[Table 2.2 & Figure 2.5]**. PAX8 expression could be characterized as diffuse and strong when positive whereas ER immunostaining was more variable in intensity. The undifferentiated components of all 20 tumors did not exhibit positive nuclear staining for either PAX8 or ER **[Table 2.2 & Figure 2.5]**. Internal positive controls of endometrial glands and corresponding differentiated components with the same tumor demonstrate that the lack of staining was not due to any technical issues during the immunohistochemistry staining process. Wildtype nuclear p53 staining was observed for both the differentiated and undifferentiated components in all but one tumor **[Table 2.2 & Figure 2.5]**. Notably, greater p53 staining was frequently observed within the undifferentiated components of the DDEC cases examined **[Figure 2.6]**. One tumor possessing a grade 3 endometrioid component uniquely also had completely lost nuclear p53 staining in both the differentiated and undifferentiated portions of the DDEC neoplasm. This tumor had intact MMR protein expression, lacked PAX8 and ER expression in both components. The loss of p53 is most likely attributable to inactivating *TP53* mutations in both the undifferentiated and differentiated components.

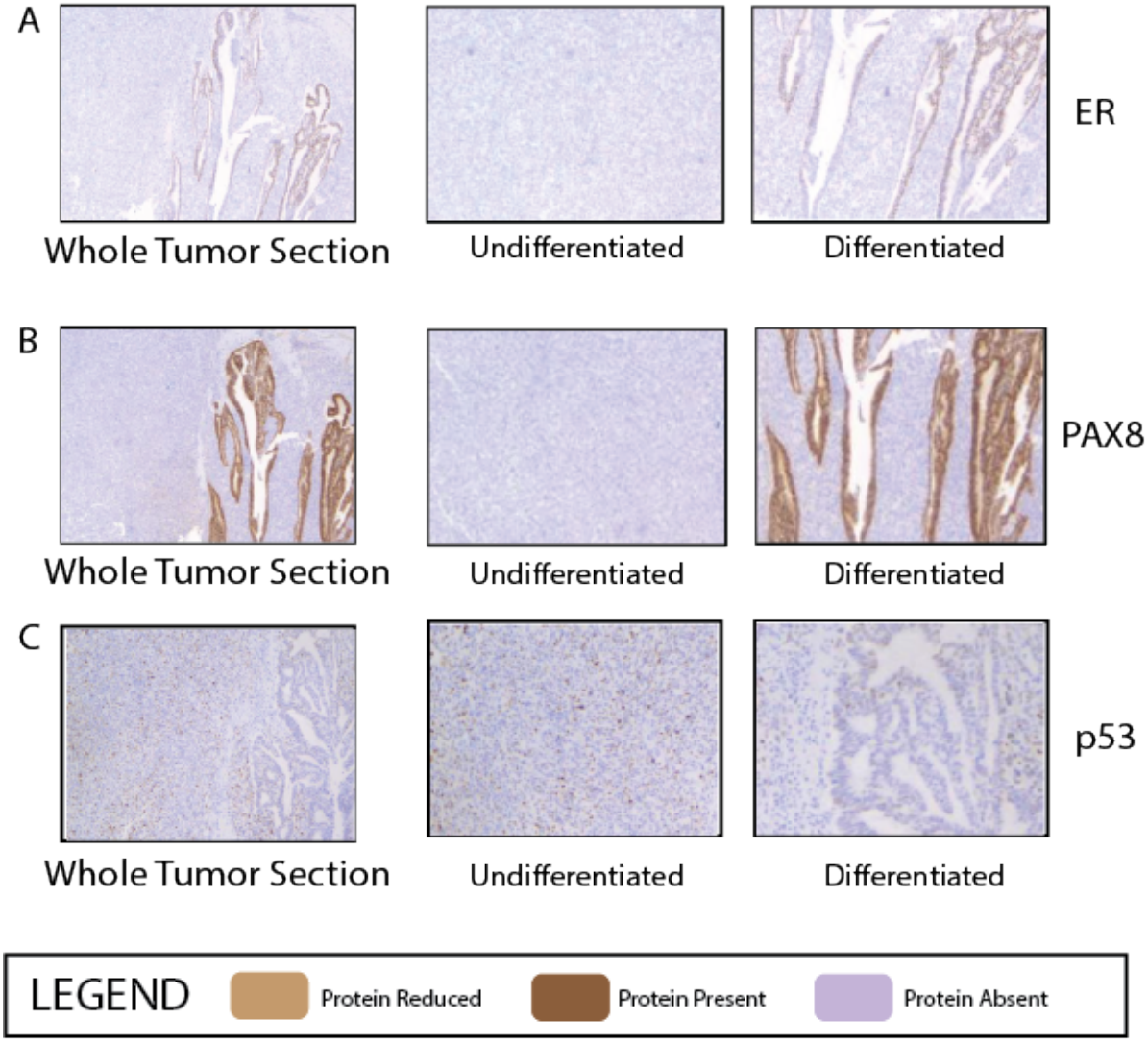


Figure 2.5. PAX8, ER and p53 immunostaining in SMARCA4 or SMARCB1-deficient dedifferentiated endometrial carcinomas. A) SMARCA4 deficient tumor showing ER expression in the endometrioid component but absent ER expression in the undifferentiated portion. **B)** SMARCA4 deficient tumor with PAX8 expression in the endometrioid component but absent PAX8 expression in the undifferentiated portion. **C)** Both the differentiated and undifferentiated components of this SMARCA4-deficient DDEC neoplasm showed wild-type p53 staining.

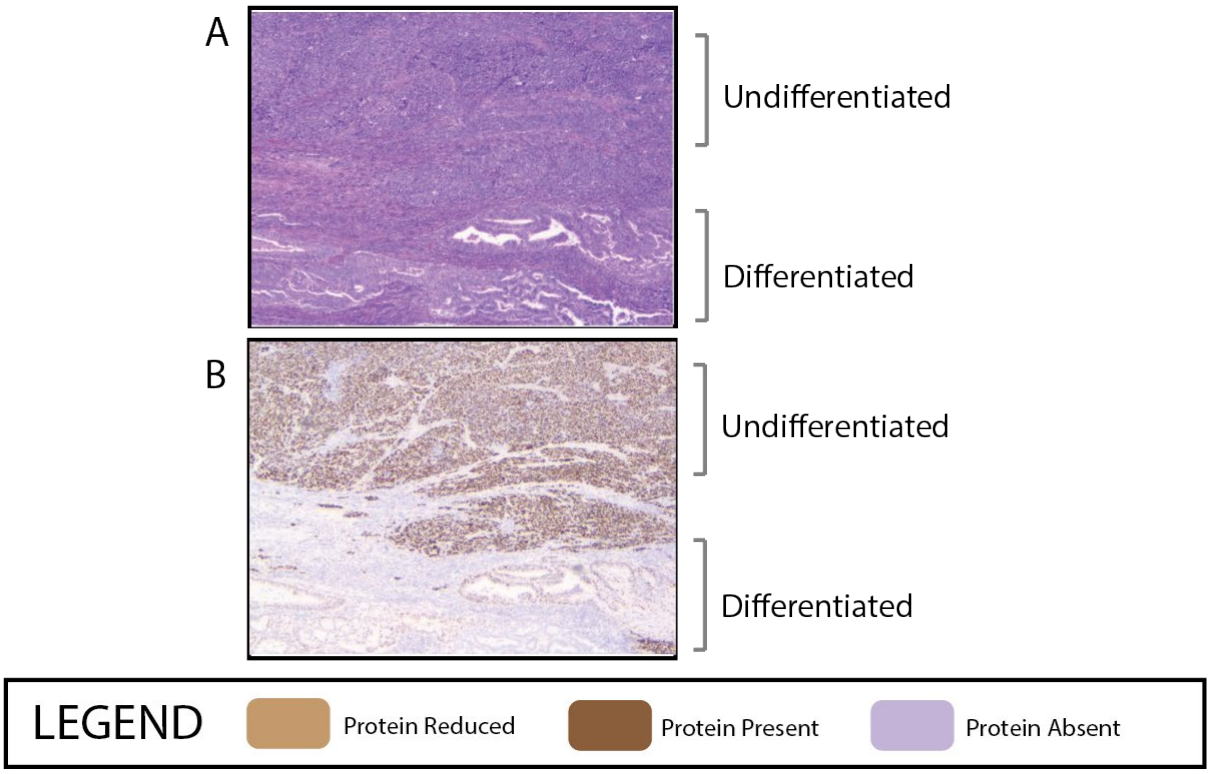


Figure 2.6. p53 immunostaining in SMARCA4/SMARCB1 intact dedifferentiated endometrial carcinomas. A) H&E stained SMARCCA4/SMARCB1 intact DDEC neoplasm. **B)** SMARCCA4/SMARCB1 intact DDEC tumor with wild-type p53 staining pattern in the endometrioid component but diffuse/strong nuclear staining pattern in the undifferentiated region.

A series of SMARCA4/SMARCB1 intact and grade 3 endometrioid carcinomas were also stained for PAX8, ER and p53. 33% of SMARCA4 and 20% of SMARCB1 intact DDEC tumors stained positive for PAX8 and ER **[Table 2.2]**. Notably, half of the ten patients with PAX8 and ER loss in the undifferentiated component of their DDEC lesions, died of their disease with four out of the five patients succumbing to disease within one year of initial diagnosis. Close to half of the SMARCA4/SMARCAB1 intact DDEC displayed a mutated p53 staining signature in contrast to the findings obtained for the SMARCA4/SMARCB1 deficient DDECs **[Table 2.2]**. The grade 3 endometrioid

carcinomas were found to have concurrent absence of PAX8 and ER in 39% of the cases assessed **[Table 2.2]**,

Table 2.2. Summary of PAX8, ER and p53 immunostaining results. P-values displayed were derived from likelihood ratio tests.

		SMARCA4/SMARCB1-deficient DDECs (n=20)	SMARCA4/SMARCB1-intact DDECs (n=15)	FIGO Grade 3 ECs (n=23)
PAX8	Positive in both D and UC	0/20 (0%)	5/15 (33%)	12/23 (52%)
	Positive in D and Negative in UC	**19/20 (95%)**	10/15 (67%)	11/23 (48%)
	Negative in both D and UC	1/20 (5%)	0/15 (0%)	0/23 (0%)
			p=0.0056	p<0.0001
ER	Positive in both D and UC	0/18 (0%)	3/15 (20%)	12/23 (52%)
	Positive in D and Negative in UC	**16/18 (89%)**	10/15 (67%)	9/23 (39%)
	Negative in both D and UC	2/18 (11%)	2/15 (13%)	2/23 (9%)
			p=0.056	p<0.0001
p53	Wildtype pattern in both D and UC	**19/20 (95%)**	8/15 (53%)	18/23 (78%)
	Wildtype pattern in D and Mutated pattern in UC	0/20 (0%)	4/15 (27%)	3/23 (13%)
	Mutated pattern in both D and UC	1/20 (5%)	3/15 (20%)	2/23 (9%)
			p=0.0053	p=0.115

2.2.3. *Concurrent inactivation of ARID1A and ARID1B in DDEC tumors demonstrates another mechanism whereby loss of SWI/SNF complex proteins likely contributes to cellular dedifferentiation.*

As only half of the DDEC tumors we examined possessed loss of chromatin remodeling proteins SMARCA4 or SMARCB1 within the regions possessing undifferentiated histology, we were curious if as has been discovered in numerous other gynecological cancers, mutations in other SWI/SNF complex subunits could explain presence of dedifferentiation in the remaining cases. The study group assessed included 40 DDEC tumors of which 18 SMARCA4 or SMARCB1 deficient had previously been examined. Previously, 18 of the tumors were found to lack SMARCA4 or SMARCB1 expression in the undifferentiated region while retaining expression in the intact component. The median age at time of diagnosis was 62 years and nearly one third of these patients had experienced extrauterine spread. Those with FIGO stage 3 or 4 disease died within one year from their initial diagnosis. The differentiated regions of all these tumors were endometrioid in type and low grade (1 or 2) in nature in 38 of the 40 endometrial cases. By targeted sequencing we examined additional genes specifically making up the SWI/SNF complex (*ARID1A, ARID1B, ARID2, PBRM1, PHF10, SMARCA2, SMARCC1* and *SMARCC2*) and inactivating mutations to ARID1A were found to be present in 12 of the abovementioned cases **[Figure 2.7 & Supplemental Table 2.1]**. From the 22 cases that were SMARCA4 and SMARCB1 intact, inactivating mutations to either *ARID1A* or *ARID1B* were observed in 15 and 10 tumors, respectively **[Figure 2.8 & Supplemental Table 2.1]**. Close to 60% of *ARID1A* mutations and 50% of *ARID1B* mutations were considered frameshift with the remaining percentages

constituted of nonsense mutations to these SWI/SNF complex subunits **[Supplemental Table 2.1]**. Nearly 50% of the DDEC cases where SMARCA4/SMARCB1 immunostaining was evident, concurrent *ARID1A* and *ARID1B* mutations were present in the undifferentiated component **[Figure 2.8]**. Markedly, if SMARCA4 or SMARCB1 deficiency existed within a tumor, no concurrent *ARID1A/B* inactivation mutations were also harboured by those regions **[Figure 2.7]**. Furthermore, none of the cases possessed *ARID1B* inactivating mutations without *ARID1A* inactivating mutations **[Figure 2.8]**.

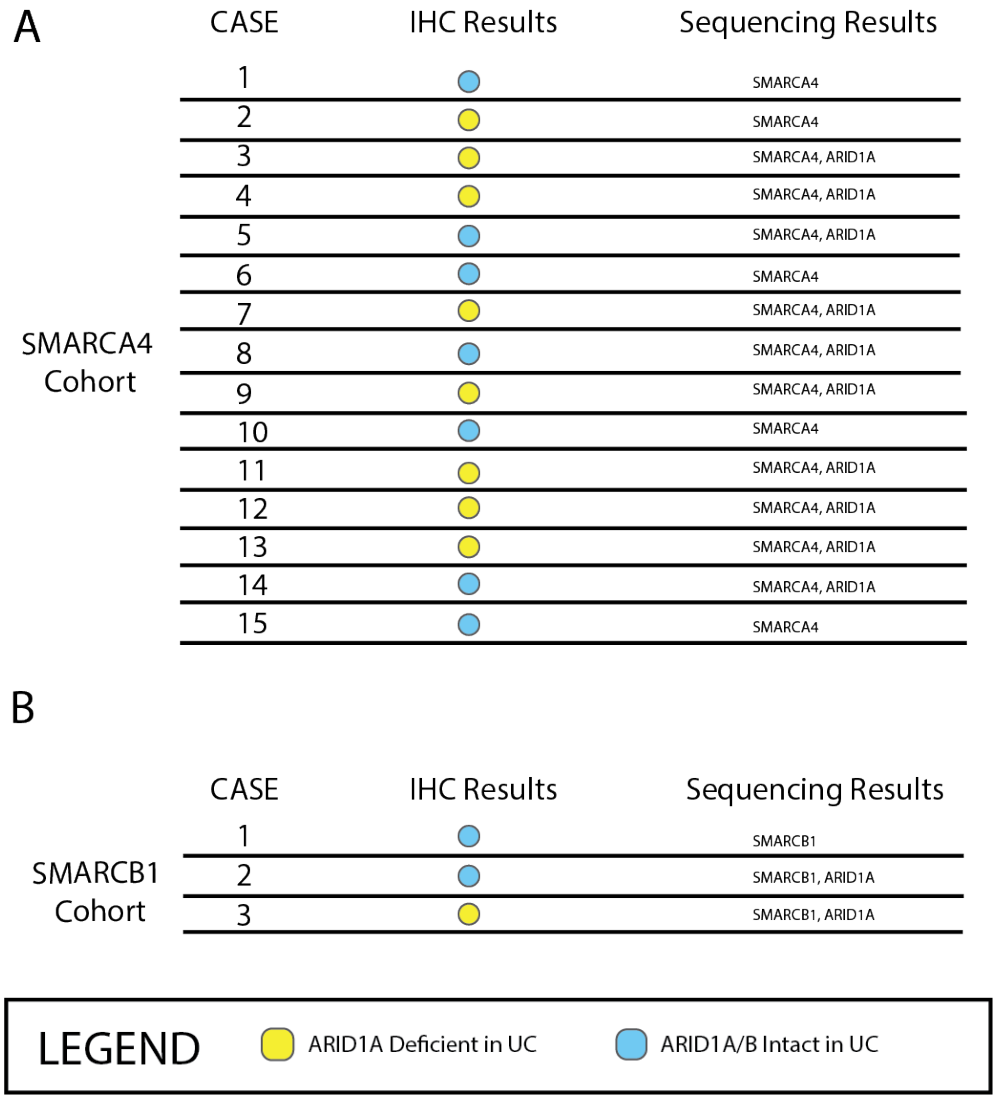


Figure 2.7. Lack of concurrent ARID1A and ARID1B co-inactivation in a subset of DDEC cases. Both mutations present or sequencing results and protein expression or IHC results are summarized here for the SMARCA4/SMARCB1 deficient cohort (n=18).

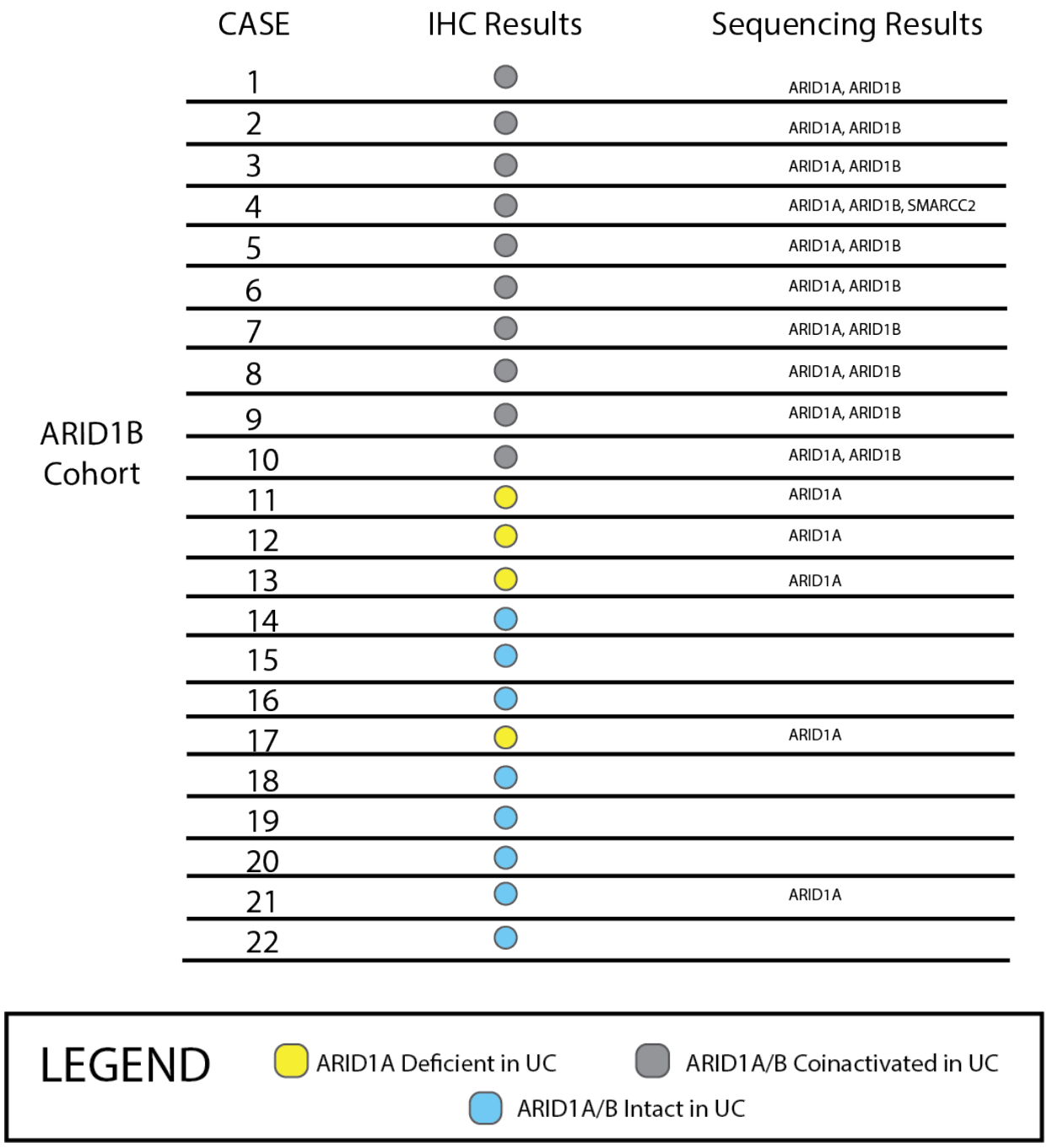


Figure 2.8. Concurrent ARID1A and ARID1B co-inactivation in a subset of DDEC cases. Both mutations present or sequencing results and protein expression or IHC results are summarized here for the SMARCA4/SMARCB1-intact cohort (n=22).

Concurrent *ARID1A* and *ARID1B* mutations whether they be frameshift or nonsense are incredibly rare. At the time this study had been published, a survey of the Catalogue of Somatic Mutations in Cancer (COSMIC) database revealed 3 of 631 endometrial carcinomas of endometrioid histotype possess coexisting *ARID1A/ARID1B* inactivating mutations. This frequent finding of co-inactivation of both *ARID1A* and *ARID1B* in the undifferentiated region of DDEC tumors, led us to evaluate their protein expression levels by immunohistochemistry. 8 of the 10 *ARID1A/ARID1B* co-mutated DDEC tumors showed absence of ARID1A nuclear immunostaining in both the endometrioid and the undifferentiated components **[Figure 2.9]**. The remaining two tumors had only lost expression of ARID1A within the undifferentiated component. ARID1B expression was found to be intact in all the endometrioid components of the 10 tumors but completely absent in the undifferentiated component **[Figure 2.9]**.

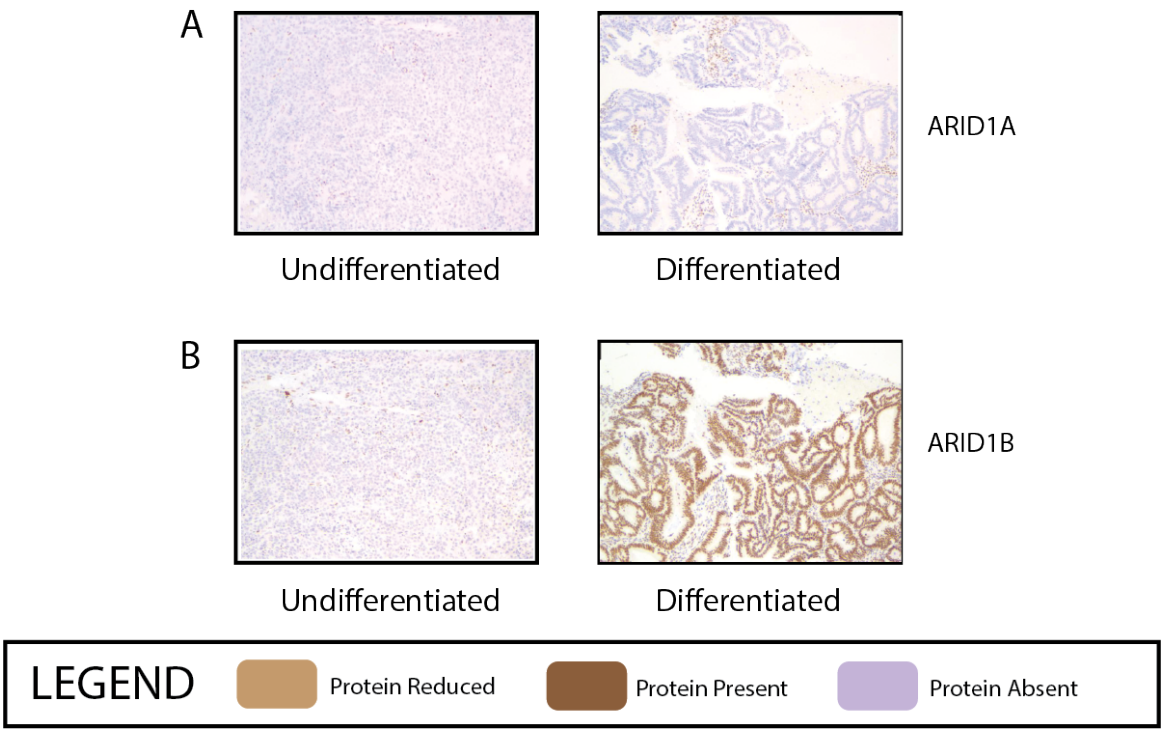


Figure 2.9. Concurrent absence of ARID1A and ARID1B expression in ARID1A/ARID1B co-mutated DDEC. A) Absent ARID1A expression in the tumor cells comprising both the undifferentiated and differentiated carcinoma components. **B)** ARID1B expression was intact in the differentiated component but was absent in the undifferentiated tumor component.

Of the 10 ARID1A/ARID1B co-deficient tumors, 8 exhibited MMR protein deficiency by immunohistochemistry. The most common being concurrent MLH1 and PMS2 loss in seven cases and 2 with MSH2 and MSH6 loss. From a clinical perspective, patients with DDEC wherein ARID1A and ARID1B were inactivated were of a median age of 57 years and 30% of those patients presented with higher stage (FIGO stage 3-4) disease. Thereby, a similar degree of aggressiveness in clinical behavior was observed for both ARID1A/ARID1B co-inactivated DDEC tumors and SMARCA4/SMARCB1 inactivated DDEC lesions **[Figure 2.10]**. Approximately 50% of patients in both groups we discovered and defined, succumbed to disease within 2 years of their initial diagnosis **[Figure 2.10]**.

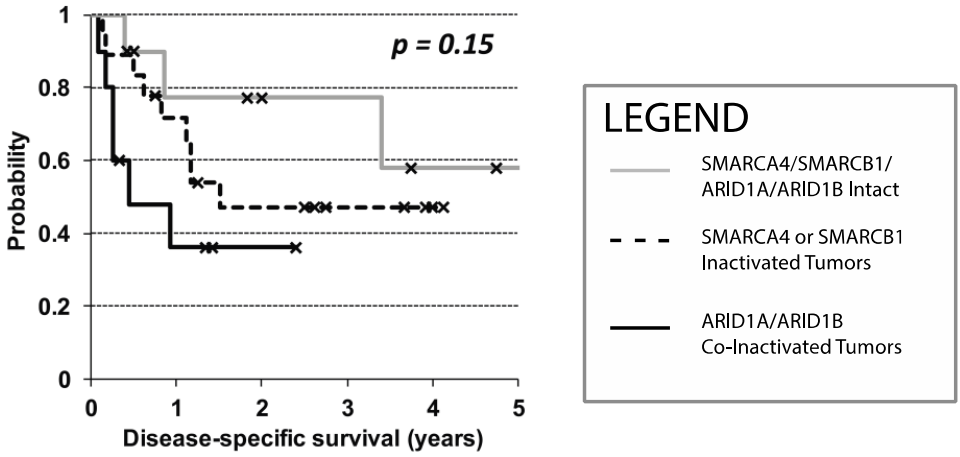


Figure 2.10. Kaplan-Meier disease-specific survival analysis comparing the different genetic subgroups of DDEC. ARID1A/B co-inactivated DDEC patients showed similarly aggressive clinical behavior compared with SMARCA4 or SMARCB1-inactivated DDEC cases (p=0.15).

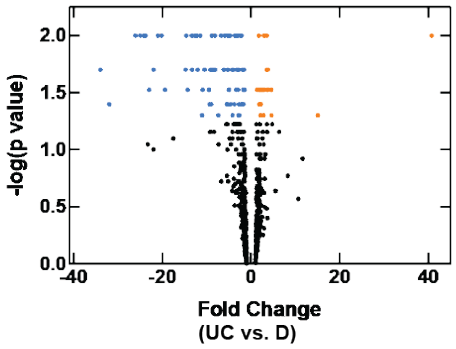
From a histological standpoint, there are no distinctive differences in tumor morphology between those tumors where ARID1A and ARID1B are lost or SMARCA4 or SMARCB1 are absent in the undifferentiated component. Additionally, the undifferentiated components in the majority of ARID1A/ARID1B coinactivated DDEC tumors also lack PAX8 and ER protein immunostaining. Only 2 of the 12 DDEC tumors showed focally retained PAX8 expression and absence of ER expression in the undifferentiated component.

2.2.4. *Emergence of epithelial-to-mesenchymal transition and stem cell like signatures in the undifferentiated portions of DDEC tumors.*

Our observation of a high percentage of cases exhibiting a loss of PAX8 and ER positive immunostaining that coincided with an absence of SWI/SNF proteins was intriguing and suggested that inactivation of core members of the SWI/SNF chromatin remodeling complex may lead to significant disruptions in transcriptional regulation.

While the number of samples limited extensive analyses, a targeted NanoString gene expression profiling assay revealed a list of genes differentially expressed between the undifferentiated and differentiated endometrioid portions of a subset of DDEC tumors. Using a nominal p-value of 0.05 as the cut-off, 109 genes were differentially expressed **[Figure 2.11.A]**. 23 genes were found to more highly expressed in the undifferentiated regions of DDEC neoplasms while 86 genes were found to be downregulated when compared to the low-grade endometrioid DDEC components **[Figure 2.11.A]**. Some of the notable genes more upregulated in the endometrioid regions of SMARCA4-deficient DDEC and therefore were found to a lesser extent in the undifferentiated portions were *CD24, CDH1, CLDN3, CLDN7, TJP3* and *LEFTY1* **[Figure 2.11.B]**. Interestingly, more present in the undifferentiated component of DDEC cases with SMARCA4 loss was Sox2, a marker of stemness that was also validated at the protein level by immunohistochemistry **[Figure 2.11C]**.

A



B

	Fold Change (UC vs. D)
CD24	-115
CDH1	-108
CLDN4	-79
ESRP1	-54
CLDN3	-34
LEFTY1	-32
TJP3	-26
FGFR2	-12
CLDN7	-10
VEFGA	-5
HIF1A	-2
TWIST1	5
ZEB1	2
ZEB2	3
SOX2	41

C

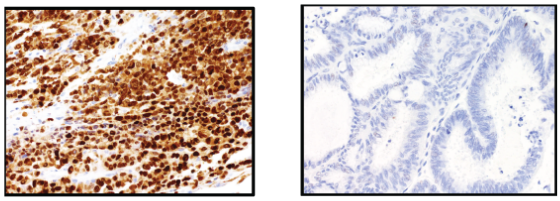


Figure 2.11 Differential gene expression observed between the undifferentiated and differentiated regions of 4 SMARCA4-deficient DDEC tumors. A) Volcano plot showing the transcriptional changes between the undifferentiated and endometrioid components of DDEC cases. **B)** Fold change values for a subset of downregulated and upregulated genes in the undifferentiated regions of DDEC neoplasms. **C)** Immunohistochemical validation of Sox2 wherein Sox2 expression was intact in the undifferentiated component but was absent in the differentiated tumor component.

To account for patient-to-patient variation, we also plotted for each individual case studied, the fold change indicative of gene expression level variations between the endometrioid and undifferentiated portions of individual tumors **[Figure 2.12]**. Several gene expression signatures associated with tumor progression particularly, migration, invasion and metastasis were evident in this way. Transcription factors linked to EMT (ZEB1/2, Twist1, Snail2) were found to be more highly expressed in the undifferentiated components of DDEC tumors lacking SMARCA4 expression than their endometrioid counterparts **[Figure 2.12A]**. Markers of a more mesenchymal phenotype such as Vimentin and N-cadherin were also upregulated in the undifferentiated regions of the

majority of DDEC tumors we examined. Additionally, Claudins 3, 4, 7 and E-cadherin critical for cell-cell contact in epithelial cells were expressed at substantially higher levels in the endometrioid components of DDEC tumors than the regions that exhibited cellular dedifferentiation **[Figure 2.12A & Figure 2.12B]**. Furthermore, several genes associated with cancer stem-like cells were discovered to be dysregulated in DDEC in addition to Sox2 upregulation in the undifferentiated regions. Sox17 and CD24 were found to be significantly downregulated in the undifferentiated portions of the four DDEC cases we examined while BMP7 was found at higher levels in the same dedifferentiated areas [**Figure 2.12C]**.

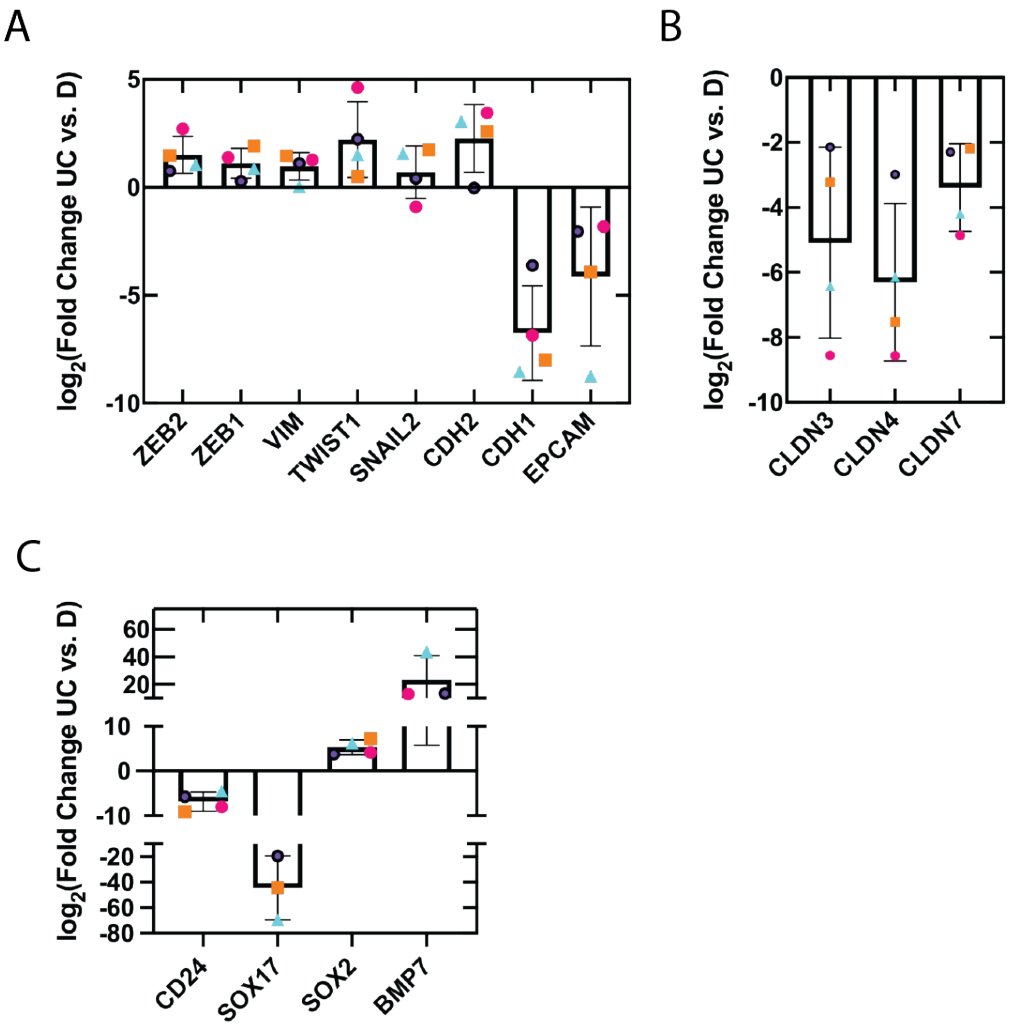


Figure 2.12. Fold change gene expression changes comparing the undifferentiated component of individual DDEC tumors to the differentiated component of the same neoplasm. A) Genes associated with EMT. **B)** Genes classified as extracellular matrix. **C)** Stem-cell associated genes.

2.3. Discussion

Through targeted sequencing and immunohistochemical validation we determined that loss of SMARCA4 or SMARCB1 is associated with histologic dedifferentiation in half of the DDEC tumors we examined. The emergence of inactivating (frameshift and/or nonsense) mutations unique to the undifferentiated component agrees with the hypothesis that undifferentiated component represents a form of tumor progression from the pre-existing low-grade endometrioid carcinoma. A report featuring a single case also

showed loss of SMARCA4 expression by immunohistochemistry in the undifferentiated component with retained SMARCA4 expression in the endometroid component (12). Stewart *et al*., examined the expression of SMARCA4, SMARCB1 and MMR protein in 17 DDEC cases (13). Within their series they observed only 3 of 17 cases (18%) with SMARCA4 loss in the undifferentiated region and 1 of 17 cases with SMARCB1 deficiency in both the differentiated and the undifferentiated region of the tumor (13). One possible explanation as to why we observed such a higher frequency of SMARCA4 loss in our cohort includes not only the difference in sample size but also the histological inclusion criteria as well. In our study, half of the cases originally diagnosed as DDEC were reclassified as FIGO grade 3 endometrioid carcinomas so that no tumors with cohesive solid architecture in the undifferentiated region or spindled epithelial components were considered as DDEC. The importance of this reclassification was highlighted in our findings that centrally reviewed cases of DDEC displayed worse disease specific survival than tumors reclassified to FIGO grade 3 endometrioid carcinomas. Improved molecular understanding of DDEC therefore is required to remove subjectivity from the diagnosis. Unfortunately, while SMARCA4/SMARCB1 deficiency may be used to confirm the diagnosis of DDEC in some cases, intact expression of either SWI/SNF protein does not exclude the possibility of a DDEC diagnosis. Abnormal MMR immunostaining also does not necessarily support the diagnosis of DDEC as 63% of the FIGO grade 3 endometrioid carcinomas initially diagnoses as DDEC in our study were found to be MMR deficient as well.

An increasing number of cancers have been reported to harbor mutations to SWI/SNF chromatin remodeling complex proteins including several rare tumor types.

These rare tumors include rhabdoid tumors of the brain, kidney, small cell carcinoma of the ovary – hypercalcemic type and a subset of pancreatic undifferentiated carcinoma all of which have been shown to possess mutations to either *SMARCA4* or *SMARCB1* and share common pathological features which include undifferentiated, highly proliferative cells (14-23). Renal cell carcinoma with dual SWI/SNF protein deficiency (SMARCA4 and SMARCB1) has been discovered and classified as a dedifferentiated tumor (24). Lung cancer exhibiting SMARCA4 loss together with SMARCA2 loss is associated with a poorly differentiated morphology and more aggressive disease behavior (25, 26). A growing number of malignancies are demonstrating that deficiency in core members of the SWI/SNF complex such as SMARCA4 or SMARCB1 may predispose pre-existing low grade tumor cells carcinomas to cellular dedifferentiation. It remains to be tested in the context of DDEC if loss of SWI/SNF components may predict their response to classes of drugs that regulate epigenetic modifications. The high frequency of MMR deficiency in our study of DDEC suggests that a hypermutating molecular background predisposes molecular events such as SMARCA4 inactivation that in turn induce dedifferentiation to take place. Further work will need to address the exact effects SMARCA4/SMARCB1 inactivation has in the context of endometrial carcinomas.

Immunohistochemistry was utilized to examine the expression patterns of PAX8, ER and p53 in SMARCA4/SMARCB1 deficient DDEC cases and compare the staining results to those of SMARCA4/SMARCB1 intact DDECs and grade 3 endometrioid carcinomas as well. While the differentiated endometrioid components displayed PAX8 and ER expression in the great majority of the cases within our series, consistent absence of PAX8 and ER immunostaining was evident in the undifferentiated components of all

DDECs. Even though, ER expression can vary across tumor subtypes, our observation of frequent PAX8 and ER expression in the differentiated portions of DDEC aligns with the fact that these two nuclear transcription factors are vital to the development and function of the endometrium, respectively (27-30). Spatially, the loss of PAX8 and ER positive immunostaining coinciding with the absence of SMARCA4 or SMARCB1 is intriguing and suggests that inactivation of core members of the SWI/SNF chromatin remodeling complex may lead to significant disruptions in transcriptional regulation. Transcriptional dysregulation could be a major contributing factor to the loss of PAX8 and ER expression in the undifferentiated components of DDEC tumors. Additionally, PAX8 and ER deficiency provides immunohistochemical support for cellular dedifferentiation such that low grade PAX8 and ER positive endometrioid tumors progress to become histologically and immunophenotypically undifferentiated in some regions. It is important to note though that the expression levels of PAX8 and ER can be influenced by a multitude of factors beyond SMARCA4 and SMARCB1 and that a large portion of the SMARCA4/SMARCB1 intact DDEC tumors and grade 3 endometrioid carcinomas lacked PAX8 and/or ER expression in either the undifferentiated components or more solid areas. Our work was the first to incorporate an additional defining molecular feature, SMARCA4/SMARCB1 inactivation, which distinguished it from previously published studies examining the expression of PAX8 and ER in DDECs that did not conclusively set a biologically definitive, objective molecular reference (5, 6). As histologic diagnosis of DDEC can be difficult, our findings in both histologically typical and molecularly defined DDEC cases propose that loss of PAX8 and ER expression is a fundamental phenomenon associated with dedifferentiation. Our findings together with those

previously reported suggest that retained PAX8 and ER expression in the undifferentiated component of DDEC are uncommon. Mutations to *TP53* are probably not involved in the development or progression of dedifferentiation in DDEC as all but one SMARCA4/SMARCB1 deficient tumor displayed wildtype p53 immunostaining in both components. Unique to the case with aberrant p53 staining was that it was the only tumor with grade 3 endometrioid carcinoma as the differentiated component. It seems that the loss of p53 staining in this case appears to have been the result of an acquisition of a *TP53* mutation prior to SMARCA4 inactivation. Prior to our work, only two other studies had evaluated *TP53* mutation status in DDEC (2, 6). Kuhn *et al.,* and Ramalingam *et al.,* found higher frequencies of mutation pattern p53 staining with 33 and 26% of DDEC cases respectively displaying aberrant p53 expression (2, 6). Our findings on SMARCA4/SMARCB1 intact DDEC wherein 47% of cases displayed mutation pattern p53 staining in the undifferentiated portions and wildtype p53 immunostaining in the differentiated component hints at the possibility that mutations to *TP53* could in part contribute to dedifferentiation or facilitate the growth of the undifferentiated component in these cases. However, additional molecular insights are needed to better understand the biology underlying both SMARCA4/SMARCB1-intact and deficient DDECs and to define their relationship with one another.

We demonstrated that concurrent *ARID1A/ARID1B* mutations and subsequent loss of protein expression account for a quarter SWI/SNF chromatin remodeling protein loss in DDEC cases. Altogether our work demonstrated that inactivation of SWI/SNF complex proteins is found in about 70% of DDEC. While mutations to *ARID1A* that result in inactivation are common particularly within the gynaecologic tract, *ARID1B* mutations

are much less prevalent (31, 32). Based on their assumed functionality, concurrent inactivation that results in loss of both ARID1A and ARID1B is expected to abrogate the chromatin remodeling function of the SWI/SNF complex due to inability to bind and target DNA in the absence of ARID subunits. It would mean that a different set of DNA-binding subunits would have to be functional in SWI/SNF complexes to support the loss of both ARID1A and ARID1B in a subset of DDEC tumors. Depletion of ARID1B in an ARID1A deficient cancer is most often reported in literature in the context of promoting synthetic lethality. In ovarian cancer lacking ARID1A, knockdown of ARID1B leads to suppressed *in vitro* growth and reduced colony formation ability (33). Synthetic lethality is not an absolute phenomenon though and is heavily dependent on the background cellular and molecular context (34). This is like other clinical observations in cancer where SMARCA4/SMARCA2 co-inactivation can be tolerated in some tumors and is in direct disagreement with data collected *in vitro (35-38).* In addition to molecular and cellular context, timeframe may be an important factor in influencing what mutations evolve and what cellular pathways become synthetically lethal. Our clinical data demonstrated that ARID1A and ARID1B co-deficiency did not impair the ability of the DDEC tumors to grow and disseminate in patients. Similar as to what was witnessed with SMARCA4/SMARCB1-inactivated DDEC, 75% of ARID1A/ARID1B co-inactivated DDEC were MMR protein deficient. This association is particularly intriguing as there are only two other tumor types where mutations to both ARID1A and ARID1B mutations are tolerated: gastric adenocarcinoma and right-sided colonic adenocarcinoma both of which are likely MMR protein-deficient/microsatellite-unstable tumors (39, 40). In DDEC, it appears as if ARID1A loss precedes ARID1B loss, leading us to the hypothesis that

ARID1A inactivation in a MMR protein deficient cancer clone might facilitate the acquisition of additional and necessary molecular aberrations to enable the emergence of ARID1A/ARID1B co-deficient undifferentiated subclones. Functional studies will need to address if and how ARID1A and ARID1B loss contribute to cellular dedifferentiation and what additional corroborating molecular alterations are needed. ARID1A and ARID1B loss though appears to represent a major alternate mechanism to SMARCA4 or SMARCB1 inactivation in the progression of endometrioid carcinoma to DDEC.

Finally, our pilot targeted gene expression profiling study comparing the undifferentiated components of clinical DDEC tumors to the differentiated endometrioid regions in the same neoplasm further supports our earlier work positing that transcription dysregulation underlays dedifferentiation in the context of endometrial carcinoma. We demonstrated for the first time, additional phenotypic features of DDEC that if in the future are validated on a larger series of cases could potentially be used in conjunction with existing histological and immunophenotypic features to further improve diagnosis. When the SMARCA4-deficient tumors were assessed together, a substantial number of genes were discovered at lower levels within the undifferentiated areas of DDEC tumors. Loss of E-cadherin, a mediator of cell-cell adhesions, has been associated with a loss of epithelial morphology and thus an increased propensity for cancer cells to metastasize (41, 42). Patients diagnosed with DDEC often exhibit extrauterine spread and loss of E-cadherin has been implicated in promoting metastasis in lung, breast and ovarian cancer (42-44). There are numerous manners in which E-cadherin expression is lost in cancer (45, 46). Many epithelial-based malignancies have been shown to have reduced E-cadherin expression through epigenetic mechanisms and transcriptional inactivation of

E-cadherin through alterations in the methylation status of the promoter region have even been implicated in the development of endometriosis (46-48). Interestingly, E-cadherin expression can also be repressed through the binding of the transcription factor ZEB1 to the E-cadherin promoter and this interaction has been shown to be mediated by SMARCA4 (49). Future work will need to examine what mechanisms are responsible for downregulation of E-cadherin in the undifferentiated regions of DDEC neoplasms. Claudin 4, another important protein involved in epithelial cell-cell contact, continually emerges as being found to a lesser extent in the undifferentiated portion when compared to the endometrioid components of DDEC tumors (50). Subtypes of cancers with very poor prognosis such as luminal breast cancer and aggressive bladder and gastric cancer are emerging which are not only characterized by low claudin-3, -4 and -7 expression but also exhibit enrichment of qualities of stemness and EMT (51, 52). We also observed an increase in various proteins involved in EMT suggesting that the undifferentiated regions of DDEC neoplasms were more mesenchymal than their more epithelial, differentiated endometrioid counterparts. In ovarian cancer, it has been demonstrated that reduction in claudin protein levels enhances tumor growth and metastasis and may influence E-cadherin expression levels (53). Again, mechanistic studies with an appropriate model of DDEC may reveal the interplay between these different molecular players and their overall contributions to the acquisition of dedifferentiation within an endometrial carcinoma.

Our comparison of the undifferentiated regions of DDEC tumors lacking SMARCA4 expression versus the SMARCA4-intact differentiated components across a small number of patient samples also strikingly revealed the emergence of a cancer stem cell

phenotype where endometrial cancer cell dedifferentiation has taken place. Upregulation of Sox2 is considered a hallmark of cancer stem cells across tissue types due to its role as a transcriptional activator and repressor that in turn influences cells' ability to reprogram themselves (54-63). Deregulation of Sox2 has also been consistently linked to EMT due to its ability as a transcription factor to regulate the expression levels of many drivers of EMT (54, 63-68). Sox2 positive tumors cells often constitute a small portion of overall heterogenous tumors as seems to be the case in DDEC too (69). Other indications of a more stem cell like state being acquired by the undifferentiated portions of DDEC was less expression of CD24 and Sox17 and higher amounts of BMP7. Low expression of the cell surface protein, CD24 has been implicated in breast and colorectal cancer progression and adoption of a more mesenchymal nature in oral squamous carcinoma especially in combination with high CD44 expression (70-73). As both high and low levels of CD24 have been associated with stem like properties continued characterization of the undifferentiated regions of DDEC will be required prior to the adoption of these markers as definitive indicators of the presence of a population of stem like cells in these heterogenous tumors. Recently, Sox17 in the context of endometrial cancer was shown to behave as a tumor suppressor capable of modulating Wnt signalling, therefore a loss of Sox17 as observed in the undifferentiated portion of DDEC neoplasms, might be associated with the increased aggressive nature of this subpopulation of cells as cellular proliferation would be less regulated in the absence of Sox17 (74). Future studies will require a closer look at Sox17 inactivation in the undifferentiated components of DDEC cases as there is evidence that it is silenced at the epigenetic level through methylation of its promoter (75-77). The bone morphogenetic protein, BMP7, a part of the TGFβ

superfamily is implicated in regulation of pluripotency (78, 79). In ovarian cancer cells, overexpression of BMP7 led not only to increased proliferation and acquisition of an EMT gene signature but reduction of BMP7 levels also reduced the migration and invasion capabilities of OC cells (80). It should also be noted that higher levels of BMP7 in OC cell lines was associated with increased resistance to chemotherapy (80). Even from this pilot gene expression profiling experiment, several possible candidates to further distinguish between the components of DDEC were discovered that warrant further investigation and validation in larger cohorts of DDEC patient samples. It will also be of interest to better understand cellular dedifferentiation in numerous cancer types to also elucidate the molecular mechanisms underlying some of the gene expression changes uncovered from this preliminary study of DDEC.

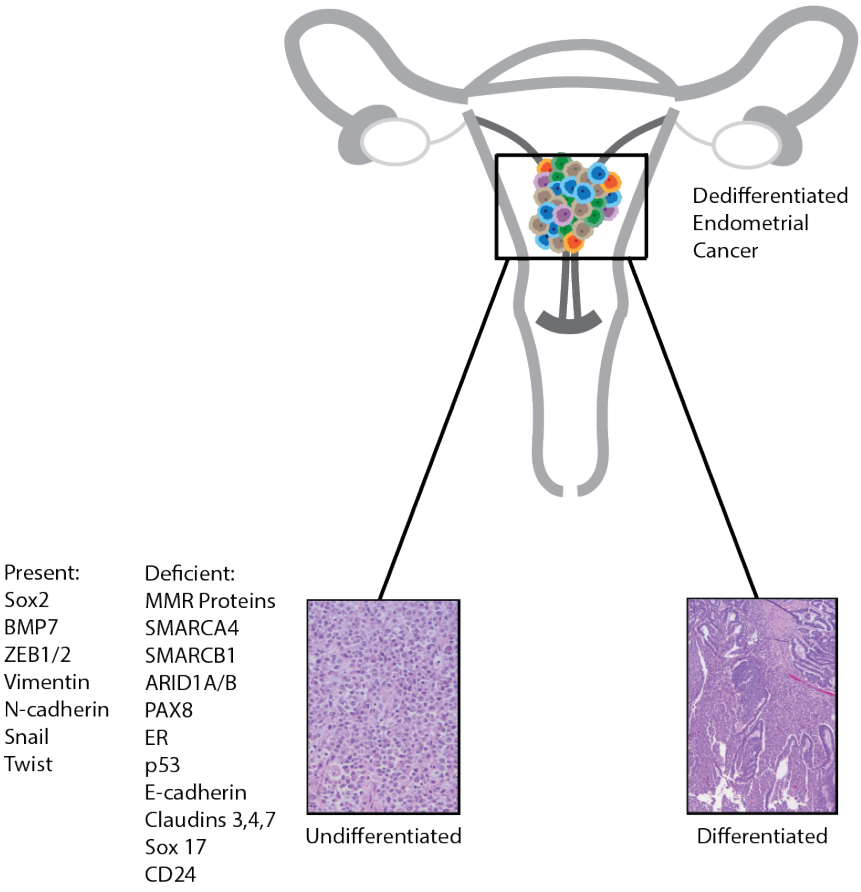


Figure 2.13. Summary of Chapter 2 results. In terms of the undifferentiated component, proteins found to either be expressed at high levels or mutated and/or absent are noted.

2.4. Methods

2.4.1. Study Samples:

For Karnezis *et al.*, 8 dedifferentiated endometrial carcinomas were included in the index series and 22 cases were studied in the validation series. The cases for Karnezis *et al.* were obtained from the pathology archives at Vancouver General Hospital (Vancouver, Canada), Calgary Laboratory Services (Calgary, Canada), Royal Alexandra Hospital (Edmonton, Alberta), The Ottawa Hospital (Ottawa, Canada) and Memorial Sloan Kettering Cancer Center (New York, United States). The Coatham *et al.* study

included 19 SMARCA4/INI1-intact dedifferentiated endometrial carcinomas and 24 SMARCA4/INI1-deficient cases. The Hoang *et al.* study had 20 DDEC cases with SMARCA4 or INI1 loss in the undifferentiated component and 15 SMARCA4/INI1 intact DDECs as determined by immunohistochemistry. Samples were acquired from Calgary Laboratory Services (Calgary, Canada), Royal Alexandra Hospital (Edmonton, Alberta), Memorial Sloan Kettering Cancer Center (New York, United States) and King Edward Memorial Hospital (Perth, Australia). All cases were found to possess the morphologic features described in (81). All the endometrial carcinomas in these studies were hysterectomy specimens.

2.4.2. Targeted Gene Panel Sequencing Analysis and Validations:

Tumor tissue cores (0.6 mm) from formalin-fixed paraffin-embedded blocks were obtained for each case from the endometrioid and the undifferentiated components separately if possible. Areas were chosen to maximize the amount of histologically viable tumor. Normal tissue distant from the endometrial carcinoma was used for comparison. The DNA from tissue cores was extracted using the formalin-fixed paraffin-embedded tissue DNA extraction kit (Qiagen) according to the manufacturer's protocol. Initially, in Karnezis *et al.,* an Illumina custom TruSeq amplicon panel was designed within Illumina's Design Studio to include 1173 amplicons covering 98% of the untranslated regions (UTRs) and exons of the following 26 recurrently mutated genes in gynecological carcinomas: *AKT1, ARID1A, FBXW7, FGR2, JAK1, KRAS, MLH1, MSH2, MSH6, NRAS, PIK3CA, PIK3R1, PIK3R2, PMS2, POLE, PPP2R1A, PTEN, RNF43, RPL22, SMARCA4, STK11, SPOP, TPF2, FOXL2, CTNNB1* and *BRAF*. In Coatham *et al.,* custom targeted sequencing panel (Illumina TruSeq) was also designed but covered 99% of the exons

and untranslated regions of 10 genes specifically making up the SWI/SNF complex (*ARID1A, ARID1B, ARID2, PBRM1, PHF10, SMARCA2, SMARCA4, SMARCB1, SMARCC1* and *SMARCC2*). Custom amplicon (533 at 175 bp) libraries were generated using 250 ng DNA and following the TruSeq Library Preparation protocol as in (82). Prior to pooling at equal concentrations, normalization was achieved by quantifying the individual libraries using the Qubit fluorometer. Pooled libraries were quantitated for amplifiable libraries with the FAST qPCR SYPR quantification kit (Kapa Biosystems). The Illumina MiSeq was used to sequence the Pooled TruSeq libraries using 300 cycle V2 kits. Analysis was completed with the MiSeq Reporter and somatic variant caller software 3.2.3.0. In Coatham *et al.,* 95% of the amplicons had coverage in the 36 tumor samples of greater than 50-fold. Frameshift, nonsense or indel mutations were identified only upon passing a quality filter with at least 10% variant allele frequency and were further validated by direct Sanger sequencing using primer sets targeting the regions surrounding the mutations and manual inspection of bam files with Integrated Genome Viewer (IGV). The corresponding genomic sequences were assessed to normal tissue to ascertain the somatic/germline status of discovered mutations.

2.4.3. Immunohistochemistry:

Analysis was performed on whole tissue sections from hysterectomy specimens. Slides were processed using the Ventana Discovery XT, Ventana Benchmark XT and Benchmark Ultra automated systems (Ventana Medical Systems). Incubation of the slides with SMARCA4 (clone EPNCIR111A, ab11064, Abcam) was done at a 1:25 dilution. Sections were scored by two pathologists with tumors scored as positive for SMARCA4 if nuclei showed diffuse, moderate or strong staining. Tumors were determined to be

SMARCA4 deficient if nuclei were absent of staining in the presence of an internal positive control (nuclear staining of stromal fibroblasts, endothelial and/or inflammatory cells. ARID1A/B immunohistochemical analysis was accomplished with a Dako Omnis Autostainer (DAKO Canada ULC) and Bond polymer refine (Leica Microsystems) was used for the detection system. Slides were incubated with antibodies specific to ARID1A at a concentration of 1:200 (HPA005456, Sigma) and ARID1B (clone 2D2, H00057492-MO1, Abnova) at a dilution of 1:100. Intact expression was claimed for ARID1A/B if nuclei were stained and expression absent if nuclei remained unstained in the presence of internal positive control immunoreactivity.

To detect PAX8 or ER, unstained slides were subjected to heat induced antigen retrieval with Cell Conditioning solution (CC1-Tris based EDTA buffer, pH 8, Ventana). The mouse monoclonal antibody to PAX8 was clone BC12 (ACI 438, Biocare Medical) while rabbit monoclonal antibody specific for ER was clone SP1 (RM-9101, Thermo Fisher Scientific). Using Ventana antibody diluents, primary antibody incubations were performed for 1 hour at 37 degrees Celsius at concentrations of 1:100 and 1:25, for PAX8 and ER, respectively. Ventana Universal Secondary Antibody was applied for 25 minutes at 37°C. The detection system utilized was the Ventana DABMap kit for ER. Only moderate to strong nuclear staining was considered positive for both PAX8 and ER immunostaining. Differentiated and undifferentiated components were assessed separately, and tumors were considered PAX8 or ER-negative only if there was adequate nuclear staining of internal positive control tissue.

For mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) the primary antibodies and staining methods were the same as that reported previously (83, 84).

2.4.4. Nanostring Gene Expression Profiling:

Gene expression profiling was conducted on 4 matched FFPE DDEC tissue samples collected from Calgary Laboratory Services (Calgary, Canada) and Royal Alexandra Hospital (Edmonton, Alberta). The PanCancer Progression panel contains 770 genes including 30 reference genes. The processes included within this panel are associated with epithelial-mesenchymal transition, extracellular matrix remodeling, metastasis, and angiogenesis. RNA was extracted from both the undifferentiated and differentiated components of formalin-fixed, paraffin embedded DDEC tumor tissue (3-6 cores) after deparaffinization using the FFPE RNA extraction kit (Roche). RNA concentrations were determined using Bioanalyzer and 100 ng of total RNA were hybridized with fluorescently barcoded 3' biotinylated capture and 5' reporter probes for each target at 65°C overnight. Samples were processed on the NanoString nCounter Digitial Analyzer (NanoString Technologies) to count the digital barcodes representing the number of transcripts. Analysis of the raw NanoString data was accomplished using nSolver Analysis Software v4.0 (NanoString Technologies). Background signals from probe was determined using the average count of the 8 negative control probes in each reaction. Raw counts were then further normalized to the geometric mean of 6 internal positive controls. Fold change estimation was achieved through two tailed Student's t-tests when cases were considered as replicates with the undifferentiated components considered sample and the differentiated regions the reference group. Differential expression was called when cases were examined individually based on the known confidence limits at that expression count level.

2.5. References

1. Giordano G, D'Adda, T., Bottarelli, L., Lombardi, M., Brigati, M., Berretta, R., Merisio, C. Two cases of low-grade endometrioid carcinoma associated with undifferentiated carcinoma of the uterus (dedifferentiated carcinoma): a molecular study. Pathol Oncol Res. 2012;2:523-8.
2. Kuhn E, Ayhan A, Bahadirli-Talbott A, Zhao C, Shih Ie M. Molecular characterization of undifferentiated carcinoma associated with endometrioid carcinoma. Am J Surg Pathol. 2014;38(5):660-5.
3. Tafe LJ, Garg K, Chew I, Tornos C, Soslow RA. Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms. Mod Pathol. 2010;23(6):781-9.
4. Vita G, Borgia L, Di Giovannantonio L, Bisceglia M. Dedifferentiated endometrioid adenocarcinoma of the uterus: a clinicopathologic study of a case. Int J Surg Pathol. 2011;19(5):649-52.
5. Li Z, Zhao C. Clinicopathologic and Immunohistochemical Characterization of Dedifferentiated Endometrioid Adenocarcinoma. Appl Immunohistochem Mol Morphol. 2016;24(8):562-8.
6. Ramalingam P, Masand RP, Euscher ED, Malpica A. Undifferentiated Carcinoma of the Endometrium: An Expanded Immunohistochemical Analysis Including PAX-8 and Basal-Like Carcinoma Surrogate Markers. Int J Gynecol Pathol. 2016;35(5):410-8.
7. Kulkarni MM. Digital Multiplexed Gene Expression Analysis Using the NanoString nCounter System. Current Protocols in Molecular Biology 2011;94(1):25B.10.1-25B.10.7.
8. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol. 2008;26(3):317-25.
9. Das K, Chan XB, Epstein D, Teh BT, Kim KM, Kim ST, et al. NanoString expression profiling identifies candidate biomarkers of RAD001 response in metastatic gastric cancer. ESMO Open. 2016;1(1):e000009.
10. Lee J, Sohn I, Do IG, Kim KM, Park SH, Park JO, et al. Nanostring-based multigene assay to predict recurrence for gastric cancer patients after surgery. PLoS One. 2014;9(3):e90133.
11. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73.
12. Strehl JD, Wachter DL, Fiedler J, Heimerl E, Beckmann MW, Hartmann A, et al. Pattern of SMARCB1 (INI1) and SMARCA4 (BRG1) in poorly differentiated endometrioid adenocarcinoma of the uterus: analysis of a series with emphasis on a novel SMARCA4-deficient dedifferentiated rhabdoid variant. Ann Diagn Pathol. 2015;19(4):198-202.
13. Stewart CJ, Crook ML. SWI/SNF complex deficiency and mismatch repair protein expression in undifferentiated and dedifferentiated endometrial carcinoma. Pathology. 2015;47(5):439-45.
14. Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, Ranish J, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. Nat Genet. 2013;45(6):592-601.

15. Masliah-Planchon J, Bieche, J., Guinebretiere, J-M., Bourdeaut, F., Delattre, O. SWI/SNF Chromatin Remodeling and Human Malignancies. Annu Rev Pathol. 2015;10(145-71).
16. Agaimy A. The Expanding Family of SMARCB1(INI1)-deficient Neoplasia: Implications of Phenotypic, Biological, and Molecular Heterogeneity. Adv Anat Pathol. 2014;21(6):394-410.
17. Hornick J, Dal Cin, P., Fletcher, CDM. Loss of INI1 Expression Is Characteristic of Both Conventional and Proximal-Type Epithelioid Sarcoma. Am J Surg Pathol. 2009;33(4):542-50.
18. Modena P, Lualdi, E., Facchinetti, F., Galli, L., Teixeira, MR., Pilotti, S., Sozzi, G. SMARCB1/INI1 Tumor Suppressor Gene Is Frequently Inactivated in Epithelioid Sarcomas. Cancer Res.65(10):4012-9.
19. Foulkes WD, Clarke, BA., Hasselblatt, M., Majewski, J., Albrecht, S., McCluggage, WG. No Small Surprise - Small Cell Carcinoma of the Ovary, Hypercalcaemic Type, Is a Malignant Rhabdoid Tumour. J Pathol. 2014;233(3):209-14.
20. Jelinic P, Mueller JJ, Olvera N, Dao F, Scott SN, Shah R, et al. Recurrent SMARCA4 mutations in small cell carcinoma of the ovary. Nat Genet. 2014;46(5):424-6.
21. Ramos P, Karnezis AN, Craig DW, Sekulic A, Russell ML, Hendricks WP, et al. Small cell carcinoma of the ovary, hypercalcemic type, displays frequent inactivating germline and somatic mutations in SMARCA4. Nat Genet. 2014;46(5):427-9.
22. Witkowski L, Carrot-Zhang J, Albrecht S, Fahiminiya S, Hamel N, Tomiak E, et al. Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. Nat Genet. 2014;46(5):438-43.
23. Agaimy A, Haller F, Frohnauer J, Schaefer IM, Strobel P, Hartmann A, et al. Pancreatic undifferentiated rhabdoid carcinoma: KRAS alterations and SMARCB1 expression status define two subtypes. Mod Pathol. 2015;28(2):248-60.
24. Rao Q, Xia QY, Shen Q, Shi SS, Tu P, Shi QL, et al. Coexistent loss of INI1 and BRG1 expression in a rhabdoid renal cell carcinoma (RCC): implications for a possible role of SWI/SNF complex in the pathogenesis of RCC. Int J Clin Exp Pathol. 2014;7(4):1782-7.
25. Matsubara D, Kishaba, Y., Ishikawa, S., Sakatani, T., Oguni, S., Tamura, T., Hoshino, H., Sugiyama, Y., Endo, S., Murakami, Y., Aburatani, H., Fukayama, M., Niki, T. Lung Cancer With Loss of BRG1/BRM, Shows Epithelial Mesenchymal Transition Phenotype and Distinct Histologic and Genetic Features. Cancer Sci. 2013;104(2):266-73.
26. Orvis T, Hepperla A, Walter V, Song S, Simon J, Parker J, et al. BRG1/SMARCA4 inactivation promotes non-small cell lung cancer aggressiveness by altering chromatin organization. Cancer Res. 2014;74(22):6486-98.
27. Yemelyanova A, Gown AM, Wu LS, Holmes BJ, Ronnett BM, Vang R. PAX8 expression in uterine adenocarcinomas and mesonephric proliferations. Int J Gynecol Pathol. 2014;33(5):492-9.
28. Laury AR, Perets R, Piao H, Krane JF, Barletta JA, French C, et al. A comprehensive analysis of PAX8 expression in human epithelial tumors. Am J Surg Pathol. 2011;35(6):816-26.

29. Lim D, Ip PP, Cheung AN, Kiyokawa T, Oliva E. Immunohistochemical Comparison of Ovarian and Uterine Endometrioid Carcinoma, Endometrioid Carcinoma With Clear Cell Change, and Clear Cell Carcinoma. Am J Surg Pathol. 2015;39(8):1061-9.
30. Mhawech-Fauceglia P, Yan, L., Liu, S., Pejovic, T. ER+/PR+/TFF3+/IMP3-immunoprofile distinguishes endometrioid from serous and clear cell carcinomas of the endometrium: a study of 401 cases. Histopathology. 2013;62(7):976-85.
31. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv. 2015;1(5):e1500447.
32. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer. 2011;11(7):481-92.
33. Helming K, Wang, X., Wilson, BG., Vazquez, F., Haswell, JR., Manchester, HE., Kim, Y., Kryukov, GV., Ghandi, M., Aguirre, AJ., Jagani, Z., Garraway, LA., Hahn, WC., Roberts, CWM. ARID1B is a specific vulnerability in ARID1A-mutant cancers. Nat Med. 2014;20:251-4.
34. Chan N, Bristow, RG. “Contextual” Synthetic Lethality and/or Loss of Heterozygosity: Tumor Hypoxia and Modification of DNA Repair. Clinical Cancer Research. 2010;16(18).
35. Hoffman G, Rahal, R., Buxton, F., Xiang, K., McAllister, G., Frias, E., Bagdasarian, L., Huber, J., Lindeman, A., Chen, D., Romero, R., Ramadan, N., Phadke, T., Haas, K., Jaskelioff, M., Wilson, BG., Meyer, MJ., Saenz-Vash, V., Zhai, H., Myer, VE., Porter, JA., Keen, N., McLaughlin, ME., Mickanin, C., Roberts, CWM., Stegmeier, F., Jagani, Z. Functional Epigenetics Approach Identifies BRM/SMARCA2 as a Critical Synthetic Lethal Target in BRG1-deficient Cancers. Proc Natl Acad Sci USA. 2014;111(8):3128-33.
36. Oike T, Ogiwara H, Tominaga Y, Ito K, Ando O, Tsuta K, et al. A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. Cancer Res. 2013;73(17):5508-18.
37. Reisman DN, Sciarrotta J, Wang W, Funkhouser WK, Weissman BE. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. Cancer Res. 2003;63(3):560-6.
38. Karnezis AN, Wang, Y., Ramos, P., Hendricks, W.Pd., Oliva, E., D’Angelo, E., Prat, J., Nucci, M.R., Nielsen, T.O., Chow, C., Leung, S., Kommoss, F., Kommoss, S., Silva, A., Ronnett, B.M., Rabban, J.T., Bowtell, D.D., Weissman, B.E., Trent, J.M., Gilks, C.B., Huntsman, D.G. Dual Loss of the SWI/SNF Complex ATPases SMARCA4/BRG1 and SMARCA2/BRM Is Highly Sensitive and Specific for Small Cell Carcinoma of the Ovary, Hypercalcaemic Type. J Pathol. 2016;238(3):389-400.
39. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, Law S, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet. 2011;43(12):1219-23.
40. Jones S, Li, M., Parsons, D.W., Zhang, Z., Wesseling, J., Kristel, P., Schmidt, M.K., Markowitz, S., Yan, H., Bigner, D., Hruban, R.H., Eshleman, J.R., Iacobuzio-Donahue, C.A., Goggins, M., Maitra, A., Malek, S.N., Powell, S. Vogelstein, B., Kinzler, K.W., Velculescu, V.E., Papadopoulos, N. . Somatic Mutations in the Chromatin Remodeling Gene ARID1A Occur in Several Tumor Types. Hum Mutat. 2012;33(1):100-3.

41. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 2008;68(10):3645-54.
42. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, et al. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. Cells-Basel. 2019;8(10).
43. Chen A, Beetham, H., Black, MA., Priya, R., Telford, BJ., Guest, J., Wiggins, GA., Godwin, TD., Guilford, PJ. . E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition. BMC cancer. 2014;14.
44. Rosso M, Majem B, Devis L, Lapyckyj L, Besso MJ, Llaurado M, et al. E-cadherin: A determinant molecule associated with ovarian cancer progression, dissemination and aggressiveness. PloS one. 2017;12(9):e0184439.
45. Venhuizen JH, Jacobs FJC, Span PN, Zegers MM. P120 and E-cadherin: Double-edged swords in tumor metastasis. Semin Cancer Biol. 2020;60:107-20.
46. Wong SHM, Fang CM, Chuah LH, Leong CO, Ngai SC. E-cadherin: Its dysregulation in carcinogenesis and clinical implications. Crit Rev Oncol Hematol. 2018;121:11-22.
47. Li Y, An D, Guan YX, Kang S. Aberrant Methylation of the E-Cadherin Gene Promoter Region in Endometrium and Ovarian Endometriotic Cysts of Patients with Ovarian Endometriosis. Gynecol Obstet Invest. 2017;82(1):78-85.
48. Yu W, Yang, L., Li, T., Zhang, Y. Cadherin Signaling in Cancer: Its Functions and Role as a Therapeutic Target. Front Oncol. 2019.
49. Sanchez-Tillo E, Lazaro A, Torrent R, Cuatrecasas M, Vaquero EC, Castells A, et al. ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. Oncogene. 2010;29(24):3490-500.
50. Tessier-Cloutier B, Soslow RA, Stewart CJR, Kobel M, Lee CH. Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas. Histopathology. 2018;73(2):299-305.
51. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010;12(5):R68.
52. Kardos J, Chai S, Mose LE, Selitsky SR, Krishnan B, Saito R, et al. Claudin-low bladder tumors are immune infiltrated and actively immune suppressed. JCI Insight. 2016;1(3):e85902.
53. Shang X, Lin X, Alvarez E, Manorek G, Howell SB. Tight junction proteins claudin-3 and claudin-4 control tumor growth and metastases. Neoplasia (New York, NY). 2012;14(10):974-85.
54. Novak D, Huser L, Elton JJ, Umansky V, Altevogt P, Utikal J. SOX2 in development and cancer biology. Semin Cancer Biol. 2019.
55. Rybak AP, Tang D. SOX2 plays a critical role in EGFR-mediated self-renewal of human prostate cancer stem-like cells. Cell Signal. 2013;25(12):2734-42.
56. Lee SH, Oh SY, Do SI, Lee HJ, Kang HJ, Rho YS, et al. SOX2 regulates self-renewal and tumorigenicity of stem-like cells of head and neck squamous cell carcinoma. Br J Cancer. 2014;111(11):2122-30.

57. Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, et al. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. Nature. 2014;511(7508):246-50.
58. Singh S, Trevino J, Bora-Singhal N, Coppola D, Haura E, Altiok S, et al. EGFR/Src/Akt signaling modulates Sox2 expression and self-renewal of stem-like side-population cells in non-small cell lung cancer. Mol Cancer. 2012;11:73.
59. Xiang R, Liao D, Cheng T, Zhou H, Shi Q, Chuang TS, et al. Downregulation of transcription factor SOX2 in cancer stem cells suppresses growth and metastasis of lung cancer. Br J Cancer. 2011;104(9):1410-7.
60. Santini R, Pietrobono S, Pandolfi S, Montagnani V, D'Amico M, Penachioni JY, et al. SOX2 regulates self-renewal and tumorigenicity of human melanoma-initiating cells. Oncogene. 2014;33(38):4697-708.
61. Basu-Roy U, Seo E, Ramanathapuram L, Rapp TB, Perry JA, Orkin SH, et al. Sox2 maintains self renewal of tumor-initiating cells in osteosarcomas. Oncogene. 2012;31(18):2270-82.
62. Yasuda K, Torigoe T, Morita R, Kuroda T, Takahashi A, Matsuzaki J, et al. Ovarian cancer stem cells are enriched in side population and aldehyde dehydrogenase bright overlapping population. PloS one. 2013;8(8):e68187.
63. Herreros-Villanueva M, Zhang JS, Koenig A, Abel EV, Smyrk TC, Bamlet WR, et al. SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells. Oncogenesis. 2013;2:e61.
64. Weina K, Utikal J. SOX2 and cancer: current research and its implications in the clinic. Clin Transl Med. 2014;3:19.
65. Han X, Fang X, Lou X, Hua D, Ding W, Foltz G, et al. Silencing SOX2 induced mesenchymal-epithelial transition and its expression predicts liver and lymph node metastasis of CRC patients. PloS one. 2012;7(8):e41335.
66. Li X, Xu Y, Chen Y, Chen S, Jia X, Sun T, et al. SOX2 promotes tumor metastasis by stimulating epithelial-to-mesenchymal transition via regulation of WNT/beta-catenin signal network. Cancer letters. 2013;336(2):379-89.
67. Xia Y, Wu Y, Liu B, Wang P, Chen Y. Downregulation of miR-638 promotes invasion and proliferation by regulating SOX2 and induces EMT in NSCLC. FEBS letters. 2014;588(14):2238-45.
68. Guo J, Wang B, Fu Z, Wei J, Lu W. Hypoxic Microenvironment Induces EMT and Upgrades Stem-Like Properties of Gastric Cancer Cells. Technol Cancer Res Treat. 2016;15(1):60-8.
69. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell. 2014;14(3):275-91.
70. Ghuwalewala S, Ghatak, D., Das, P., Dey, S., Sarkar, S., Alam, N., Panda, CK., Roychoudhury, S. CD44highCD24low molecular signature determines the Cancer Stem Cell and EMT phenotype in Oral Squamous Cell Carcinoma. Stem Cell Research 2016;16(2):405-17.
71. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133(4):704-15.

72. Choi D, Lee HW, Hur KY, Kim JJ, Park GS, Jang SH, et al. Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. World J Gastroenterol. 2009;15(18):2258-64.
73. Ponti D, Zaffaroni N, Capelli C, Daidone MG. Breast cancer stem cells: an overview. Eur J Cancer. 2006;42(9):1219-24.
74. Zhang Y, Bao, W., Wang, K., Lu, W., Wang, H., Tong, H., Wan, X. SOX17 is a tumor suppressor in endometrial cancer. Oncotarget. 2016;7(46):76036-46.
75. Zhang W, Glockner SC, Guo M, Machida EO, Wang DH, Easwaran H, et al. Epigenetic inactivation of the canonical Wnt antagonist SRY-box containing gene 17 in colorectal cancer. Cancer Res. 2008;68(8):2764-72.
76. Yin D, Jia Y, Yu Y, Brock MV, Herman JG, Han C, et al. SOX17 methylation inhibits its antagonism of Wnt signaling pathway in lung cancer. Discov Med. 2012;14(74):33-40.
77. Fu D, Wang, ZM., Chen-Li, Wang, BL., Shen, ZZ., Huang, W., Shao, ZM. . Sox17, the canonical Wnt antagonist, is epigenetically inactivated by promoter methylation in human breast cancer. Breeast Cancer Research and Treatment 2009;119:601-12.
78. Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop Relat Res. 1998(346):26-37.
79. Graff J. Embryonic patterning: To BMP or not to BMP, that is the question. Cell. 1997;89(2):171-4.
80. Guan H, Li J, Sun R, Liu W, Feng M, Ma H, et al. Expression Of BMP7 In Ovarian Cancer And Biological Effect Of BMP7 Knockdown On Ovarian Cancer Cells. Onco Targets Ther. 2019;12:7897-909.
81. Silva EG, Deavers, M.T., Bodurka, D.C., Malpica, A. Association of Low-Grade Endometrioid Carcinoma of the Uterus and Ovary With Undifferentiated Carcinoma: A New Type of Dedifferentiated Carcinoma? Int J Gynecol Pathol. 2006;25(1):52-8.
82. Kobel M, Meng, B., Hoang, L.N., Almadani, N., Li, X., Soslow, R.A., Gilks, C.B., Lee, C.H. . Molecular Analysis of Mixed Endometrial Carcinomas Shows Clonality in Most Cases. Am J Surg Pathol. 2016;40(2):166-80.
83. Hoang L, Han, G., McConechy, M., Lau, S., Chow, C., Blake Gilks, C., Huntsman, DG., Kobel, M., Lee, CH. Immunohistochemical characterization of prototypical endometrial clear cell carcinoma-diagnostic utility of HNF-1beta and oestrogen receptor. Histopathology. 2014;64(6):585-96.
84. Hoang LN, McConechy MK, Meng B, McIntyre JB, Ewanowich C, Gilks CB, et al. Targeted mutation analysis of endometrial clear cell carcinoma. Histopathology. 2015;66(5):664-74.

3
Characterization of cell-line based models with chromatin remodeling protein deficiencies that recapitulate dedifferentiated endometrial cancer

3.1. Introduction

DDEC while a rare neoplasm, constituting less than 10% of all endometrial cancer cases, possesses a nearly 100% fatality rate when diagnosed as stage III or IV disease (1). These dismal outcomes have been exasperated by a lack of cell line and xenograft models requisite to illuminate therapeutic targets in DDEC. Moreover, models wherein one may test the dependencies between the differentiated and dedifferentiated components have not been developed. The establishment of model systems amenable to experimental manipulation is necessary to ascertain the roles of SWI/SNF proteins in cellular dedifferentiation in the context of endometrial carcinoma. Engineered bacterial nucleases have spurred the development of genome editing technologies with the capability of directly targeting and modifying genomic sequences in eukaryotic cells (2, 3). The advent of this technology has allowed for the determination of the contribution of genetics to disease by bringing about the creation of more cellular and animal models of pathological processes (2, 3). All applications begin with the generation of DSBs by nucleases and in the case of functional gene knockouts, NHEJ-mediated repair is desired due to its tendency to introduce errors (3-7). Much like RNA interference, delivery of editing machinery *in vitro* to immortalized cell lines in concert with the action of NHEJ, can generate loss of function mutations and permanent inactivation of single or multiple genes (3, 8). Most prevalent of the genome editing technologies as of late is the type II CRISPR/Cas9 system (9). Several delivery strategies have been devised for genome editing with the CRISPR/Cas9 platform (3). Firstly, a plasmid based CRISPR/Cas9

strategy was utilized wherein a plasmid encoding for both the Cas9 protein and single guide RNA (sgRNA) is designed and subsequently transfected (10-12). Later developments, taking advantage of the fact that gene editing results in permanent changes in the genome, sought to accomplish CRISPR-mediated editing though Cas9 protein/sgRNA ribonucleoprotein (RNP) complexes (13). It should be noted that regardless of CRISPR gene editing methodology, constitutive expression or high stability of Cas9 nuclease and/or sgRNA and therefore subsequent accumulation of off-target activity may be undesirable when generating clonal cell lines for a phenotypical study of a specific gene knockout (13). Within this chapter, we perform extensive characterization of cell line derived models of DDEC that were generated in two MMR protein deficient endometrial cancer cell lines using Cas9 and sgRNA delivered via plasmid and RNP methods. We ascertained how loss of SMARCA4 in DNA MMR-deficient HEC 116 and HEC 59 EC cells contributes to their functional characteristics both in *in vitro* and *in vivo.* We show how we recapitulated the clinical phenotype of cellular dedifferentiation in endometrial cancer and the potential role of SMARCA4 as a chromatin remodeling protein in this process. Taken as a whole, this work represents the first models of DDEC generated and the comprehensive analyses of these SMARCA4 intact and SMARCA4 deficient EC cell lines aids in piecing together the mechanisms underlying the acquisition of plasticity and heterogeneity in this subset of lethal endometrial cancer cases.

3.2. Results

3.2.1. Mismatch repair protein deficient endometrial cancer cells exhibit hallmarks of well-differentiated carcinomas and possess intact SMARCA4 protein expression.

In 2013, extensive characterization at the genomic, transcriptomic, and proteomic level of hundreds of endometrial carcinomas using array and sequencing based technologies, resulted in the eventual categorization of neoplasms of the endometrium into four categories including microsatellite instable hypermutated (MSI-H) EC (14). MMR deficiency was predominant in the clinical DDEC cases previously examined by our group (1, 15, 16). To choose the most appropriate genetic background in which to develop cell-line based models that would most closely resemble DDEC in patients, we characterized several commercially available endometrial cancer cell lines. Interestingly, MMR was also extremely prevalent amongst the twelve endometrial cancer cell lines we examined with eight of the twelve displaying characteristics of microsatellite instability. The two cell lines, HEC 116 and HEC 59, we chose to derive SMARCA4 deficient cell line models of DDEC in shared several features with the MSI endometrioid tumors reported earlier (14). Specifically, they lacked mutations in *FBXW7* and *PP2R1A* **[Figure 3.1A]**. Additionally, HEC 59 EC cells possessed mutations in *ARID1A* and *TP53* **[Figure 3.1A]**. By immunohistochemistry, even in the absence of mutations, HEC 116 does not express ARID1A **[Figure 3.1B]**.

We further examined HEC 116 and HEC 59 cells in the context of cellular proliferation and survival through phospho-kinase arrays wherein activation states of various signalling pathways could be ascertained. Upon serum starvation, in both MMR-

deficient EC cell lines but especially HEC 59, proteins associated with the Wnt signalling pathway exhibited elevated phosphorylation levels [**Figure 3.2A]**. Phosphorylation levels measured as relative immunoblot intensities, of various STAT transcription factors were high in both EC cell lines, specifically STAT3 in HEC 116 cells subjected to serum starvation [**Figure 3.2B]**. Observed to some extent with both EC cell lines but particularly with HEC 116 cells was that serum starvation induced increased phosphorylation levels of several kinases within the AKT and ERK signalling pathways **[Supplemental Figure 3.1]**.

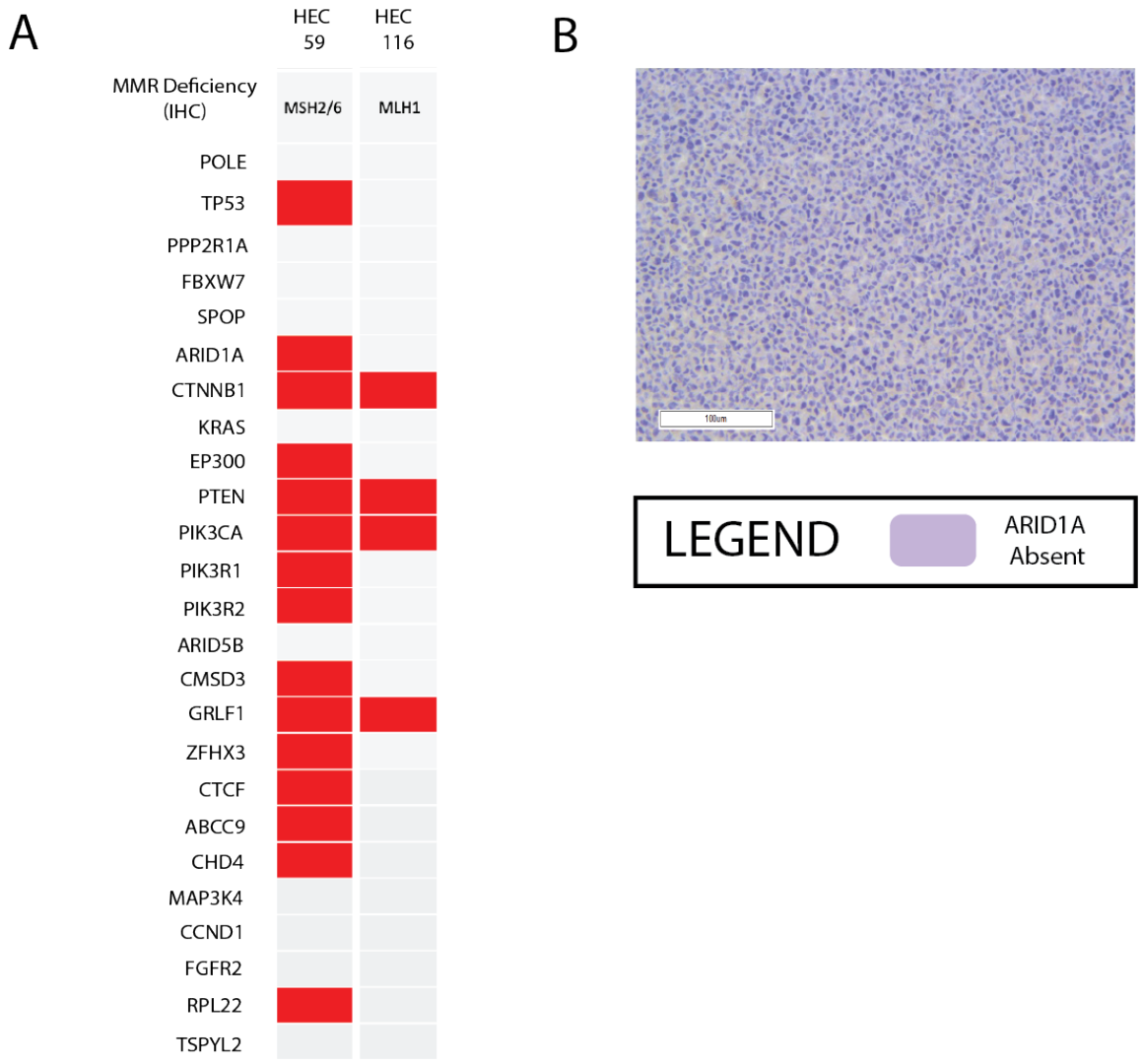


Figure 3.1. Summary of the molecular features and mutational profiles of commercial endometrial cancer cell lines. A) Mutations present in HEC 59 or HEC 116 EC cells discovered by targeted sequencing are highlighted in red. **B)** Absent ARID1A expression in parental HEC 116 EC cells. Scale bar = 100 μM.

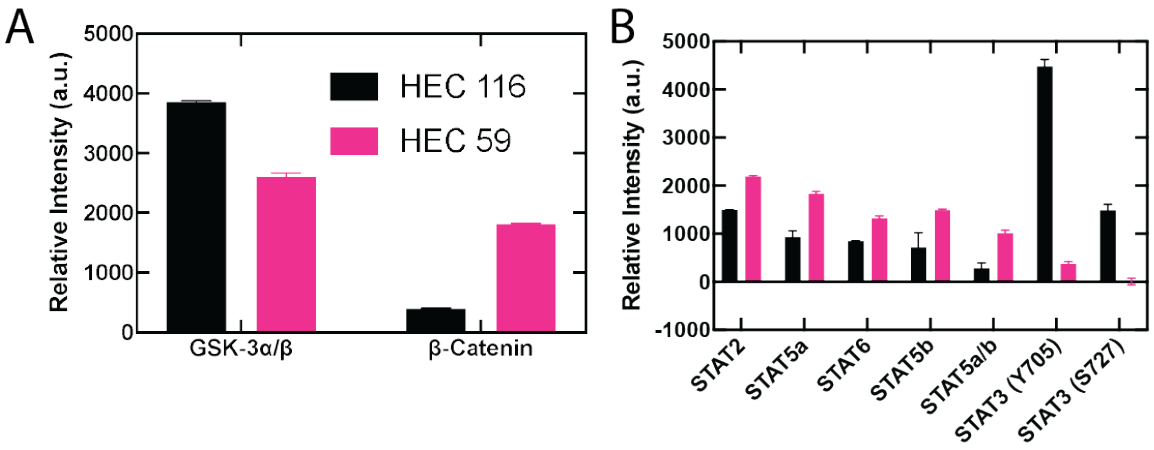


Figure 3.2. Activation of Wnt and STAT family kinases in mismatch repair protein deficient endometrial cancer cell lines HEC 116 and HEC 59. Quantitative analysis of phosphokinase antibody array blots using lysates of serum starved HEC 116 and HEC 59 EC cells. Data consists of two technical replicates. Activated proteins in these EC cell lines following serum starvation included **A)** Wnt related signaling molecules and **B)** STAT3.

Having established that HEC 116 and HEC 59 EC cell lines exhibited activation of numerous signalling pathways known to play a role in endometrial cancer development, we also demonstrated the high-grade nature of these endometrial cancer cell lines through immunostaining for the gynecological differentiation marker, PAX8 (17). Both HEC 116 and HEC 59 EC cells have retained PAX8 expression in addition to the SWI/SNF CRC subunit, SMARCA4 **[Figure 3.3]**.

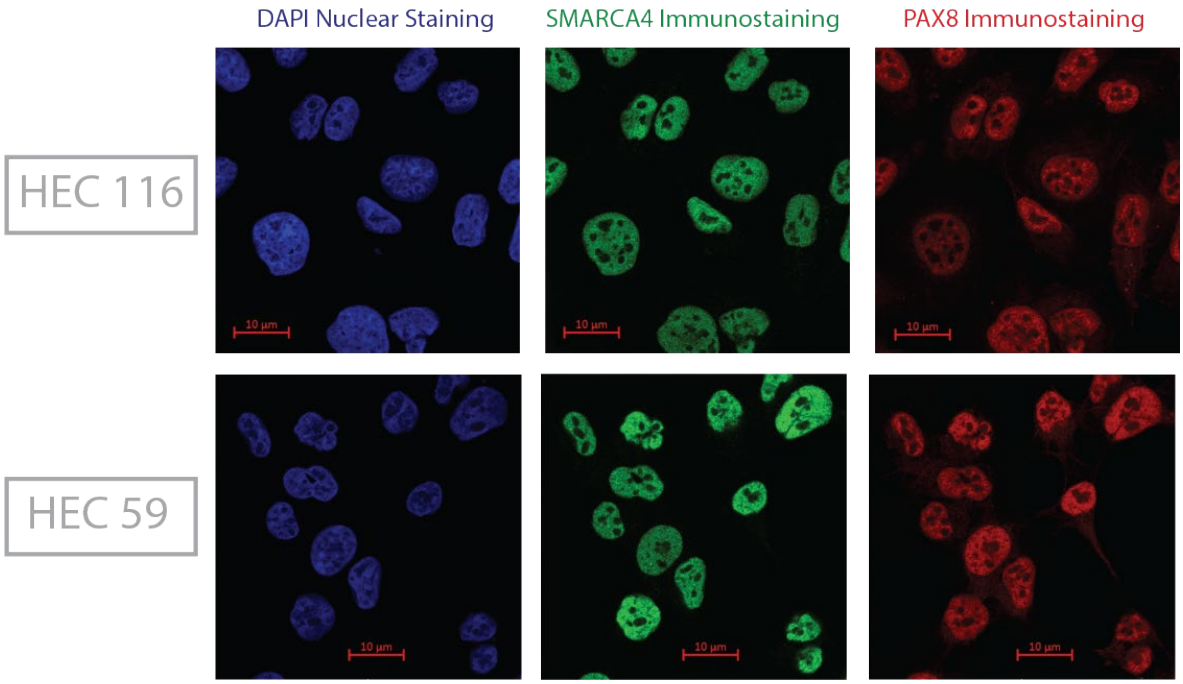


Figure 3.3. Mismatch repair protein deficient endometrial cancer cell lines HEC 116 and HEC 59 possess high levels of both SWI/SNF CRC subunits and gynecological differentiation markers. Immunofluorescent staining followed by confocal microscopy revealed ample expression of both SMARCA4 and PAX8. Scale bar = 10 µM.

3.2.2. Generation of SMARCA4-deficient endometrial cancer cell lines by CRISPR gene editing.

Two different CRISPR methodologies were employed to induce frameshift mutations in SMARCA4 that would lead to loss of protein in endometrial cancer cells through the introduction of premature stop codons, thereby modelling what we observe in clinical cases of DDEC. Targets within the second exon of SMARCA4 were chosen as the multitude of isoforms reported for this protein appear to retain this region of the gene **[Supplemental Figure 3.2]**. Initially, an "all-in-one" plasmid was used which contains both the genetic code for Cas9 as well as the guide RNA sequence specific to SMARCA4 **[Figure 3.4]**. Single cell-derived clones were generated and screened for mutations by droplet digital PCR (ddPCR) as described in Findlay *et al.* prior to further validation by

cloning and sequencing of the target region **[Figure 3.4]** (18). HEC 116 Plasmid Derived SMARCA4 knockout (KO) cells consisted of 2 clones with biallelic mutations and a wildtype (WT) clone that possessed no mutations to the target site but had been through the gene editing workflow **[Figure 3.5]**. The SMARCA4 status of the mixed KO clones was validated at the protein level by immunohistochemistry **[Figure 3.8A]**. The development of a gene editing tool from IDT, claiming to reduce off-target effects was explored as another way to obtain functional knockouts of SMARCA4 **[Figure 3.4]**. Briefly, ribonucleoprotein complexes containing recombinant Cas9 protein, trans-activating CRISPR RNA (tracrRNA) and CRISPR guideRNA (cRNA) specific to SMARCA4 were utilized, leading to the attainment of two RNP-derived SMARCA4 deficient cell line models in HEC 116 and HEC 59 **[Figure 3.4]**. Two wildtype clones make up both the HEC 116 and HEC 59 SMARCA4 intact populations while one clone and four clones with biallelic inactivation of SMARCA4 were found in HEC 59 and HEC 116 endometrial cancer cells, respectively **[Figure 3.6 & Figure 3.7]**. Confirmation of these RNP-derived cell line models at the protein level was accomplished in a similar manner as with the HEC 116 Plasmid Derived SMARCA4 KO and WT cells **[Figure 3.8B & Figure 3.8C]**. All knockout clones are predicted to have premature stop codons introduced as a direct result of frameshift mutations to SMARCA4, located in the disordered region preceding the HSA domain **[Supplemental Figure 3.2].**

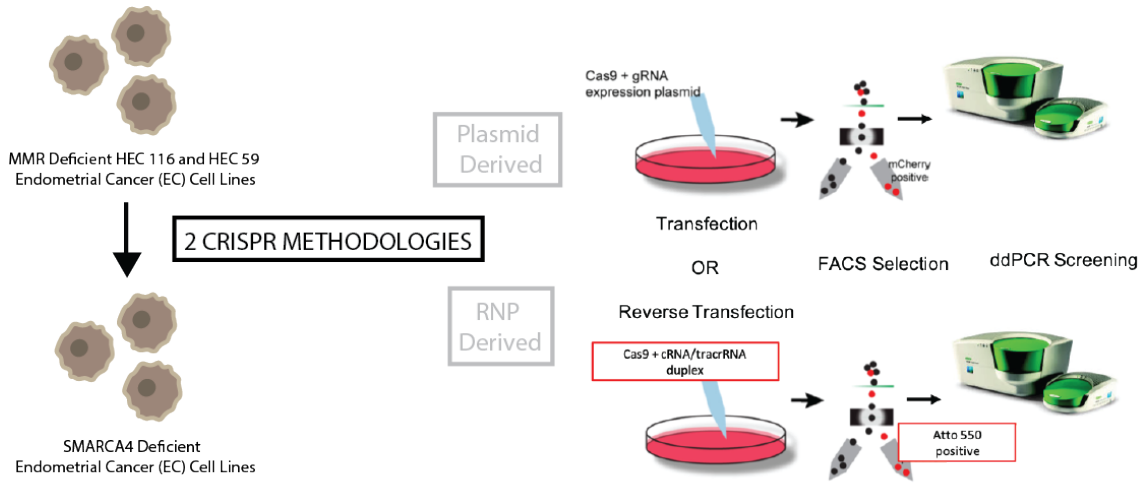


Figure 3.4. Schematic outlining the CRISPR workflows employed to derive EC cell lines lacking SMARCA4 expression. Firstly, target cell lines were transfected with either the Cas9+gRNA expression plasmid or an RNP complex consisting of Cas9 plus a cRNA/tracRNA duplex. Next, gene edited cells were enriched by cell sorting and expanded as single clones prior to screening by ddPCR. Adapted from (18).

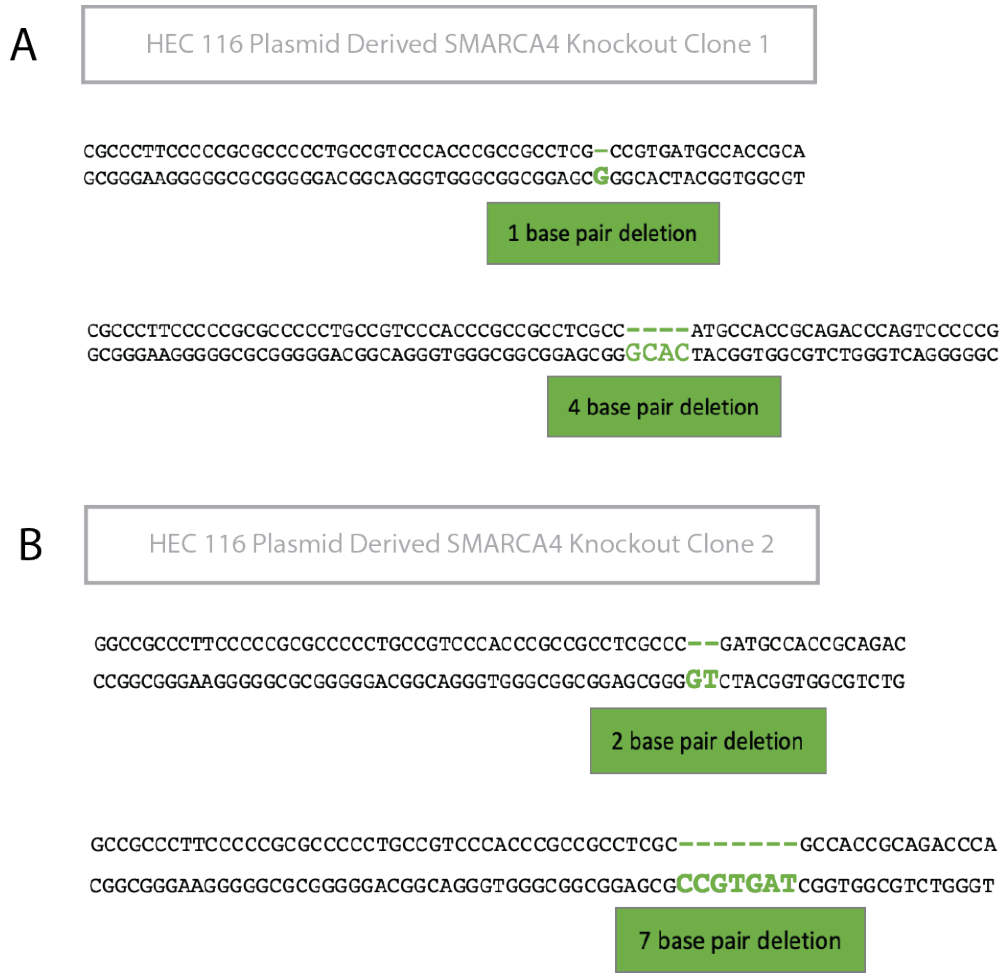
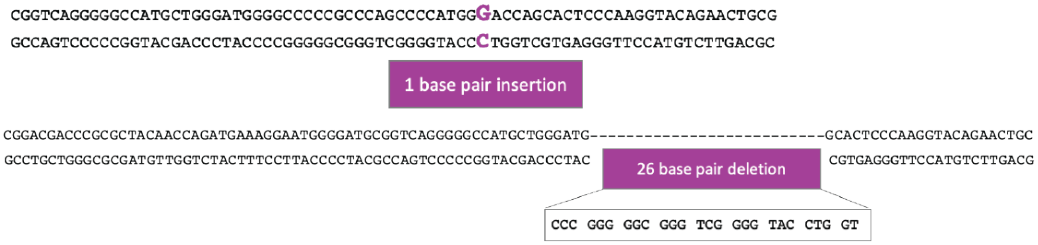


Figure 3.5. HEC 116 Plasmid Derived SMARCA4 knockout EC cells contain frameshift mutations upon CRISPR gene editing. A) One of the two clones possessed alleles with either one or four nucleotides missing. **B)** The other clone constituting the mixed HEC 116 Plasmid Derived SMARCA4 deficient cells also has alleles that when repaired by NHEJ resulted in two or seven nucleotide deletion events.

A

HEC 116 RNP Derived SMARCA4 Knockout Clone 1



B

HEC 116 RNP Derived SMARCA4 Knockout Clone 2

C

HEC 116 RNP Derived SMARCA4 Knockout Clone 3

D

HEC 116 RNP Derived SMARCA4 Knockout Clone 4

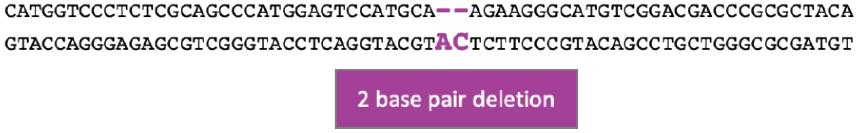


Figure 3.6. HEC 116 RNP Derived SMARCA4 knockout EC cells contain frameshift mutations upon CRISPR gene editing. A) Unlike the plasmid-derived cell line model of DDEC, one of the four clones had alleles when sequenced that were found to have undergone either a one nucleotide insertion event or a twenty-six-nucleotide deletion. **B)** Similar to the knockout clone 1 comprising the HEC 116 RNP Derived SMARCA4 deficient cells, this clone was discovered to harbor alleles with either one nucleotide inserted, or thirteen nucleotides deleted. **C)** HEC 116 SMARCA4 deficient knockout clone 3 that is RNP derived is unique in that by NHEJ it was repaired to have either a one nucleotide insertion or deletion in SMARCA4 introduced. **D)** Both alleles for knockout clone 4 were revealed by sequencing to harbor deletions of two nucleotides within the coding region of SMARCA4.

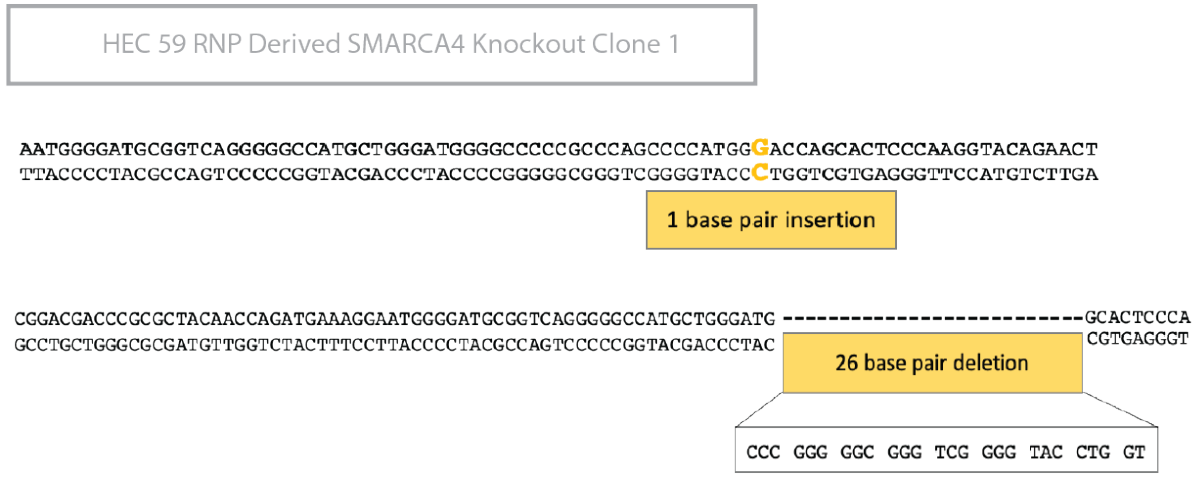


Figure 3.7. HEC 59 RNP Derived SMARCA4 knockout EC cells contain frameshift mutations upon CRISPR gene editing. A single clone possessing similar genetic alterations to one of the HEC 116 RNP Derived SMARCA4 knockout clones was discovered and found to have one allele containing a single nucleotide insertion and a twenty-six nucleotide deletion on the second allele.

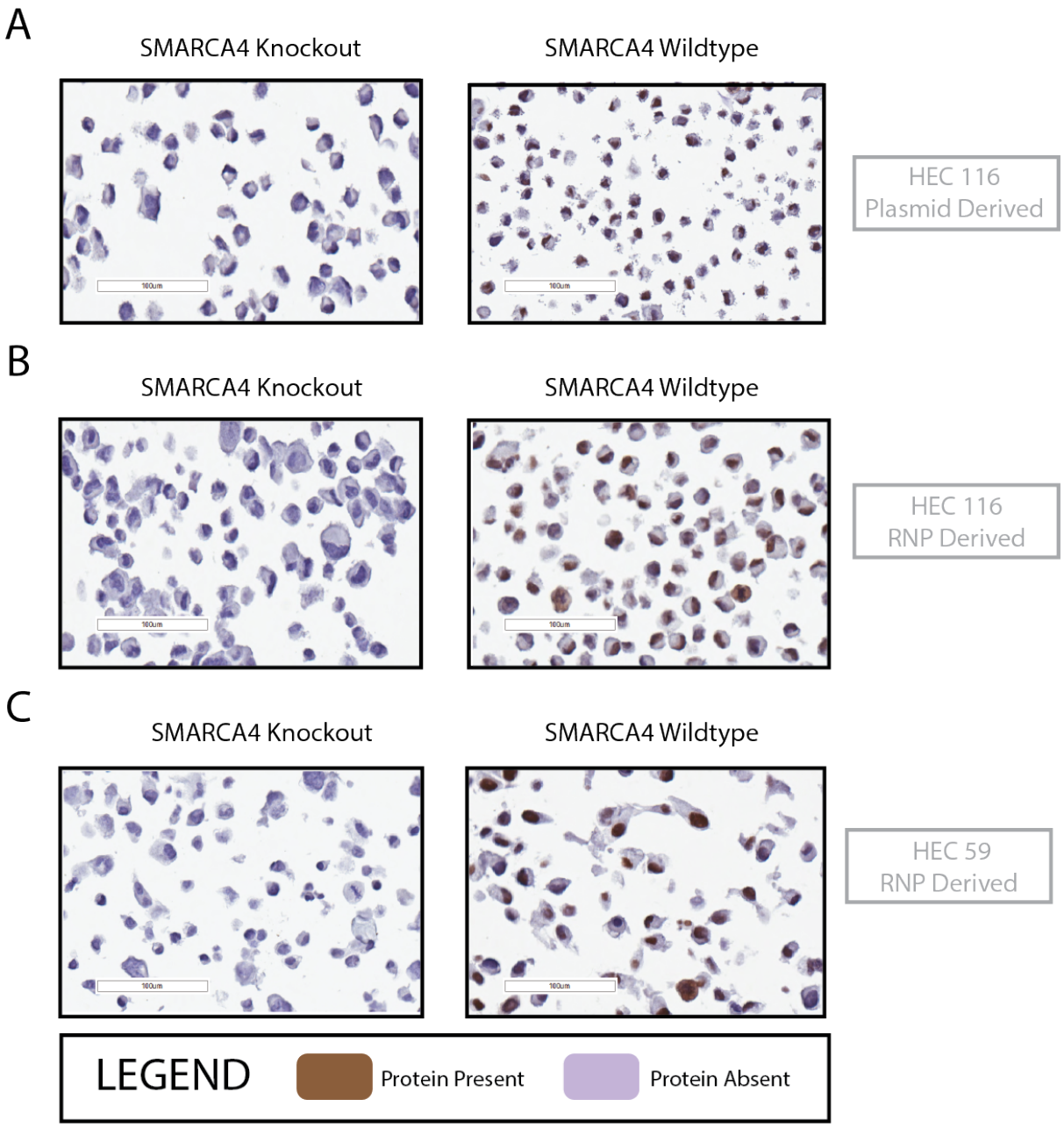


Figure 3.8. SMARCA4 immunostaining verified absence of protein upon introduction of frameshift mutations by CRISPR gene editing. Absence of nuclear SMARCA4 signal is evident in the mixed SMARCA4 KO clones when compared to their mixed SMARCA4 WT counterparts. Similar phenomenon was observed for all three of the following sequence confirmed derivations of DDEC: **A)** HEC 116 Plasmid Derived, **B)** HEC 116 RNP Derived, and the **C)** HEC 59 RNP Derived model. Scale bar = 100 µM.

3.2.3. In vitro SMARCA4 deficiency in MMR deficient EC cells recapitulates some features of clinical DDEC.

Having successfully obtained endometrial cancer cell lines lacking SMARCA4 expression, we began extensive functional characterization of all three models particularly focused on hallmarks of cancer such as proliferation, invasion, and plasticity (19). First, we examined the expression of several genes between the paired SMARCA4 knockout and wildtype cell lines with an emphasis on those associated with epithelial-to-mesenchymal transition, stemness, and endometrial development. Regardless of the CRISPR gene editing methodology utilized, in HEC 116 EC cells lacking SMARCA4 expression, levels of E-cadherin expression are reduced **[Figure 3.9A & Figure 3.9B]**. Additionally, gene expression levels of Oct4 increase in the absence of SMARCA4 protein in HEC 116 EC cells **[Figure 3.9A & Figure 3.9B]**. ER loss, a feature of clinical DDEC cases was only witnessed in the HEC 116 Plasmid Derived model with SMARCA4 knocked out and the same phenomenon was not apparent in the HEC 116 RNP Derived SMARCA4 KO cells **[Figure 3.9A & Figure 3.9B]**. The HEC 59-derived model on the other hand did not exhibit partial EMT nor acquisition of stemness in a similar fashion as the gene edited HEC 116 EC cells, even though examination of cycle threshold (C_T) values indicated baseline expression levels of E-cadherin to be within the same range. Baseline expression of ER on the other hand, was determined to be undetectable or well below 35 in HEC 59 EC cells regardless of SMARCA4 status **[Figure 3.9C]**. The decrease in N-cadherin and Sox2 expression present in HEC 59 EC cells lacking SMARCA4 expression though indicates that absence of SMARCA4 substantially alters the gene expression program of these cancer cells **[Figure 3.9C]**.

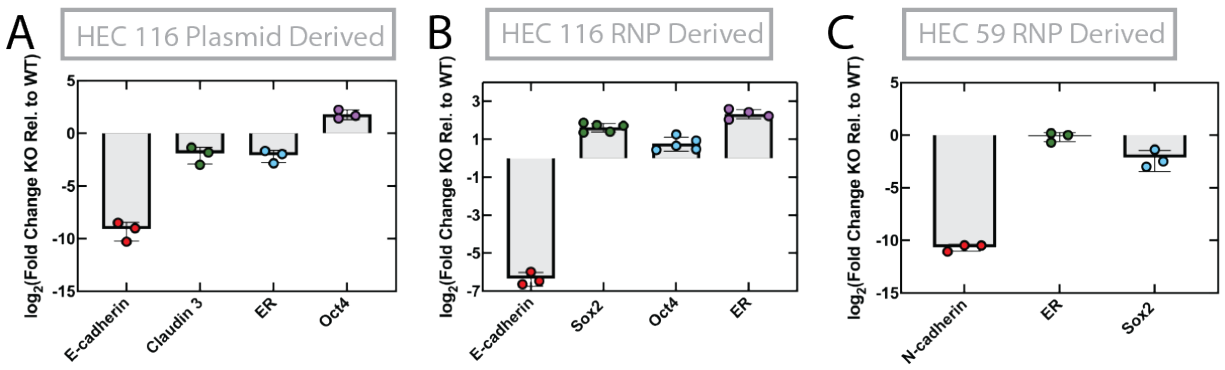


Figure 3.9. HEC 116 EC cells lacking SMARCA4 expression exhibit gene expression changes resembling the undifferentiated SMARCA4 deficient regions of DDEC patient neoplasms. Quantitative real time PCR analysis of **A)** HEC 116 Plasmid Derived **B)** HEC 116 RNP Derived and **C)** HEC 59 RNP Derived EC cells for select markers of EMT (E-cadherin, N-cadherin), gynecological function (ER), and stemness (Oct4, Sox2). At least three SMARCA4 KO biological replicates depicted as colored circles were normalized to paired WT samples and represented as log2 fold changes.

Functional examination of the derived DDEC cell line models was executed next particularly focusing on their ability to form tumorspheres and self-renew as well as their capacity to grow independent of anchorage. Sphere formation assays were carried out with both SMARCA4 intact and SMARCA4 deficient EC cell lines. Regardless of the cell line and the way it was propagated, cells lacking SMARCA4 expression were less able to form spheres than their wildtype counterparts **[Figure 3.10]**. The spheres formed from SMARCA4 knockout HEC 116 and HEC 59 EC cells in minimal media were significantly smaller in size. They also manifested an altered morphology, being more grape-like than spheroidal in appearance **[Figure 3.10]**. While HEC 116 cells form fewer first-generation spheres regardless of the CRISPR methodology used to derive them originally, HEC 59 cells could form enough first generation spheres that self-renewal could be tested by second and tertiary sphere formation experiments. While the number of spheres formed

from HEC 59 WT EC remained relatively consistent with each passing generation, the number of spheres formed from HEC 59 RNP Derived SMARCA4 KO EC cells increased in later generations, particularly when fewer cells were plated **[Figure 3.11]**. Colony formation in agar was undertaken to quantify the tumorgenicity of our cell-line models of DDEC and cell proliferation was measured using trypan blue exclusion assays (20, 21). Both HEC 116 and HEC 59 EC cells lacking SMARCA4 expression formed fewer colonies than EC cells retaining SMARCA4 **[Figure 3.12]**. Notably, only endometrial cancer cells lacking SMARCA4 expression derived using RNP-based CRISPR methodology consistently proliferated slower than their paired SMARCA4 intact EC cells when grown in conventional 2D cultures **[Figure 3.13]**.

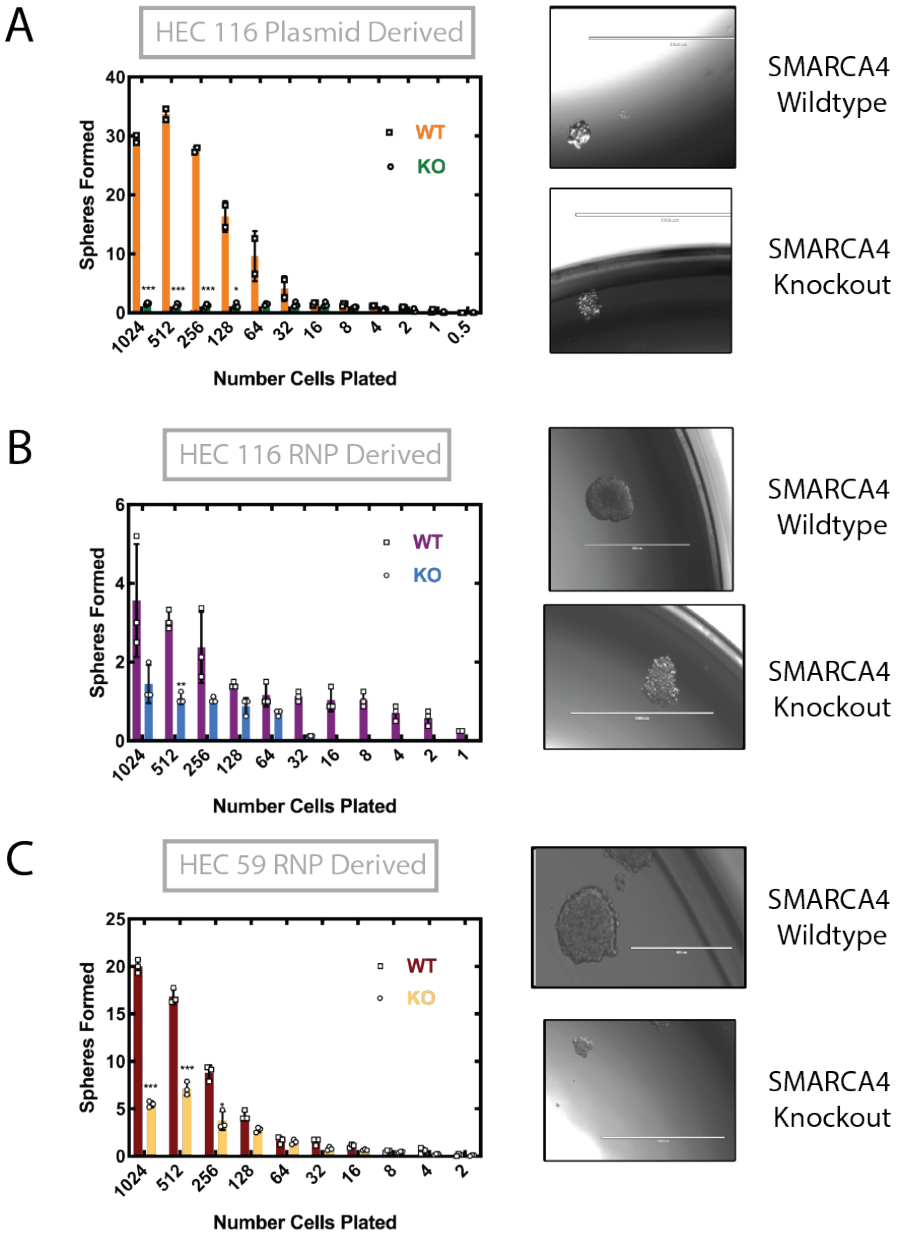


Figure 3.10. Endometrial cancer cells with absent SMARCA4 expression are significantly less capable of sphere formation than EC cells with intact SMARCA4 protein levels. Limiting dilution sphere formation assays were carried out using **A)** HEC 116 Plasmid Derived, **B)** HEC 116 RNP Derived, and **C)** HEC 59 RNP Derived SMARCA4 KO and WT EC cells. Decreasing concentrations of single cell suspensions were seeded in low attachment plates and grown in minimal media for up to two weeks. Multiple t-tests confirmed the presence of any significant difference in sphere forming capabilities (***= $p < 0.001$. ** = $p < 0.01$. * = $p < 0.05$). Data consists of eight technical replicates from three independent biological replicates depicted as white-colored shapes. Representative images of spheres are featured on the right. Scale bar = 1000 µM.

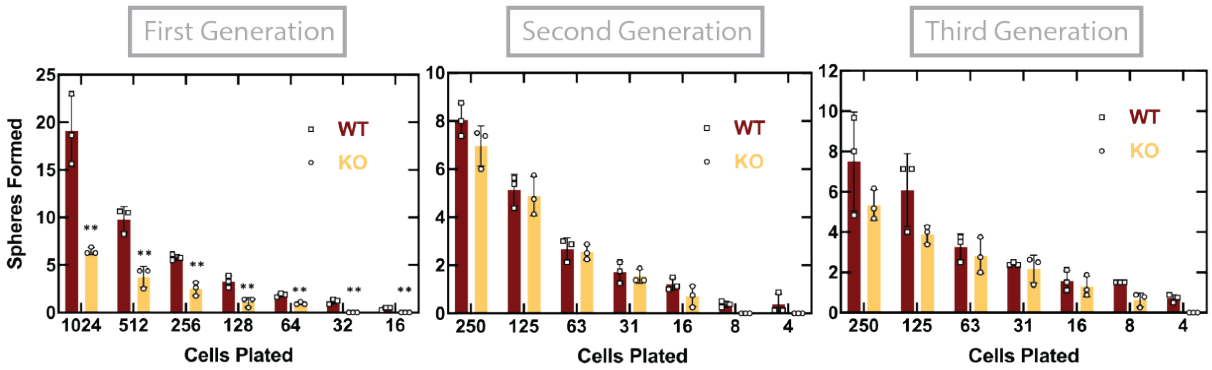


Figure 3.11. HEC 59 endometrial cancer cells with absent SMARCA4 expression are more capable of self-renewal upon secondary and tertiary sphere formation. Limiting dilution sphere formation assays were carried out using HEC 59 RNP Derived SMARCA4 KO and WT EC cells. Decreasing concentrations of single cell suspensions were seeded in low attachment plates and grown in minimal media for up to two weeks. Second and third generation spheres were formed by dissociating the spheres from the previous generation into single cells. Multiple t-tests confirmed the presence of any significant difference in sphere forming capabilities (** = $p < 0.01$. * = $p < 0.05$). Data consists of eight technical replicates from three independent biological replicates depicted as white-colored shapes.

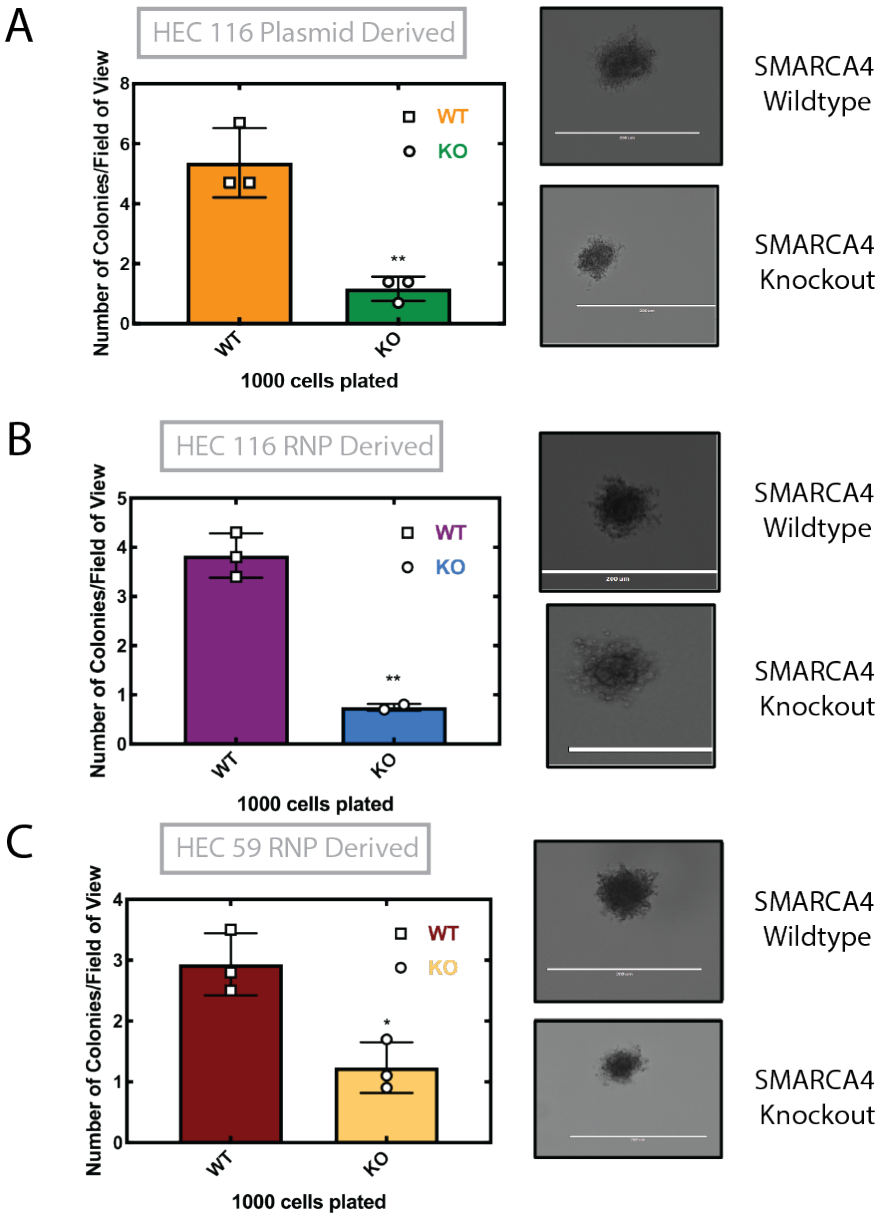
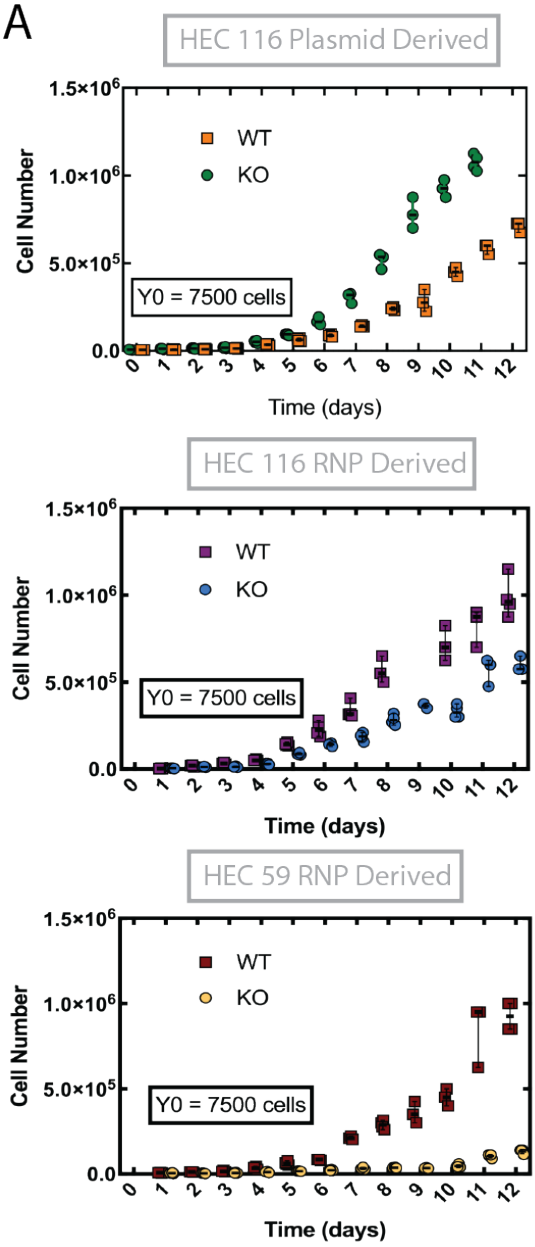


Figure 3.12. Endometrial cancer cells with absent SMARCA4 expression are less capable of anchorage independent growth. Soft agar experiments were carried out utilizing **A)** HEC 116 Plasmid Derived, **B)** HEC 116 RNP Derived, and **C)** HEC 59 RNP Derived SMARCA4 KO and WT EC cells. 1000 cells/well were seeded in a layer of 0.7% agarose-containing media and incubated for up to two weeks. Multiple t-tests confirmed the presence of any significant difference in colony forming capabilities (** = $p < 0.01$. * = $p < 0.05$). Representative images of colonies are shown on the right. Values in the graphs represent the mean and standard deviation from ten fields of view across three replicate experiments depicted as white-colored shapes. Scale bar = 200 μm.

A



B

	Doubling Time (hour)
HEC 116 Plasmid Derived DDEC Model	
SMARCA4 Wildtype	43 (42-43)
SMARCA4 Knockout	36 (35-36)
HEC 116 RNP Derived DDEC Model	
SMARCA4 Wildtype	36 (36-38)
SMARCA4 Knockout	41 (38-41)
HEC 59 RNP Derived DDEC Model	
SMARCA4 Wildtype	41 (40-41)
SMARCA4 Knockout	72 (71-75)

Figure 3.13. RNP Derived but not Plasmid Derived SMARCA4 deficient EC cells grow slower than their wildtype counterparts. A) 7500 SMARCA4 KO and WT cell lines were plated and counted by Trypan Blue daily over a growth period lasting 12 days. Data consists of three technical replicates represented as individual-colored shapes from two independent biological replicates. Filled black rectangles with error bars represent the mean and standard deviation of the SMARCA4 KO and WT technical replicates, respectively. **B)** Doubling times with 95% confidence intervals were calculated upon fitting the cell number data with non-linear regression curve fits, specifically the exponential growth equation in GraphPad Prism.

3.2.4. In vitro SMARCA4 deficient cell line models of DDEC exhibit properties of senescence.

Based on the clinical evidence that SMARCA4 alters differentiation-associated gene expression programs, we had hypothesized that that loss of SMARCA4 would induce a more aggressive stem-like phenotype, thus our findings in terms of a lack of self-renewal, proliferation and anchorage independent growth were surprising. To better understand the behavior of SMARCA4 knockout EC cells *in vitro,* we compared the transcriptomes and secretomes of HEC 116 and HEC 59 SMARCA4 knockouts to those obtained from their WT counterparts. Bulk RNA-sequencing found a significant shift in the transcriptome upon loss of SMARCA4 in serum starved HEC 116 Plasmid Derived cells. The bulk transcriptomic data clustered distinctly based on their SMARCA4 status **[Supplemental Figure 3.3]**. Notably, a relatively even number of transcripts were up and down-regulated in the absence of the chromatin remodeling protein, 1680 and 1761, respectively **[Figure 3.14A]**. Transcripts downregulated upon SMARCA4 knockout include those that would encode proteins associated with estrogen response as well as the extracellular matrix and adherens junctions of epithelial cells **[Figure 3.14B]**. Corroborating with some of our earlier qRT-PCR findings, some of the most dysregulated genes in the absence of SMARCA4 were downstream targets of Zeb1 like *CDH1, CDH3 and EPCAM* **[Figure 3.14]**. Genes upregulated in SMARCA4 knockout EC cells were found to be related to cell cycle progression, response to hypoxia and properties of stemness. Many of the upregulated transcripts in HEC 116 SMARCA4 deficient EC cells though, were proteins that are considered hallmarks of cellular senescence, such as interferon responsive genes in addition to TNF signalling **[Figure 3.14B].**

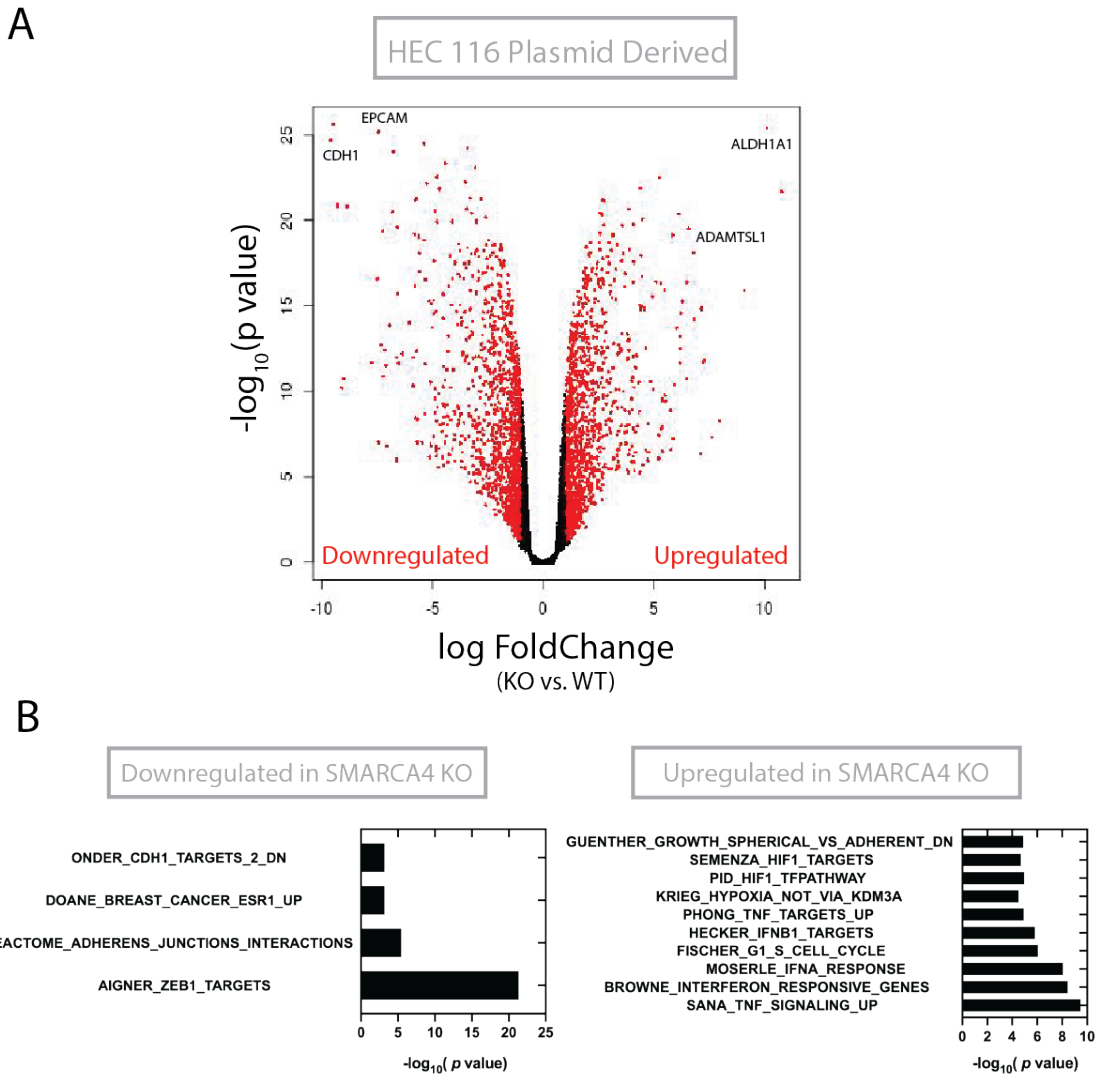
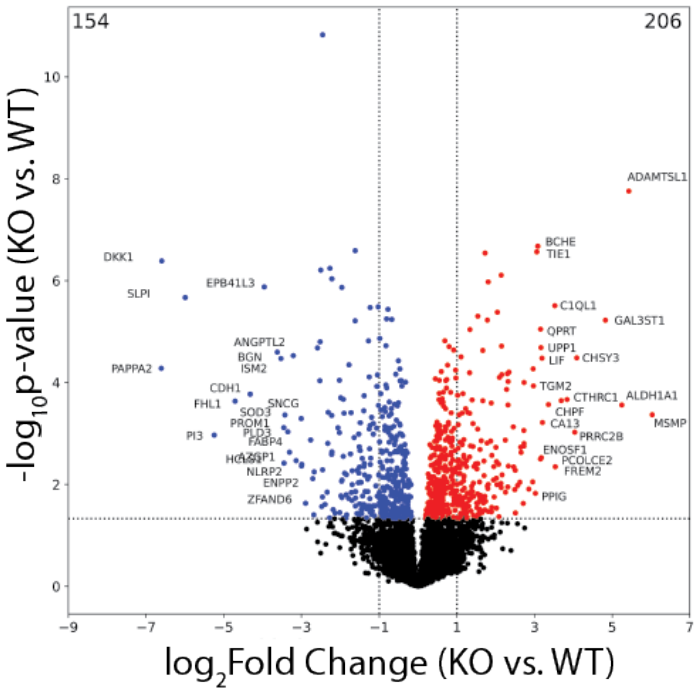


Figure 3.14. Lack of SMARCA4 expression in endometrial cancer cells leads to upregulation of gene expression programs associated with cellular senescence. A) Volcano plot analysis of modulated cellular pathways wherein log fold change comparing HEC 116 SMARCA4 KO EC cells to HEC 116 SMARCA4 WT EC cells was plotted against the log of the p-values. Filled black dots are not significant while filled red circles indicate a fold change greater than 1 and significance less than 0.05. **B)** Correlation Adjusted Mean Rank (Camera) gene set testing confirmed activation of a senescence signature in conjunction with a reduction in expression of ZEB1 pathway components such as E-cadherin.

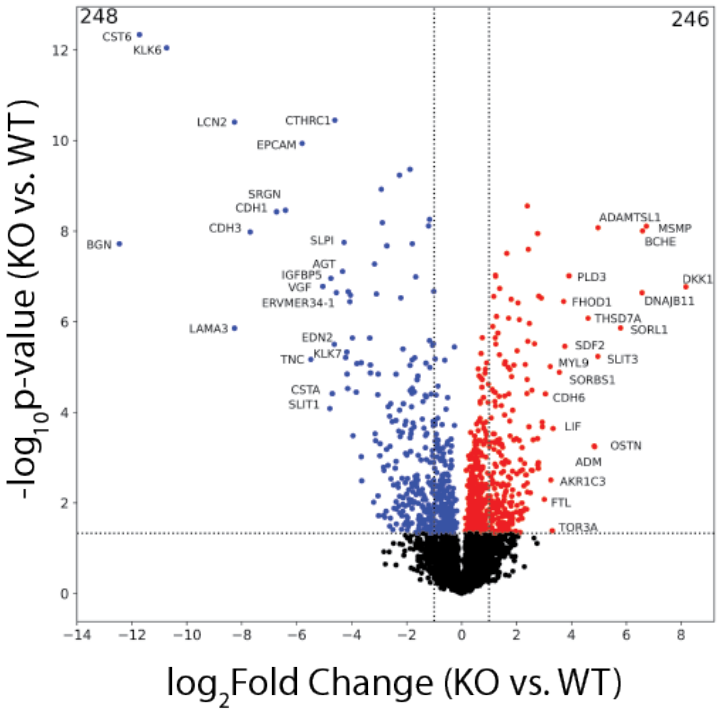
Next, due to the pathways that we expected to change based on our transcriptome data, we examined the secretome rather than the whole proteome of gene edited EC cells. Mass spectrometry performed on serum-free conditioned media from our various cell line models of DDEC identified on average over 300 proteins that were significantly different between SMARCA4 knockout and wildtype conditioned media. A relatively similar number of proteins were found to be upregulated or downregulated regardless of the pair of EC cell lines studied **[Figure 3.15]**. Within the proteomics data of both HEC 116 cell line models of DDEC, we detected significantly lower E-cadherin expression, a marker also shown to be reduced in clinical DDEC **[Figure 3.15]** (22). ALDH1A1, a stemness associated marker in cancers of the breast and ovary, was discovered at elevated levels within the conditioned media from HEC 116 Plasmid Derived SMARCA4 KO EC cells **[Figure 3.15]** (23, 24). To evaluate which pathways and processes differ when SMARCA4 is lost in endometrial cancer cells, gene set enrichment analysis was executed using the Molecular Signatures Database (MSigDB). Several gene sets with nominal p-values ≤0.05 were identified, with a subset also within the recommended false discovery rate (FDR) cut-off of 0.25 **[Tables 3.1-3.3]**. Among the gene sets found to be enriched (positive normalized enrichment score (NES)) were inflammatory response (Hallmark) in HEC 116 Plasmid Derived SMARCA4 KO EC cells and interferon response and signaling (Browne and Reactome, respectively) in HEC 59 RNP Derived SMARCA4 KO EC cells **[Table 3.1 & Table 3.3]**. Proteins included in these gene sets include integrins, interleukins, TIMP1 among others and have been reported to characterize the SASP of epithelial cells (25). Other notable gene sets significantly downregulated in both HEC 116 SMARCA4 KO DDEC cell line models, was CDH1 targets (Onder) and unique

to the RNP Derived HEC 116 SMARCA4 KO EC cells, estrogen response late gene sets (Hallmark) **[Table 3.1 & Table 3.2]**.

HEC 116 Plasmid Derived



HEC 116 RNP Derived



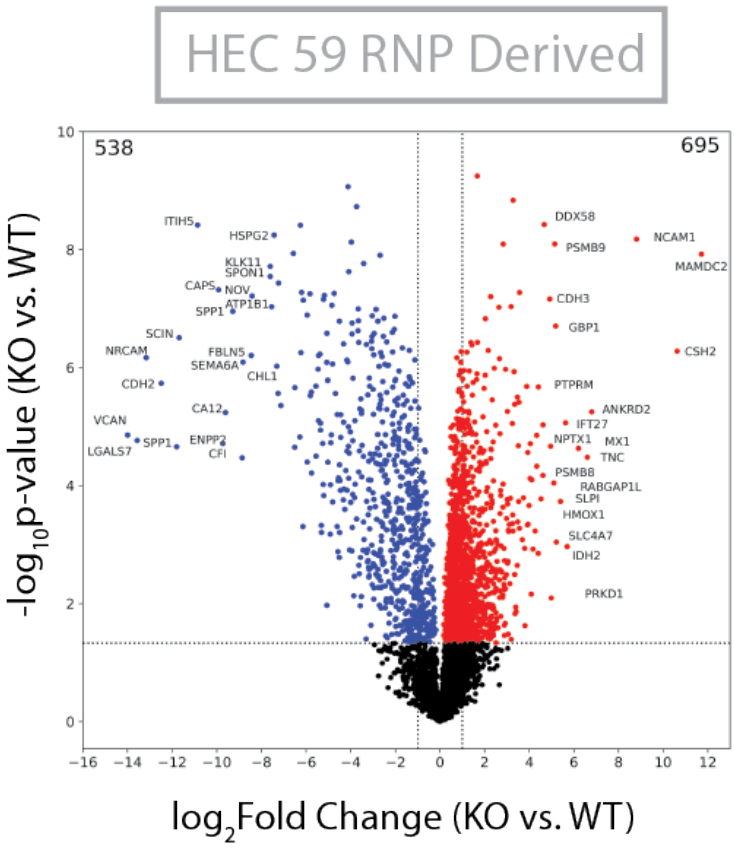


Figure 3.15. Substantial differences in the secretomes of EC cells based on their SMARCA4 status was observed across all cell line models of DDEC. Volcano plot of log2 fold changes in label free quantification (LFQ) intensities (KO vs. WT) revealing a substantial number of differentially expressed proteins. Proteins significantly elevated in the conditioned media of SMARCA4 KO cells are colored in red while proteins found at significantly reduced levels in the conditioned media of SMARCA4 KO cells are shown in blue. The top 20 differently expressed genes are labeled in black.

Table 3.1. Gene sets enriched in HEC 116 Plasmid Derived SMARCA4 deficient and intact endometrial cancer cell lines. Red bolded rows indicate limited gene sets with FDR q-values less than 0.25.

HEC 116 Plasmid Derived

Upregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
KEGG_LYSOSOME	64	1.90	0.000	0.400	0.348
HALLMARK_INFLAMMATORY_RESPONSE	29	1.87	0.000	0.339	0.522
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	**13**	**1.87**	**0.002**	**0.238**	**0.536**
REACTOME_PEPTIDE_CHAIN_ELONGATION	**77**	**1.87**	**0.000**	**0.178**	**0.536**
KEGG_RIBOSOME	**75**	**1.85**	**0.000**	**0.176**	**0.606**
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	**13**	**1.84**	**0.004**	**0.175**	**0.675**
REACTOME_INFLUENZA_VIRAL_RNA_ TRANSCRIPTION_AND_REPLICATION	**89**	**1.84**	**0.000**	**0.164**	**0.717**
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROTIN_SULFATE	**10**	**1.84**	**0.000**	**0.148**	**0.729**
KARAKAS_TGFB1_SIGNALING	**10**	**1.84**	**0.002**	**0.132**	**0.732**
TONKS_TARGETS_OF_RUNX1_RUNX1T1 _FUSION_HSC_UP	**62**	**1.82**	**0.000**	**0.152**	**0.818**

Downregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
ONDER_CDH1_TARGETS_3_DN	**11**	**-2.15**	**0.000**	**0.005**	**0.005**
SENGUPTA_NASOPHARYNGEAL_CARCINOMA	**52**	**-2.08**	**0.000**	**0.014**	**0.026**
JAEGER_METASTASIS_DN	**61**	**-2.08**	**0.000**	**0.010**	**0.029**
CHARAFE_BREAST_CANCER_BASA:_VS_MESENCHYMAL_DN	**25**	**-2.05**	**0.000**	**0.012**	**0.045**
NADLER_OBESITY_UP	**35**	**-2.00**	**0.002**	**0.031**	**0.142**
MCDOWELL_ACUTE_LUNG_INJURY_DN	**16**	**-1.92**	**0.000**	**0.097**	**0.424**
HOFMAN_MYELODYSPLASTIC_SYNDROM_RISK_UP	**10**	**-1.91**	**0.000**	**0.094**	**0.468**
HUPER_BREAST_BASAL_LUMINAL_DN	**29**	**-1.91**	**0.000**	**0.085**	**0.482**
ZHANG_ANTIVIRAL_RESPONSE_TO_RIBAVIRIN_UP	**11**	**-1.89**	**0.002**	**0.105**	**0.593**
YAO_TEMPORAL_RESPONSE_TOPROGESTERONE_CLUSTER_0	**25**	**-1.88**	**0.000**	**0.110**	**0.648**

Table 3.2. Gene sets enriched in HEC 116 RNP Derived SMARCA4 deficient and intact endometrial cancer cell lines. Red bolded rows indicate limited gene sets with FDR q-values less than 0.25.

HEC 116 RNP Derived

Upregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
WAKASUGI_HAVE_ZNF143_BINDING_SITES	19	1.89	0.000	0.318	0.300
KAUFFMANN_DNA_REPAIR_GENES	82	1.83	0.000	0.459	0.642
KEGG_CELL_CYCLE	42	1.82	0.000	0.371	0.717
PUJANA_BREAST_CANCER_LIT_INT_NETWORK	46	1.79	0.000	0.420	0.837
PID_ATM_PATHWAY	14	1.77	0.002	0.440	0.914
REACTOME_PHOSPHOLIPID_METABOLISM	39	1.75	0.000	0.502	0.965
PID_PS1_PATHWAY	19	1.70	0.000	0.812	1
BIOCARTA_G2_PATHWAY	10	1.69	0.002	0.791	1
FARDIN_HYPOXIA_11	14	1.69	0.004	0.740	1
CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_DN	191	1.67	0.000	0..790	1

Downgregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
ONDER_CDH1_TARGETS_2_DN	**129**	**-2.33**	**0.000**	**0.000**	**0.000**
COLDREN_GEFITINIB_RESISTANCE_DN	**59**	**-2.32**	**0.000**	**0.000**	**0.000**
JAEGER_METASTASIS_DN	**64**	**-2.23**	**0.000**	**0.000**	**0.000**
CHARAFE_BREAST_CANCER_BASAL_VS_MESENCHYMAL_UP	28	**-2.21**	**0.000**	**0.000**	**0.000**
CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_UP	111	**-2.17**	**0.000**	**0.000**	**0.001**
HOLLERN_EMT_BREAST_CANCER_DN	34	**-2.16**	**0.000**	**0.000**	**0.001**
SESTO_RESPONSE_TO_UV_C1	44	**-2.13**	**0.000**	**0.000**	**0.002**
LIEN_BREAST_CARCINOMA_METAPLASTIC_VS_DUCTAL_DN	23	**-2.10**	**0.000**	**0.001**	**0.005**
SATO_SILENCED_BY_METHYLATION_IN_PANCREATIC_CANCER_1	88	**-2.07**	**0.000**	**0.002**	**0.018**
HALLMARK_ESTROGEN_RESPONSE_LATE	64	**-2.06**	**0.000**	**0.003**	**0.028**

Table 3.3. Gene sets enriched in HEC 59 SMARCA4 deficient and intact endometrial cancer cell lines. Red bolded rows indicate limited gene sets with FDR q-values less than 0.25.

HEC 59 RNP Derived

Upregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
BROWNE_INTERFERON_RESPONSIVE_GENES	**18**	**2.04**	**0.000**	**0.066**	**0.070**
DAUER_STAT3_TARGETS_DN	**16**	**1.95**	**0.000**	**0.158**	**0.295**
XU_AKT1_TARGETS_6HR	**10**	**1.91**	**0.002**	**0.178**	**0.438**
PID_NECTIN_PATHWAY	20	1.86	0.002	0.290	0.724
DING_LUNG_CANCER_EXPRESSION_BY_COPY_NUMBER	48	1.84	0.000	0.304	0.818
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	14	1.84	0.000	0.257	0.825
KEGG_RIBOSOME	75	1.79	0.000	0.383	0.948
DAWSOM_METHYLATED_IN_LYMPHOMA_TCL1	11	1.79	0.002	0.345	0.952
REACTOME_METABOLISM_OF_MRNA	178	1.77	0.000	0.422	0.987
DER_IFN_ALPHA_RESPONSE_UP	39	1.76	0.002	0.393	0.988

Downregulated SMARCA4 Knockout

MSigDB	Gene Hits	NES	p-value	FDR q-value	FWER p-value
NABA_MATRISOME	**261**	**-2.77**	**0.000**	**0.000**	**0.000**
NABA_CORE_MATRISOME	**97**	**-2.76**	**0.000**	**0.000**	**0.000**
NABA_ECM_GLYCOPROTEINS	**76**	**-2.63**	**0.000**	**0.000**	**0.000**
REACTOME_GLYCOSAMINOGLYCAN_METABOLISM	**50**	**-2.56**	**0.000**	**0.000**	**0.000**
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	**103**	**-2.52**	**0.000**	**0.000**	**0.000**
WANG_SMARCE1_TARGETS_UP	**78**	**-2.43**	**0.000**	**0.000**	**0.000**
RIGGI_EWING_SARCOMA_PROGENITOR_DN	**50**	**-2.41**	**0.000**	**0.000**	**0.000**
VECCHI_GASTRIC_CANCER_ADVANCED_VS_EARLY_UP	**59**	**-2.39**	**0.000**	**0.000**	**0.000**
LIU_PROSTRATE_CANCER_DN	**143**	**-2.38**	**0.000**	**0.000**	**0.000**
NABA_MATRISOME_ASSOCIATED	**164**	**-2.36**	**0.000**	**0.000**	**0.000**

To validate the senescence associated changes to the secretome and transcriptome we discovered by omics approaches we examined various other features of senescence. Cellular senescence is defined as a state of proliferative arrest that is not only characterized by inhibition of cell proliferation, but also increased β-galactosidase activity, and cell cycle arrest due to the accumulation of cyclin-dependent kinase inhibitors like p21 (26-28). Higher numbers of cells were found to be positively stained for β-galactosidase within all three of the DDEC models lacking SMARCA4 expression **[Figure 3.16].** Another marker of senescence, p21 was found to be more highly expressed but only within the HEC 59 RNP Derived SMARCA4 KO EC cells **[Figure 3.17].** Another common trait of senescent cells is widespread epigenetic resetting most notably the formation of areas of heterochromatin referred to as senescence-associated heterochromatin foci (SAHF) that are necessary for cellular adaptation (28, 29). SAHF exhibit enrichment of histone H3 lysine 9 trimethylation (29, 30). Western blot analysis revealed higher levels of H3K9me3 in all the models of DDEC we derived in HEC 116 EC cells **[Figure 3.17].** Analysis of histone mark expression by immunofluorescence corroborated the western blot studies with H3K9me3 levels found to be elevated in both HEC 116 plasmid and RNP based SMARCA4 KO EC cells **[Figure 3.18A & Figure 3.18B].**

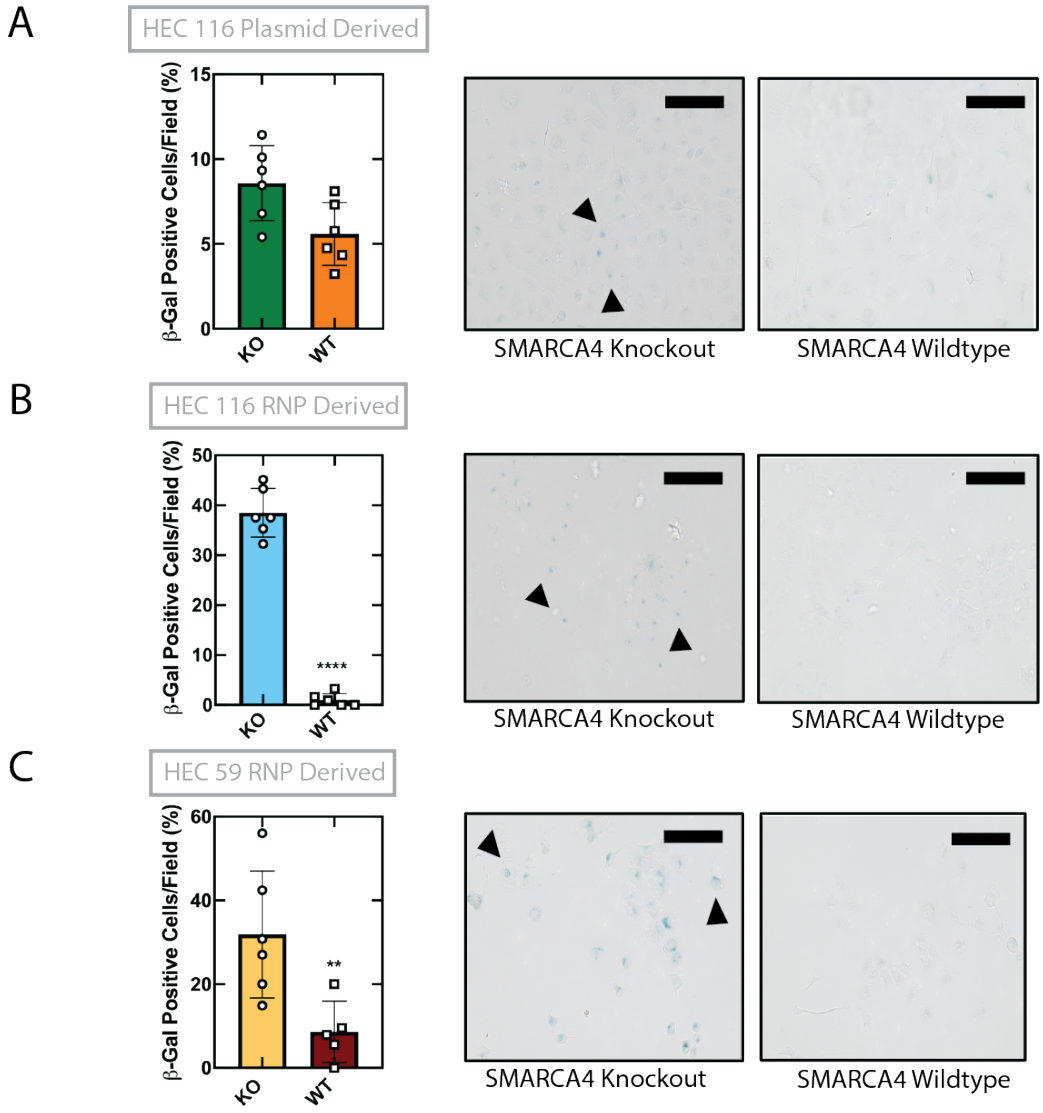


Figure 3.16. Endometrial cancer cells with absent SMARCA4 expression are more senescent than their SMARCA4 intact counterparts. Senescence associated β-galactosidase staining of serum-starved **A)** HEC 116 Plasmid Derived, **B)** HEC 116 RNP Derived, and **C)** HEC 59 RNP Derived SMARCA4 KO and WT EC cells. Percentage of positive cells from 6 randomly selected fields is depicted as white-colored shapes with error bars indicating standard deviation. Multiple t-tests revealed significant discoveries with ****= p <0.0001. ***= p < 0.001. ** = p < 0.01. * = p < 0.05. Representative images with a few positive cells highlighted by arrows are shown on the right. Scale bar = 50 μm.

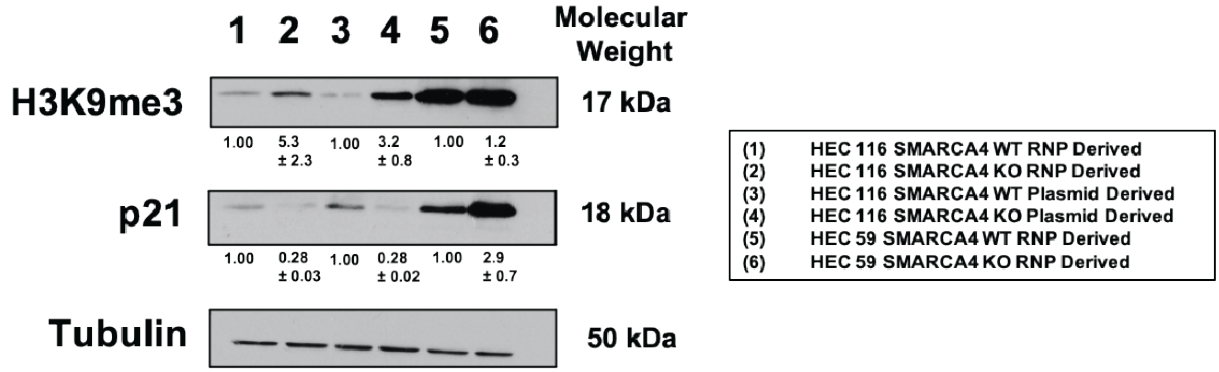


Figure 3.17. Lack of SMARCA4 expression in endometrial cancer cells increases levels of proteins associated with senescence. Western blot analysis revealed increased amounts of H3K9me3 in HEC 116 SMARCA4 KO cells generated by CRISPR gene editing. HEC 59 SMARCA4 deficient EC cells possess higher levels of p21 than SMARCA4 intact HEC 59 cells. Blots are representative of three independent biological replicates. Densitometry allowed for the obtainment of ratios of band intensity relative to SMARCA4 WT samples (mean ± standard deviation).

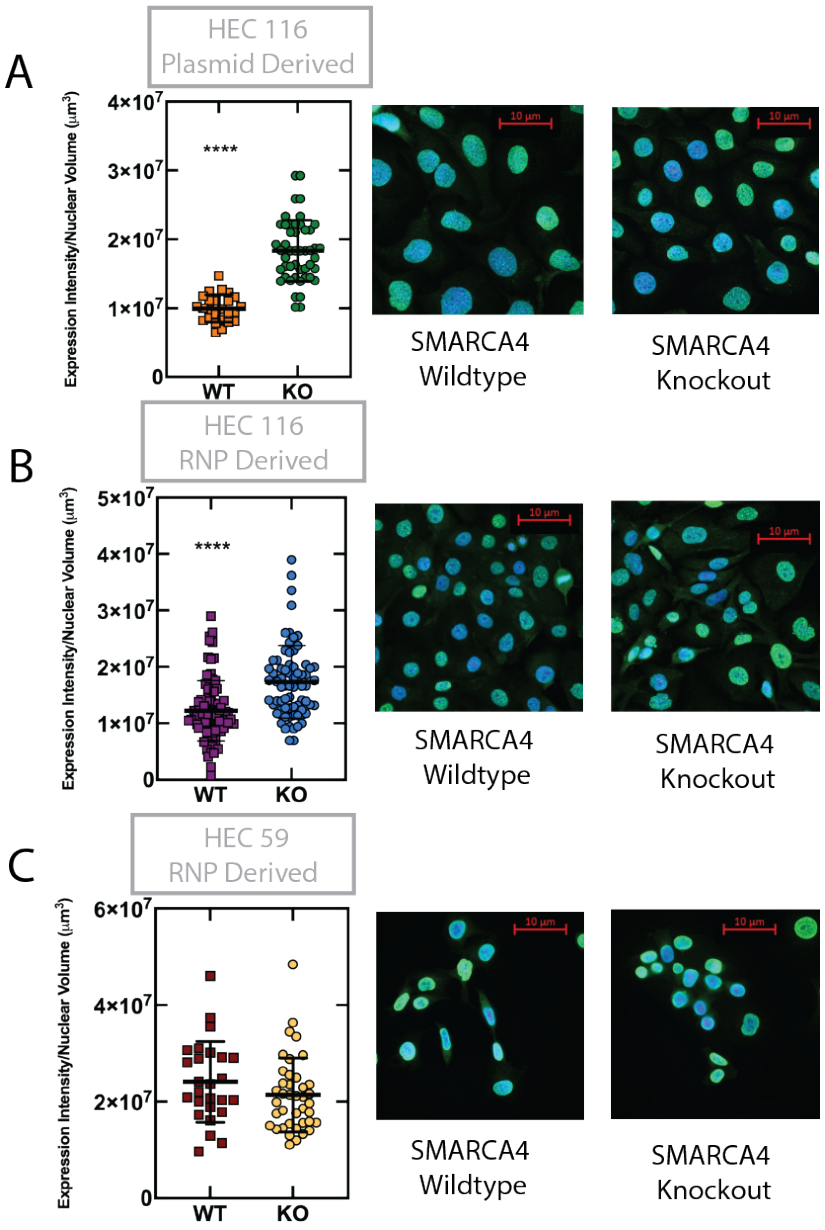


Figure 3.18. Lack of SMARCA4 expression in HEC 116 endometrial cancer cells leads to higher expression of the repressive chromatin mark H3K9me3. Merged confocal images of **A)** HEC 116 Plasmid Derived, **B)** HEC 116 RNP Derived, and **C)** HEC 59 RNP Derived EC cells stained with H3K9me3 (green) and DAPI (blue). Quantitative analysis of immunostaining was achieved through Imaris by dividing the sum of H3K9m3 signal intensity of each cell over the volume of the cell within two fields of view. Multiple t-tests revealed significant discoveries with ****= p <0.0001. Scale bar = 10 µm.

3.2.5. Serial passaging of SMARCA4 deficient EC cells in vivo results in formation of DDEC-like tumors with mixed histology.

As SMARCA4 loss alone *in vitro* was insufficient in bringing about the more aggressive phenotype observed in clinical DDEC, we turned to *in vivo* studies to examine both tumor growth and tumor histology from our cell line models of DDEC. Multiple experimental tumor systems have demonstrated increased malignancy upon serial transfers (31, 32). Prolonged exposure of cancer cells to selective forces within an organism such as hormones and growth factors is believed to be influential (32). To select for more stem-like populations of cells, we employed a serial passaging technique, where tumors were dissociated into single cell populations and reinjected into a new generation of mice **[Figure 3.19]**.

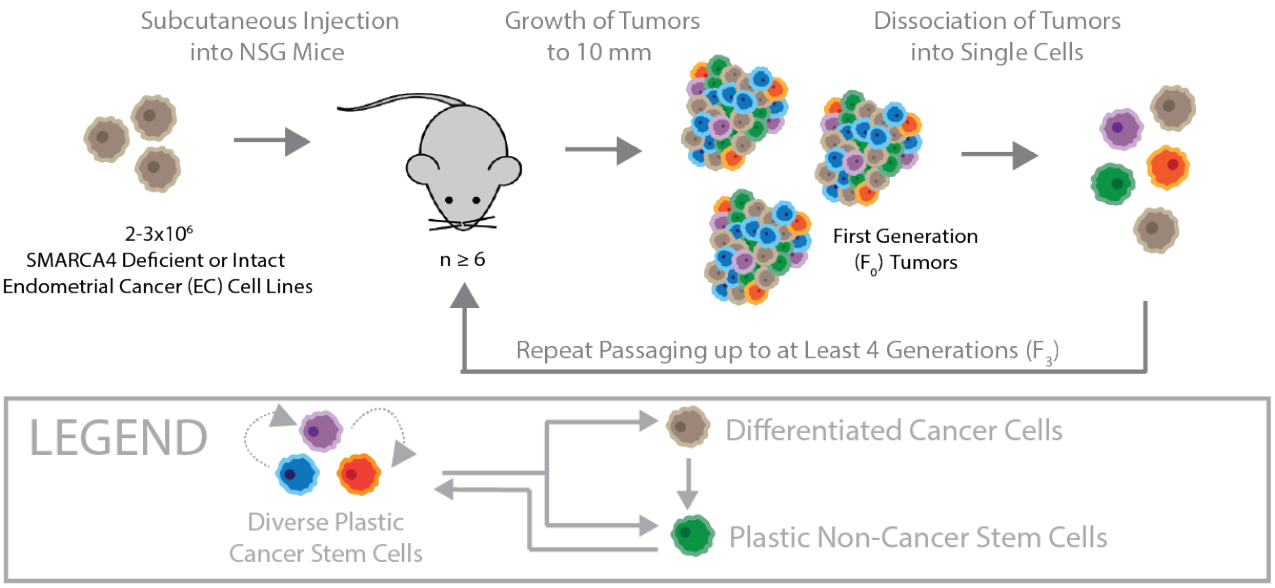


Figure 3.19. Schematic outlining *in vivo* serial passaging process that resulted in formation of DDEC tumors with mixed histology from SMARCA4 deficient EC cell lines.

Following the first injection, the absence of SMARCA4 typically resulted in slower tumor growth **[Figure 3.20]**. This especially was evident with the HEC 59 model wherein 10 millimeter neoplasms were only reached with HEC 59 SMARCA4 deficient EC cells six months post implantation compared to the two months observed with SMARCA4 intact EC cells. With each passing generation though, the rate of tumor formation from EC cells lacking SMARCA4 became more like their wildtype counterparts **[Figure 3.20]**. Upon only two generations of serial passaging, differences in tumor histology could be witnessed with HEC 116 Plasmid Derived SMARCA4 KO tumors forming tumors with both endometrioid and undifferentiated components **[Figure 3.21]**. On the other hand, HEC 116 Plasmid Derived SMARCA4 WT tumors could be characterized as being of a fully differentiated histology **[Figure 3.21]**. Accordingly, the undifferentiated regions of the second generation HEC 116 Plasmid Derived SMARCA4 KO displayed reduced levels of PAX8 and an absence of E-cadherin **[Figure 3.21]**. In contrast, the well differentiated regions retained PAX8 and E-cadherin to levels observed in wild type HEC 116 tumors **[Figure 3.21]**. It should be noted that RNP-derived tumors regardless of cell line did not form any appreciable glands or ducts and HEC 59 RNP Derived SMARCA4 KO cells never quite caught up to HEC 59 RNP Derived SMARCA4 WT tumor growth **[Supplemental Figure 3.4]**. Accumulation of mutant p53 in pancreatic cancer has been linked to tumor cells overcoming growth arrest or senescence and an increased propensity to metastasize (33). Immunohistochemical staining patterns in terms of percent positive cells were not quantified but observationally nuclear p53 staining was witnessed for both the early and late SMARCA4 intact HEC 116 Plasmid Derived tumors

but elevated p53 staining was observed within the undifferentiated components of third generation HEC 116 Plasmid Derived SMARCA4 deficient tumors **[Figure 3.22]**.

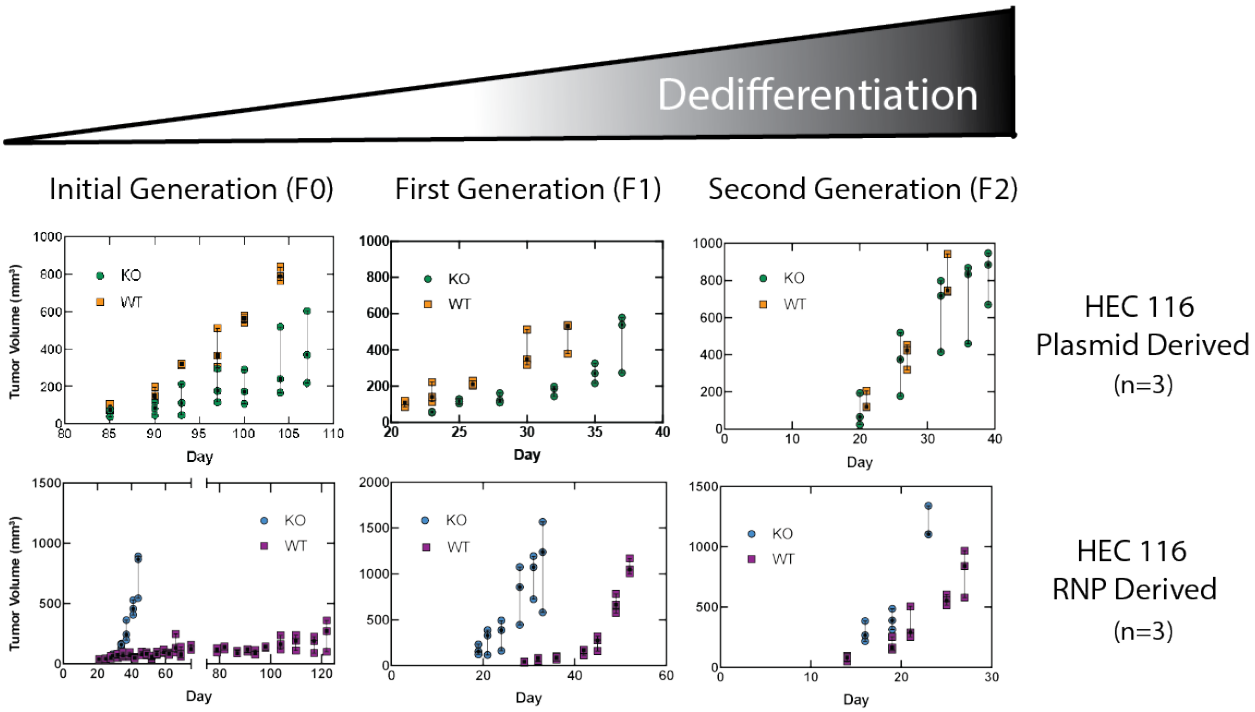


Figure 3.20. HEC 116 Plasmid Derived EC cells lacking SMARCA4 expression initially form tumors at a slower rate but with serial passaging the difference in tumor growth between cells regardless of SMARCA4 status was negligible. Three million cells were implanted subcutaneously in NOD.Cg-Prkdcscidll2rg (NSG) mice and allowed to grow to 10 mm prior to serial passaging. Tumor size was determined from caliper measurements. Filled black rectangles with error bars represent the median tumor volume and standard deviation of the SMARCA4 KO and WT replicates, respectively. Data consists of three mice represented as individual-colored shapes.

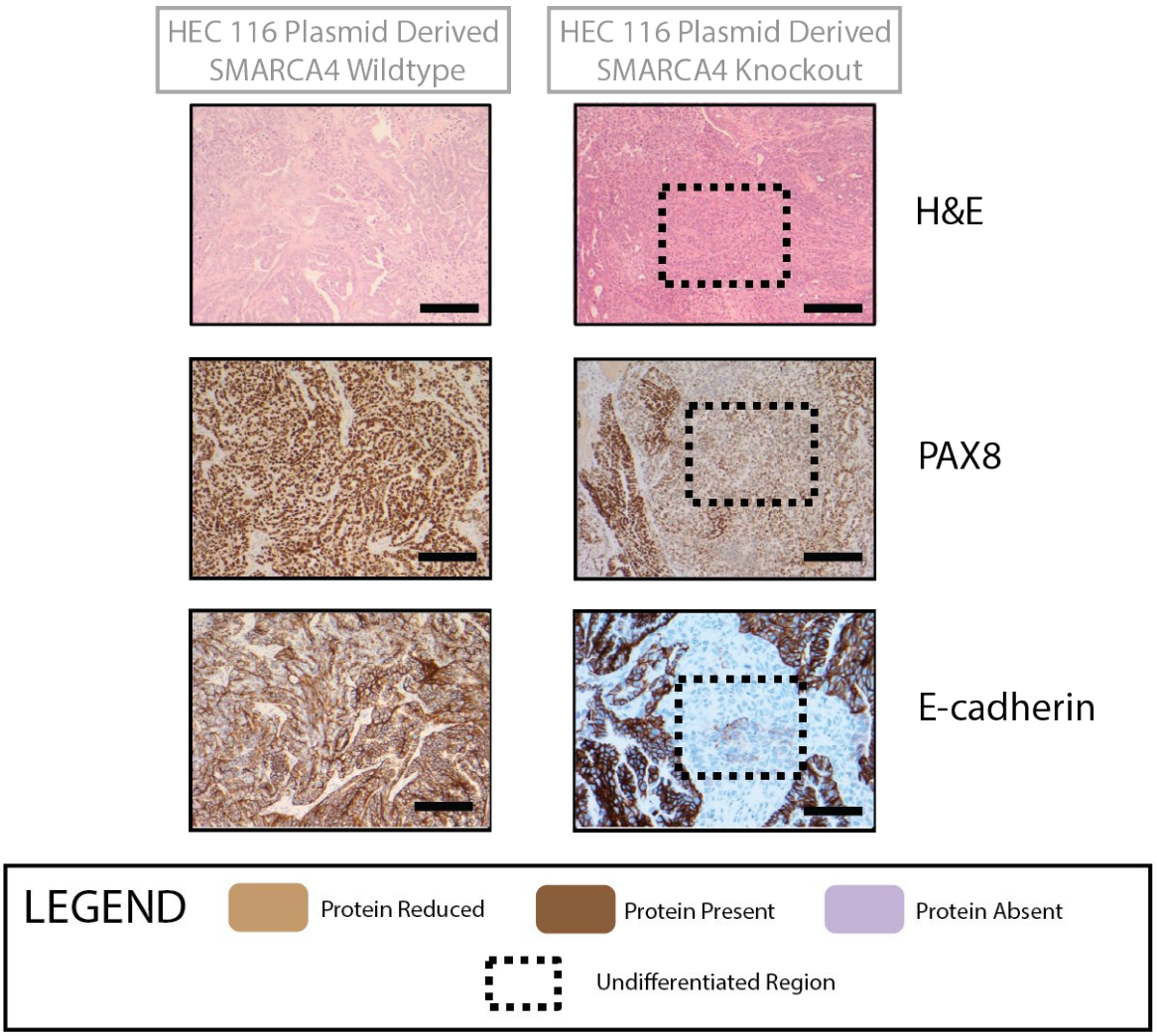


Figure 3.21. PAX8 and E-cadherin immunostaining in HEC 116 Plasmid Derived tumor tissue. H&E stained SMARCA4 KO EC tumor highlighting a region where glandular and ductal formation is absent. Reduced levels of nuclear PAX8 expression were evident in tumors formed from HEC 116 Plasmid Derived SMARCA4 KO EC cells. Clear absence of cytoplasmic E-cadherin within the undifferentiated regions of HEC 116 Plasmid Derived tumors originating from SMARCA4 deficient cells. Scale bar = 200 μm.

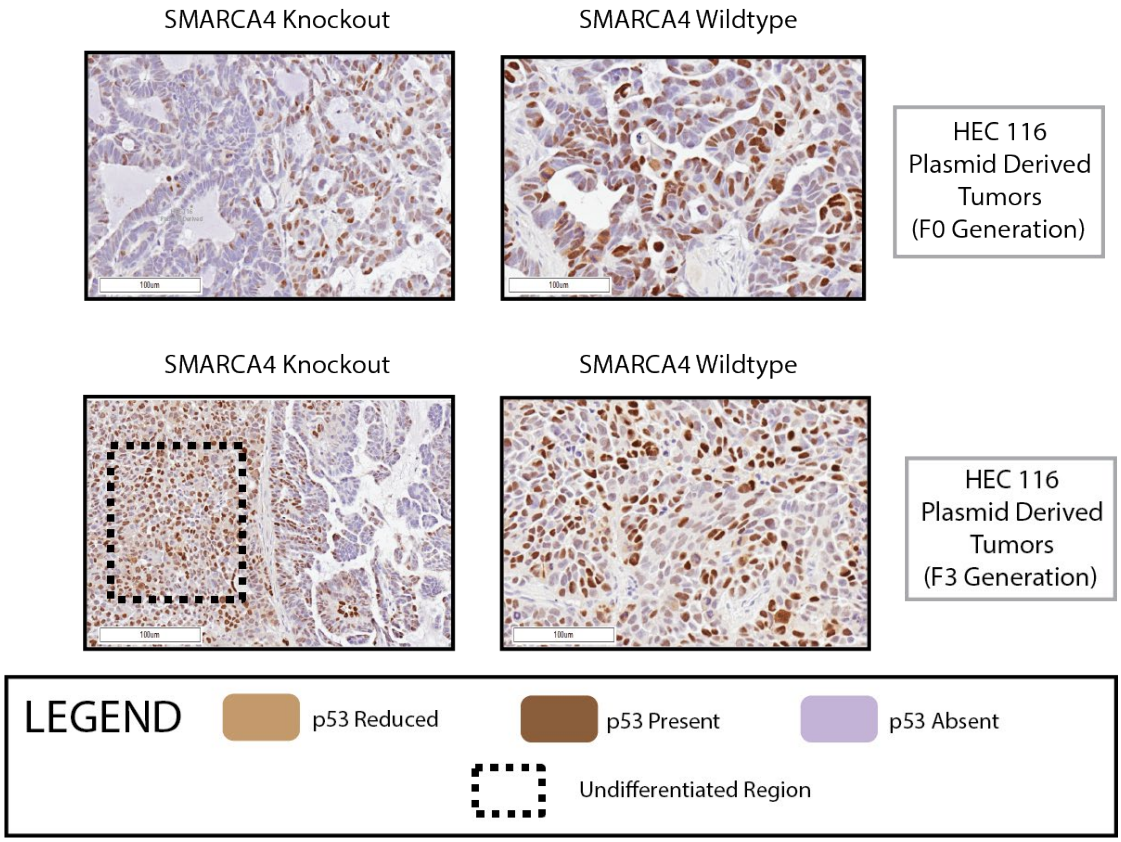


Figure 3.22. Alterations in p53 expression levels occur upon dedifferentiation of plasmid derived SMARCA4 deficient endometrial cancer cells *in vivo.* SMARCA4 KO EC tumor highlighting an undifferentiated region where higher levels of nuclear p53 expression was evident after successive serial passaging.

Cultured cells from third generation HEC 116 Plasmid Derived SMARCA4 KO tumors (KO F3 cell-line derived xenograft (CDX)) also exhibited less expression of PAX8 and ER transcripts than the SMARCA4 deficient tumor cells originating from early generations in the serial passaging process **[Supplemental Figure 3.5]**. When these third generation serially passaged tumor cells were assessed for their sphere forming capabilities, the cells lacking SMARCA4 expression displayed inconsistent results with one occasion showing no difference in sphere formation despite SMARCA4 status and another round demonstrating reduced sphere formation with SMARCA4 KO **[Figure 3.23]**. It should be noted though that SMARCA4 intact HEC 116 Plasmid Derived EC

also presented varying degrees of sphere formation upon exposure to serial passaging. This work suggests that the very act of serial passaging can increase sphere forming capacity regardless of SMARCA4 status. While the grape-like morphology of the SMARCA4 knockout spheres formed from cultured serially passaged tumor cells did not change substantially from that of the spheres originating from unpassaged HEC 116 Plasmid Derived EC cells, irrespective of the attempt, KO F3 CDX cells were more capable of sphere formation than unpassaged HEC 116 SMARCA4 deficient EC cells.

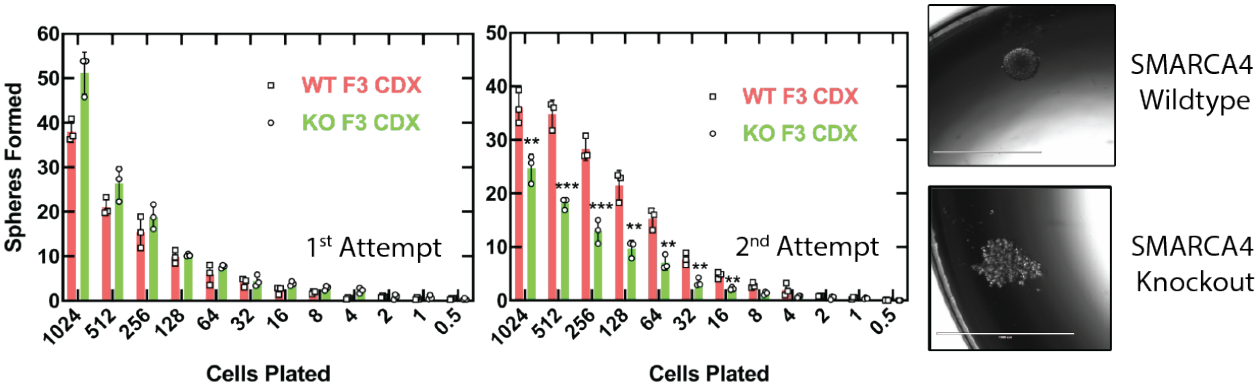


Figure 3.23. Serially passaged SMARCA4 deficient tumor cells from a model of DDEC generated by plasmid-based CRISPR form variable amounts of spheres across experiments. Limiting dilution sphere formation assays were carried out using third generation HEC 116 Plasmid Derived SMARCA4 KO and WT EC cell lines that had been cultured *in vitro*. Decreasing concentrations of single cell suspensions were seeded in low attachment plates and grown in minimal media for up to two weeks. Multiple t-tests confirmed the presence of any significant difference in sphere forming capabilities (** = $p < 0.01$. * = $p < 0.05$). Data consists of eight technical replicates from three independent biological replicates depicted as white-colored shapes. Representative images of spheres are featured on the right. Scale bar = 1000 µM.

Another feature of SWI/SNF deficient DDEC in patients, is that these tumors with undifferentiated components are frequently refractory to conventional chemotherapy such as carboplatin (1). To assess the response of our gene edited cell line models of DDEC to platinum-based therapies, mice harbouring either HEC 116 Plasmid Derived

SMARCA4 knockout or wildtype CDX tumors were treated with vehicle (phosphate buffered saline (PBS)) or carboplatin (60 mg/kg). Mice were dosed every week for a total of five weeks apart from one week break, starting 14 days post CDX implantation. In addition to final tumor weight being significantly lower, tumor growth of carboplatin treated SMARCA4 intact tumors was reduced, albeit not significantly compared to vehicle treatment **[Figure 3.24]**. SMARCA4 deficient tumors exposed to carboplatin in the end, weighed as much as their vehicle treated counterparts and grew faster than wildtype tumors suggesting SMARCA4 KO tumor bearing mice were less responsive to carboplatin **[Figure 3.24]**. Collectively, all this data suggests the emergence of the clinical DDEC phenotype as characterized by mixed histology, gene expression changes to markers of epithelial differentiation, increased sphere forming abilities and therapy resistance, are a direct result of selective pressure *in vivo.*

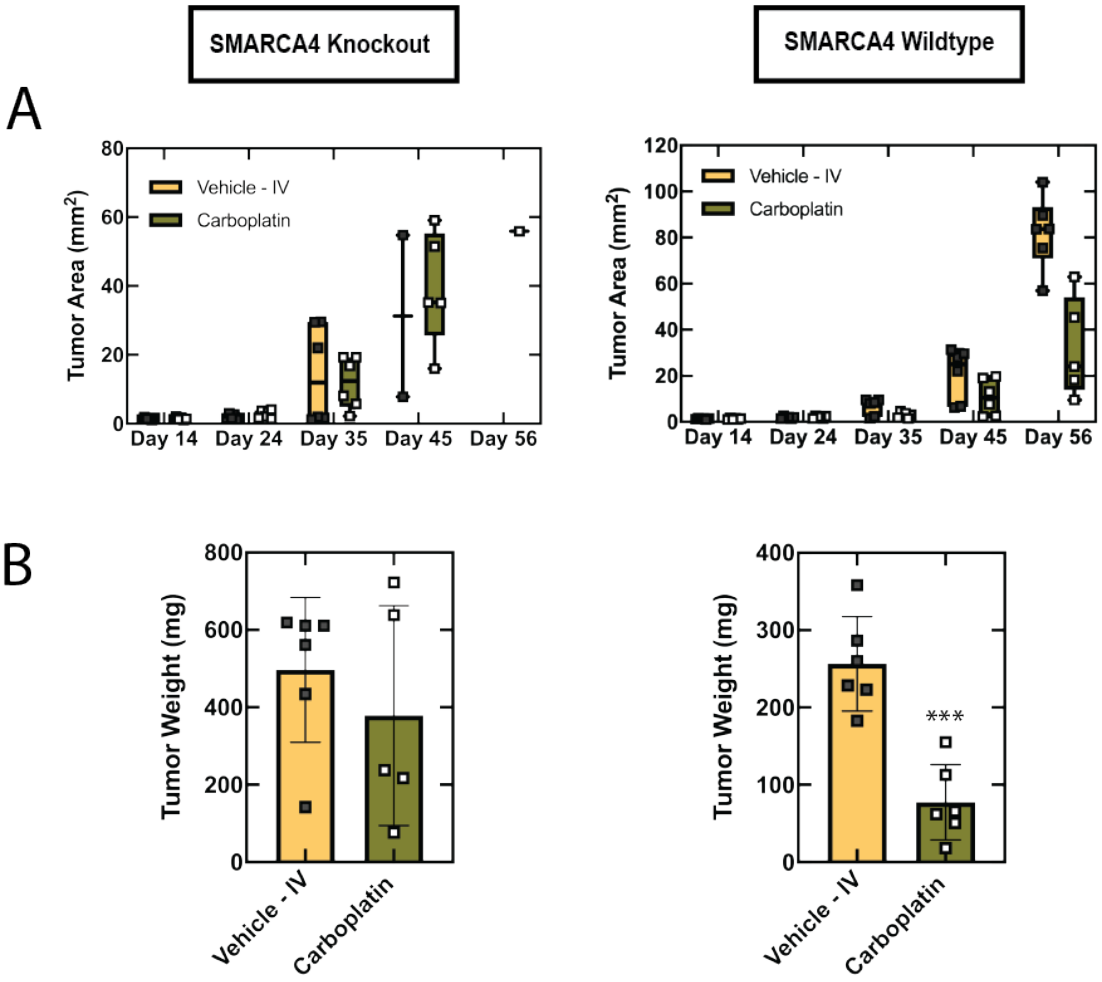


Figure 3.24. Effect of carboplatin treatment on HEC 116 Plasmid Derived DDEC tumor growth and tumor weight. A) Tumor area of HEC 116 Plasmid Derived SMARCA4 KO and SMARCA4 WT tumors and subjected to carboplatin treatment over time. Data consists of one independent round of experimentation (n=6) with white-colored shapes representing any of the tumors originating from the carboplatin treated group and filled black shapes for tumors from the vehicle treated mice. Box plots shown in have whiskers that indicate variability outside the upper and lower quartiles. Statistical differences between the tumor growth curves on various days were calculated in GraphPad Prism using nonparametric conditions and Kolmogorov-Smirnov t-tests. **B)** HEC 116 Plasmid Derived SMARCA4 KO and SMARCA4 WT tumor weight upon excision at experimental endpoint. Data consists of one independent round of experimentation (n=6) with white-colored shapes representing any of the tumors originating from the carboplatin treated group and filled black shapes for tumors from the vehicle treated mice. T-tests revealed no significant difference between treatment groups except for SMARCA4 WT tumor volume upon treatment with carboplatin. ***= $p < 0.001$.

Finally, in more well-characterized SMARCA4 deficient neoplasms such as SCCOHT and non-small-cell lung cancer (NSCLC), concurrent inactivation of SMARCA2 is prevalent (15, 34). SMARCA2 is mutually exclusive with SMARCA4, acting as an ATPase catalytic subunit within the SWI/SNF chromatin remodeling complex (35). We used immunoblotting to assess SMARCA2 levels in the HEC 116 Plasmid Derived model of DDEC. Examination of lysates from both frozen tumors and cultured dissociated tumor cells revealed consistently higher SMARCA2 protein levels in SMARCA4 deficient samples when compared to their SMARCA4 intact counterparts irrespective of the extent of passaging **[Figure 3.25]**. In the HEC 116 Plasmid Derived model of DDEC, SMARCA2 appears to compensate for the absence of SMARCA4, leaving the question remaining as to what the remaining defining features or drivers of DDEC are and if they are shared with other SWI/SNF deficient neoplasms.

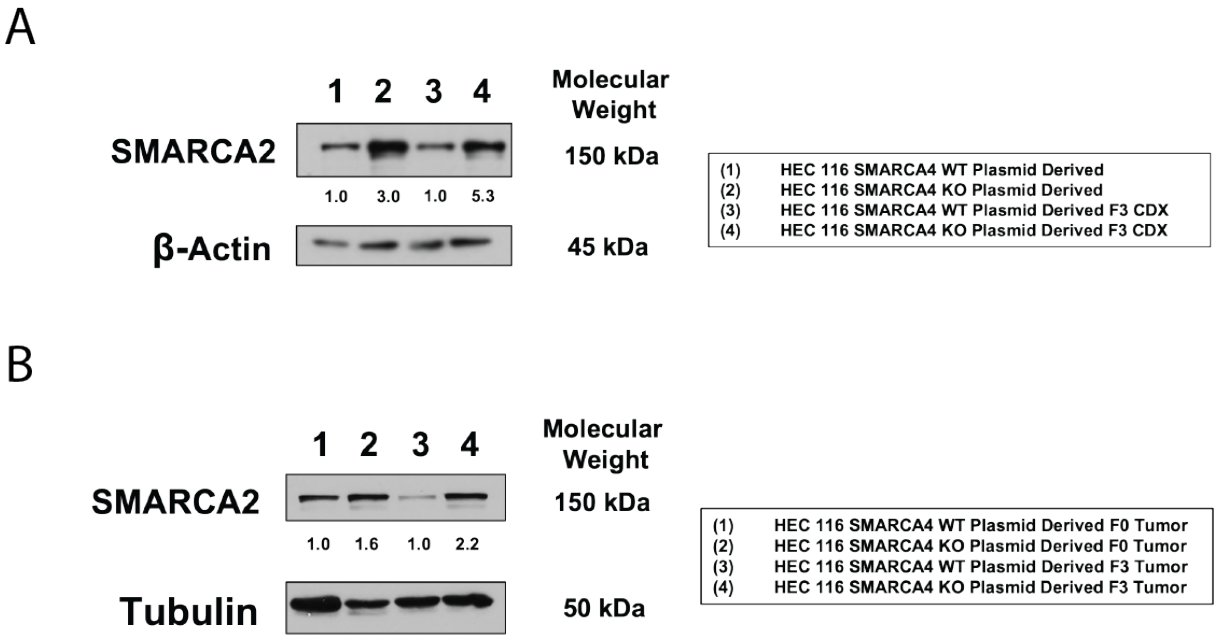


Figure 3.25. Increased SMARCA2 expression levels in SMARCA4 deficient EC cells are irrespective of passaging. A) Western blot analysis showed a marked increase in SMARCA2 levels when comparing either SMARCA4 KO unpassaged or cultured third generation tumor cells to similarly treated cells with absent SMARCA4 expression. **B)** Higher levels SMARCA2 expression were seen with SMARCA4 deficient EC cells when immunoblotting was performed on tumor lysates from both early and later generations. Blots are representative of one independent biological replicate. Densitometry allowed for the obtainment of ratios of band intensity relative to SMARCA4 WT samples.

3.2.6. SMARCA4 deficient endometrial cancer cells undergo a shift in chromatin accessibility and gene expression profiles with loss of AP-1 transcription factors acting as a potential driver of dedifferentiation in vivo.

Having demonstrated *in vivo* that SMARCA4 deficient ECs can recapitulate properties of clinical DDEC, we were curious as to what expression programs might be accompanying the histological changes we observed. It is already well established that clinical DDEC with its mixed phenotype exhibits a degree of cellular heterogeneity therefore we employed single-cell RNA (scRNA) and single-cell Assay of Transposase Accessible Chromatin (scATAC) sequencing approaches to study the heterogeneity

within the HEC 116 Plasmid Derived cell line model of DDEC. Major contributors to heterogeneity in the context of cancer usually include genetic diversity and epigenetic plasticity (36). Tumors are comprised of many genetic states which give rise to numerous epigenetic landscapes, which in turn can be subjected to phenotypic transitions (36). Our use of CRISPR gene editing also provided the unique opportunity to study tumor evolution from a relatively homogenous starting population. To capture the dedifferentiating cell population within our SMARCA4 deficient EC cells, we analyzed HEC 116 Plasmid Derived tumor cells from later generations in the serial passaging workflow comparing them to unpassaged cells and profiling them with the 10X Genomics Chromium system **[Figure 3.26 & Figure 3.27]**.

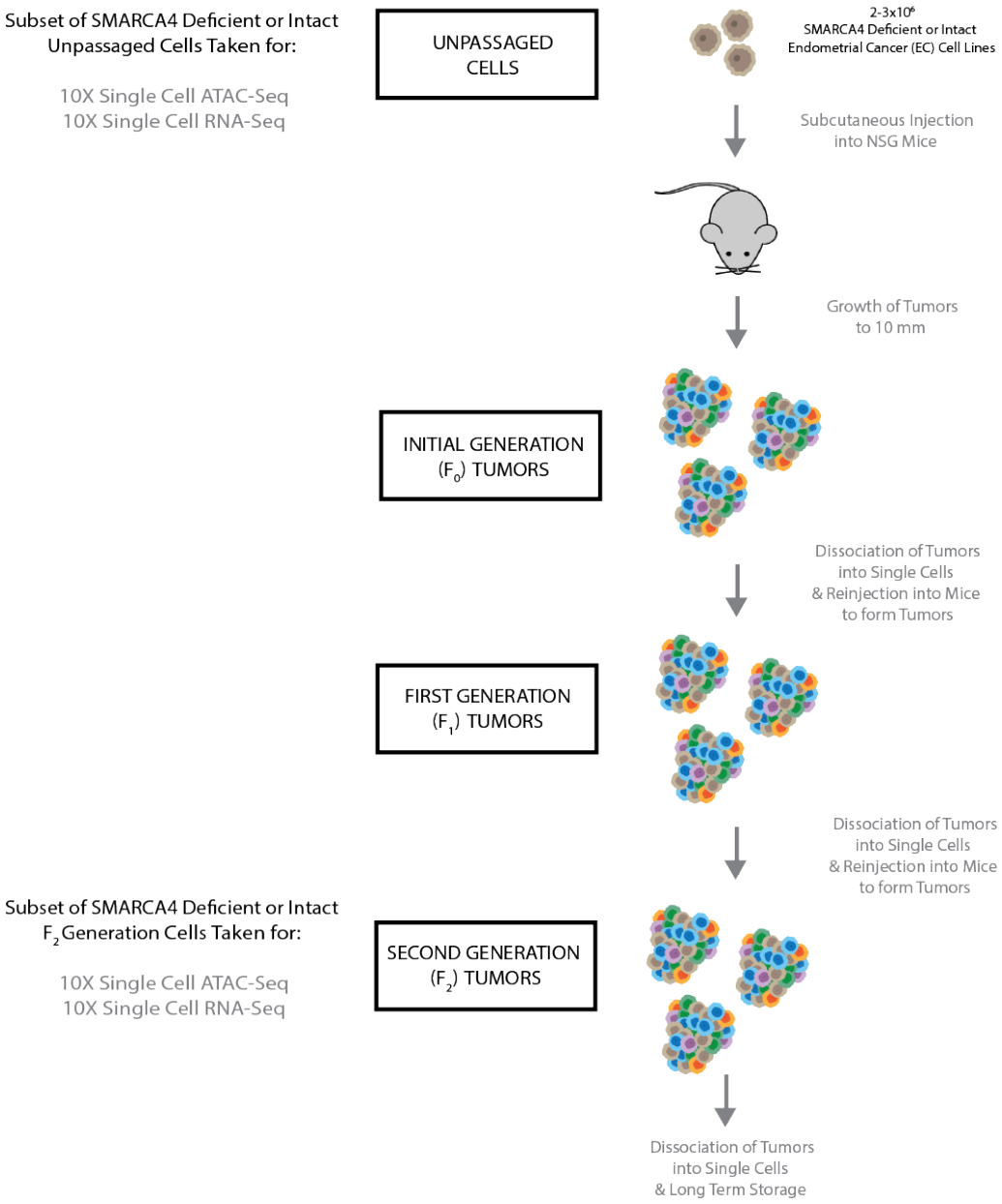


Figure 3.26. Schematic outlining the timepoints within the *in vivo* serial passaging process SMARCA4 deficient and intact EC cell lines were taken for single cell sequencing.

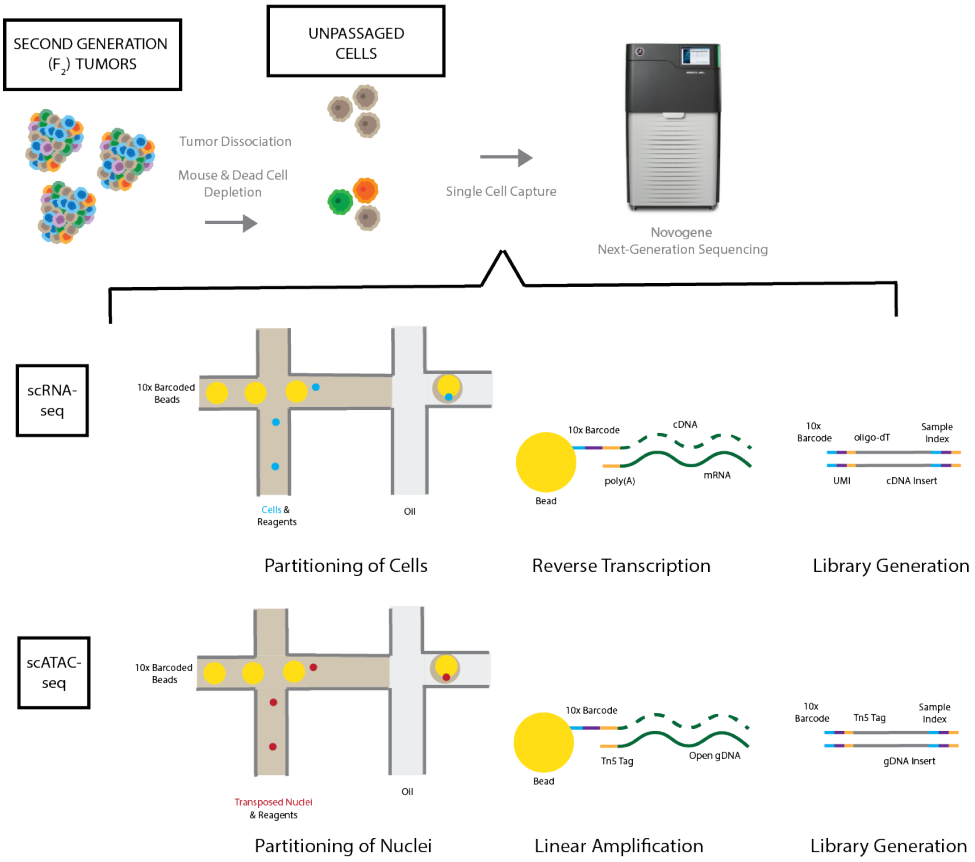


Figure 3.27. Schematic outlining the Chromium droplet-based platform for single cell sequencing from 10X Genomics. Briefly, droplet-based instruments can encapsulate single cells (shown in blue) for RNA-sequencing or for ATAC-sequencing, nuclei subjected to transposition by Tn5 (featured in red) in individual partitions. Within these Gel Bead-In Emulsions (GEMs) all the necessary reagents are present for either reverse transcription or linear amplification depending on the type of assay, in addition to molecular tagging. Third generation tumor cells were acquired by dissociation of multiple F2 EC tumors followed by mouse and dead cell depletion.

On average for each of the 4 samples we examined, approximately 6000 cells passed extensive quality control post data processing using the Cell Ranger pipeline. Upon unbiased clustering using Seurat, distinct cell states or types were not easily identifiable based on expression levels of markers amongst the unpassaged non-tumor HEC 116 EC cells regardless of SMARCA4 status **[Figure 3.28A & Figure 3.28B]**. Our single-cell transcriptomic profile of HEC 116 SMARCA4 KO EC cells that were not

subjected to serial passaging matches closely with our bulk RNA-seq findings wherein genes associated with senescence like *TIMP1 and IL8*, were found to expressed across most of the cells analysed **[Figure 3.29A]**. Strikingly, serial passaging up to two generations *in vivo* led to the distinct clustering of cells based on gene expression within the SMARCA4 deficient HEC 116 EC tumor cells **[Figure 3.28D]**. This cluster of 113 cells displayed features of clinical DDEC that we have described previously including, loss of *CLDN4* and *PAX8* in addition to other markers of epithelial cells such as *CLDN3, KRT8* and *MUC1* **[Figure 3.29B]**. This contrasts with the expression levels of some of these markers of dedifferentiation and more specifically DDEC, among the unpassaged HEC 116 SMARCA4 KO cells **[Supplementary Figure 3.6]**. It should be noted that within both the SMARCA4 deficient and intact HEC 116 EC tumor samples, a subset of cells within two less distinctive clusters also exhibited lesser expression of the genes encoding epithelial cell markers: *CLDN3, CLDN4, PAX8, KRT8,* and *MUC1* **[Supplementary Figure 3.7A].** This suggests that regardless of SMARCA4 status, the selective pressure of serial passaging pushes a proportion of cells into a state of EMT.

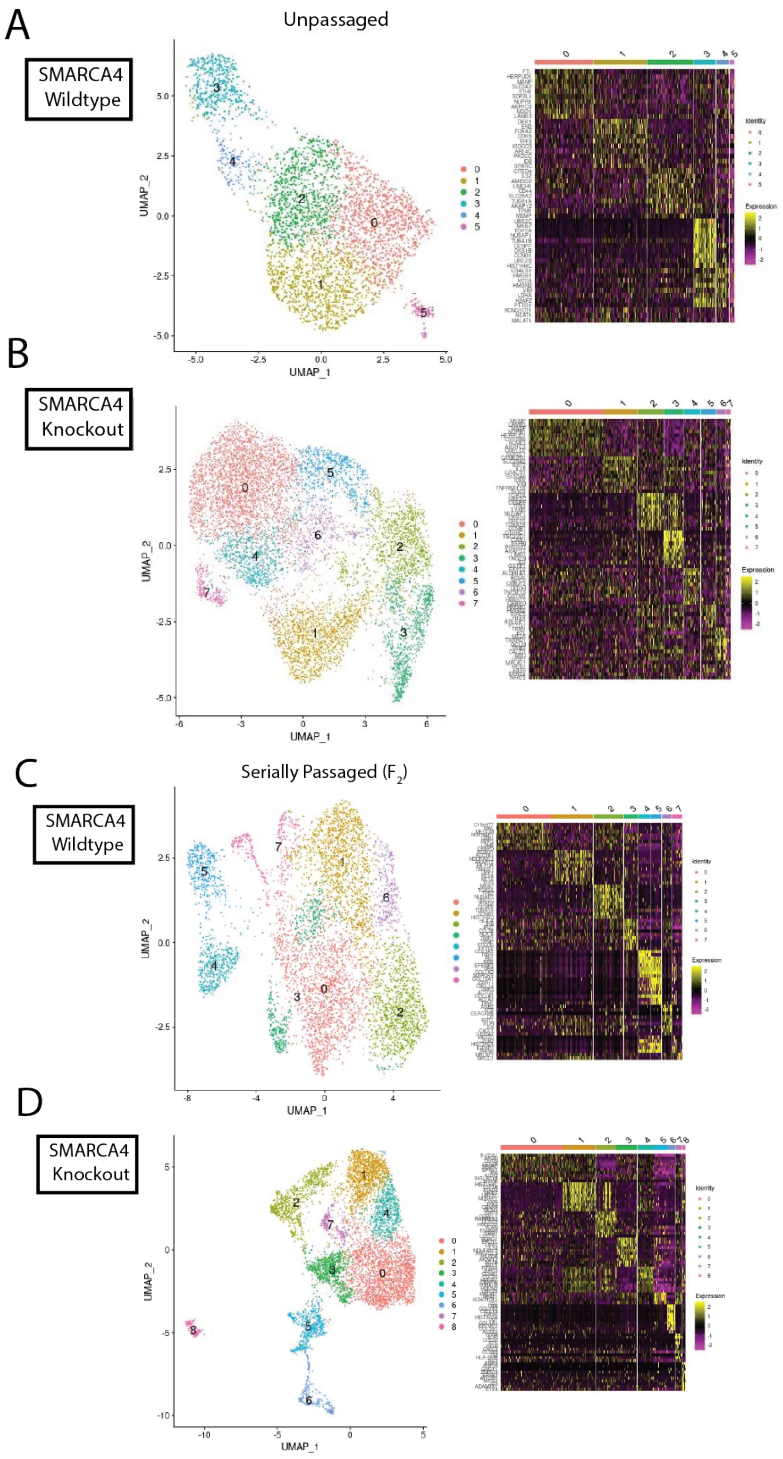

A
Unpassaged
SMARCA4
Wildtype
B
SMARCA4
Knockout
C
Serially Passaged (F2)
SMARCA4
Wildtype
D
SMARCA4
Knockout
UMAP_1
UMAP_2

Figure 3.28. Single cell transcriptomic analysis of the HEC 116 Plasmid Derived model of DDEC reveals the emergence of a novel cluster of SMARCA4 KO cells. UMAP plots of scRNA-seq data from **A)** unpassaged SMARCA4 WT cells, **B)** unpassaged SMARCA4 KO cells, **C)** serially passaged SMARCA4 WT cells, and **D)** serially passaged SMARCA4 KO cells. Paired heatmaps denote the top 10 differentially expressed genes within each cluster at the lowest possible resolution and following cell-cycle regression. Examination of the heatmap produced upon the clustering of the F2 SMARCA4 KO tumor scRNA sequencing data reveals the smallest overlap in differentially expressed genes amongst all the samples tested.

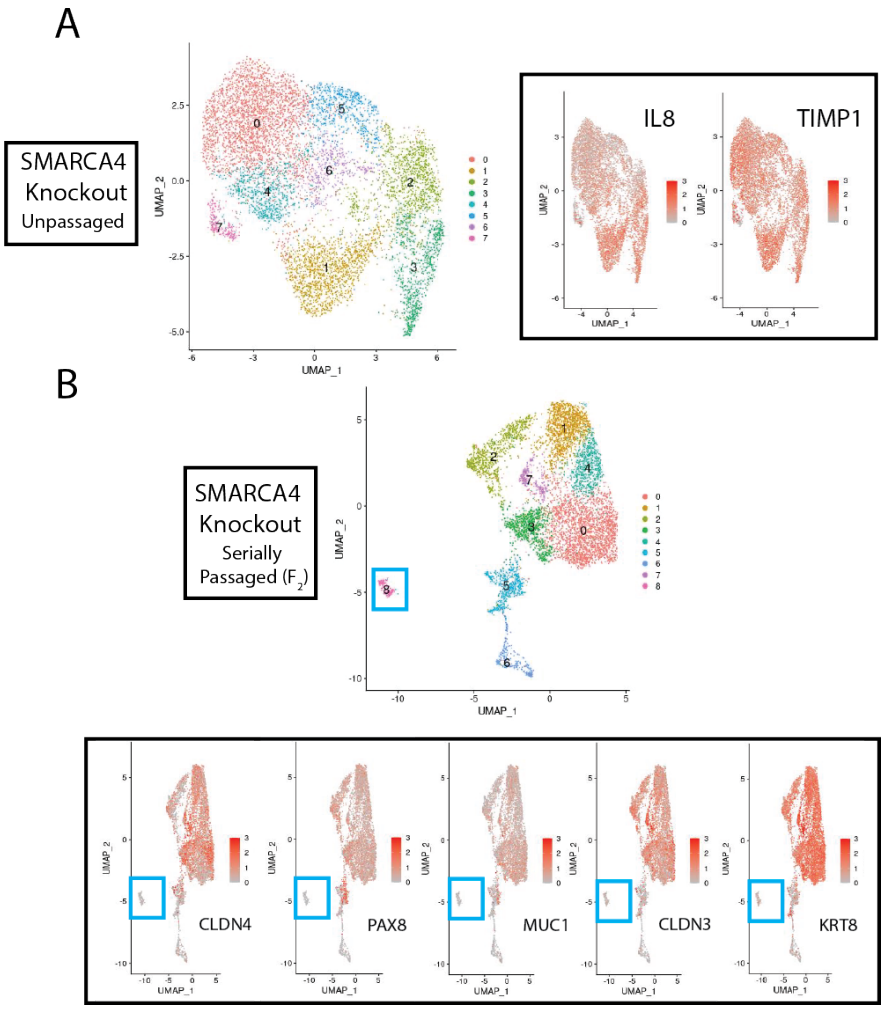


Figure 3.29. SMARCA4 deficient EC cells are characterized by markers of senescence *in vitro* and gene expression programs associated with endometrial cellular dedifferentiation *in vivo.* A) Uniform Manifold Approximation and Projection (UMAP) plots depicting gene expression patterns for select senescence associated genes: *TIMP1* and *IL8* in SMARCA4 KO cells not subjected to *in vivo* selective pressure. **B)** UMAP plots depicting gene expression patterns for select makers of undifferentiated regions in clinical DDEC tumors: *CLDN3, CLDN4, PAX8, MUC1, and KRT8* in SMARCA4 KO cells subjected to *in vivo* selective pressure. Colored boxes highlight the unique cluster of interest.

Integration of the HEC 116 SMARCA4 intact and deficient F2 generation tumor cell datasets led to the discovery of a cluster of 102 cells, composed only of SMARCA4 KO EC cells. Gene set enrichment analysis with topGO revealed that this cluster was enriched with genes associated with endometrial dedifferentiation as well as chromatin

related processes and cell cycle progression **[Figure 3.30 & Figure 3.31]**. Notably the transcription factors *POU3F2* and *DLX1* were upregulated in this cluster as were *CTCFL* and *BMP4* **[Figure 3.32]**. Interestingly, an absence of expression of AP-1 transcription factor family members, *FOS* and *JUN*, was evident among the cells constituting the F2 generation SMARCA4 KO specific cluster **[Figure 3.32]**. No SMARCA4 intact HEC 116 Plasmid Derived serially passaged F2 generation tumor cells were found that had altered expression of these factors known to be critical to the transcription of oncogenes **[Supplemental Figure 3.7B].**

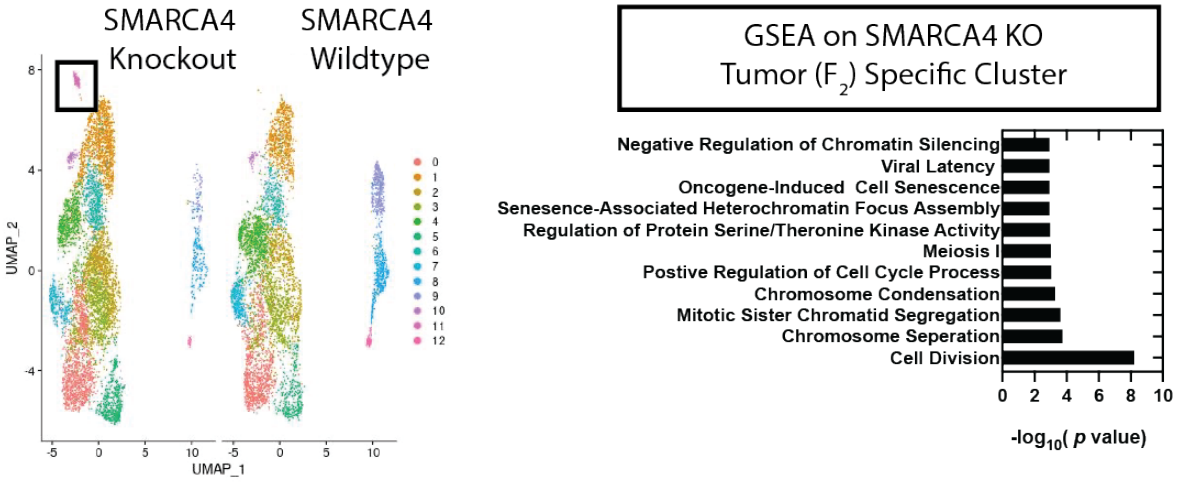


Figure 3.30. Integration of HEC 116 Plasmid Derived EC tumor cell scRNA-seq datasets leads to the retainment of a unique SMARCA4 KO cluster of cells enriched for gene sets associated with cellular senescence, cell cycle and cell division. UMAP plots of integrated scRNA-seq data from serially passaged HEC 116 Plasmid Derived tumor cells separated by SMARCA4 status. Gene set testing with topGO confirmed activation of a senescence signature in conjunction with a several other higher order chromatin related processes.

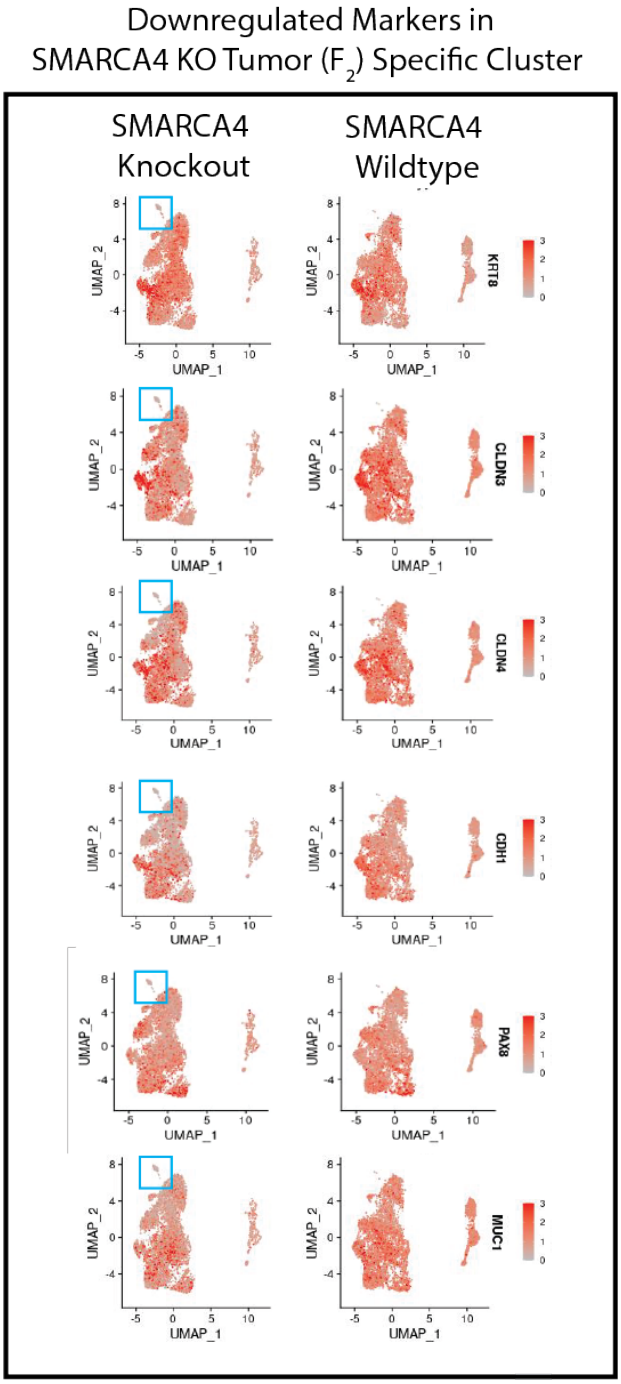


Figure 3.31. Integration of HEC 116 Plasmid Derived EC tumor cell scRNA-seq datasets leads to the retainment of a unique SMARCA4 KO cluster of cells with gene expression programs associated with endometrial cellular dedifferentiation. UMAP plots depicting gene expression patterns for select makers of undifferentiated regions in clinical DDEC tumors: *CLDN3, CLDN4, CDH1, PAX8, MUC1, and KRT8* in SMARCA4 KO cells subjected to *in vivo* selective pressure. Colored boxes highlight the unique cluster of interest.

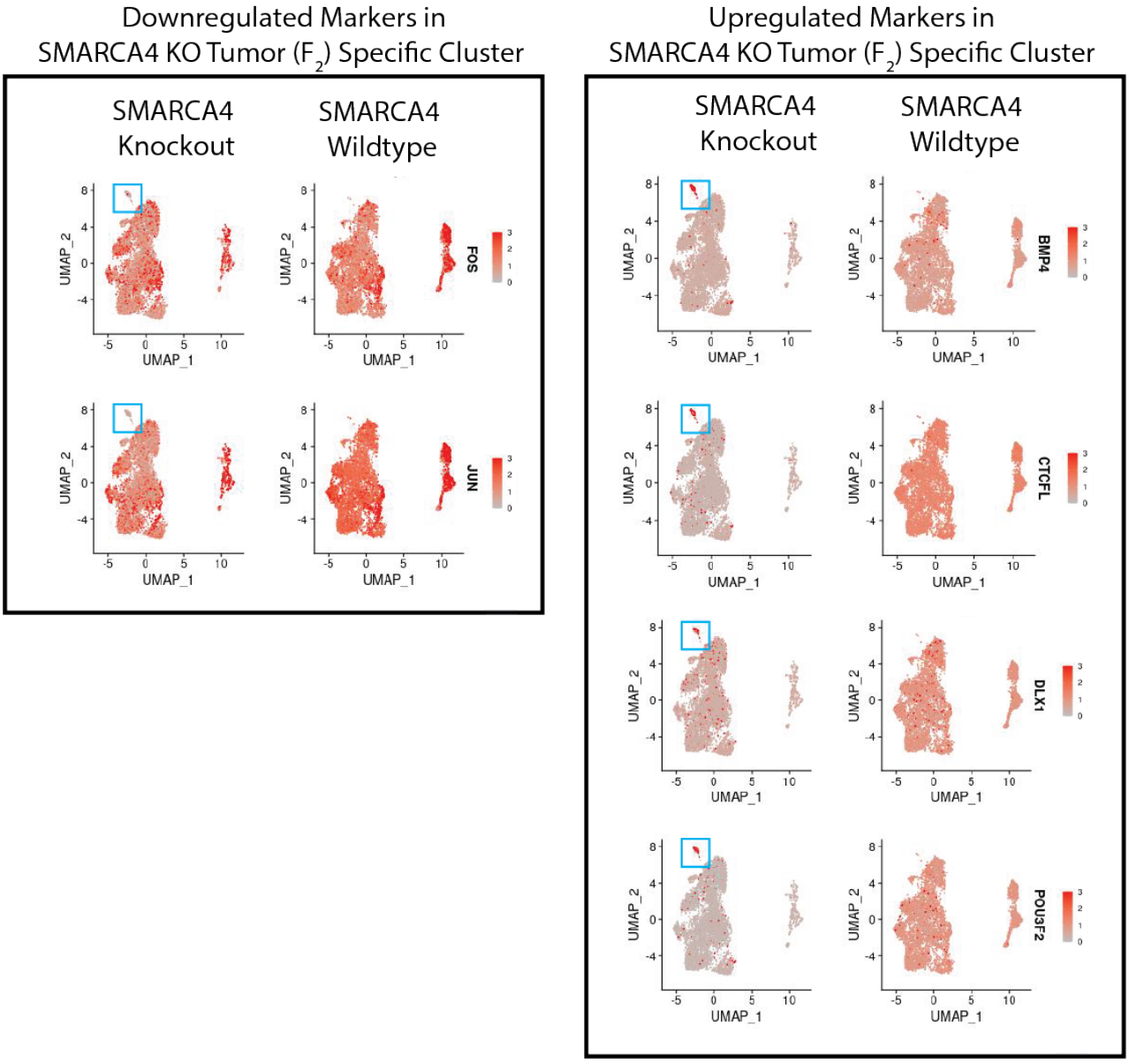
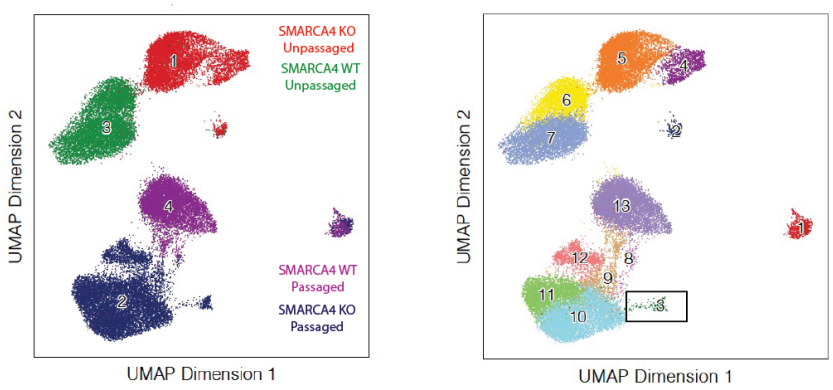


Figure 3.32. Integration of HEC 116 Plasmid Derived EC tumor cell scRNA-seq datasets leads to the retainment of a unique SMARCA4 KO cluster of cells with gene expression programs associated with phenotype switching. UMAP plots depicting gene expression patterns for select makers of phenotype switching: *JUN, FOS, CTCFL, DLX1, POU3F2,* and *BMP4* in SMARCA4 KO cells subjected to *in vivo* selective pressure. Colored boxes highlight the unique cluster of interest.

Having observed potential alterations in transcription factor activity, single cell ATAC-sequencing was carried out and identification of 13 clusters was possible upon integration of both unpassaged and passaged datasets using Analysis of Regulatory Chromatin in R (ArchR) **[Figure 3.33A]**. Visualization of the single-cell ATAC profiles with UMAP revealed tighter clustering of cells based on chromatin accessibility due to the origin of the cells rather than their SMARCA4 status **[Figure 3.33A].** Like with the single cell transcriptomics, it is evident how much chromatin accessibility shifted upon exposure of endometrial cancer cells to *in vivo* forces. A cluster of 257 cells was discovered to consist of only serially passaged HEC 116 SMARCA4 KO tumor cells **[Figure 3.33B]**. We scrutinized this SMARCA4 KO tumor specific cluster in two ways: first, through examination of the ATAC-seq peaks or chromatin accessibility of cis-elements, and secondly, by the accessibility of transcription factor binding sites in single cells.

The SMARCA4 KO cluster emerging only upon serial passaging *in vivo*, demonstrated reduced accessibility near the following genes: *PAX8, CDH1,* and *CLDN4*, which are phenotypical traits we previously documented with FFPE clinical samples **[Figure 3.33C]**. Enhanced accessibility at cis elements neighboring the *POU3F2, DLX1, BMP4,* and *CTCFL* genes was paired with lesser accessibility of regulatory regions governing expression of AP-1 transcription factors, *FOS* and *JUN* **[Figure 3.34]**. These results mirror the single cell level gene expression changes to these same factors we previously found by scRNA-seq within a similar sized population of SMARCA4 KO tumor cells.

A



B

Cluster	Unpassaged KO	Unpassaged WT	Serially Passaged KO	Serially Passaged WT
1		2	220	414
2	195	26	7	8
3			257	
4	1059	9	4	4
5	6196	54	6	11
6	59	2972	6	44
7	16	5045	1	42
8			120	132
9	7	16	707	264
10		1	5747	13
11		1	3184	20
12	1	1	1331	33
13		4	27	6662

C

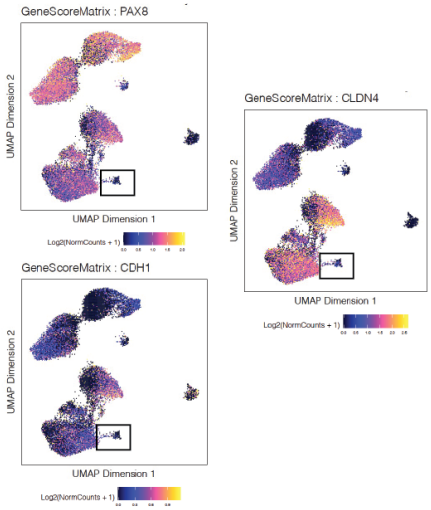


Figure 3.33. Integration of HEC 116 Plasmid Derived EC scATAC-seq datasets resulted in the discovery of a unique SMARCA4 KO cluster of cells with reduced chromatin accessibility at markers of an epithelial phenotype. UMAP plots of scATAC-seq data indicating **A)** the sample type in which cells within clusters originate and the clusters called by ArchR. **B)** Table highlighting the number of cells constituting each cluster that called based on similar chromatin accessibility patterns. **C)** UMAP plots depicting chromatin accessibility patterns for select makers of undifferentiated regions in clinical DDEC tumors: *CLDN4, CDH1,* and *PAX8* in SMARCA4 KO cells subjected to *in vivo* selective pressure. Boxes highlight the unique cluster of interest.

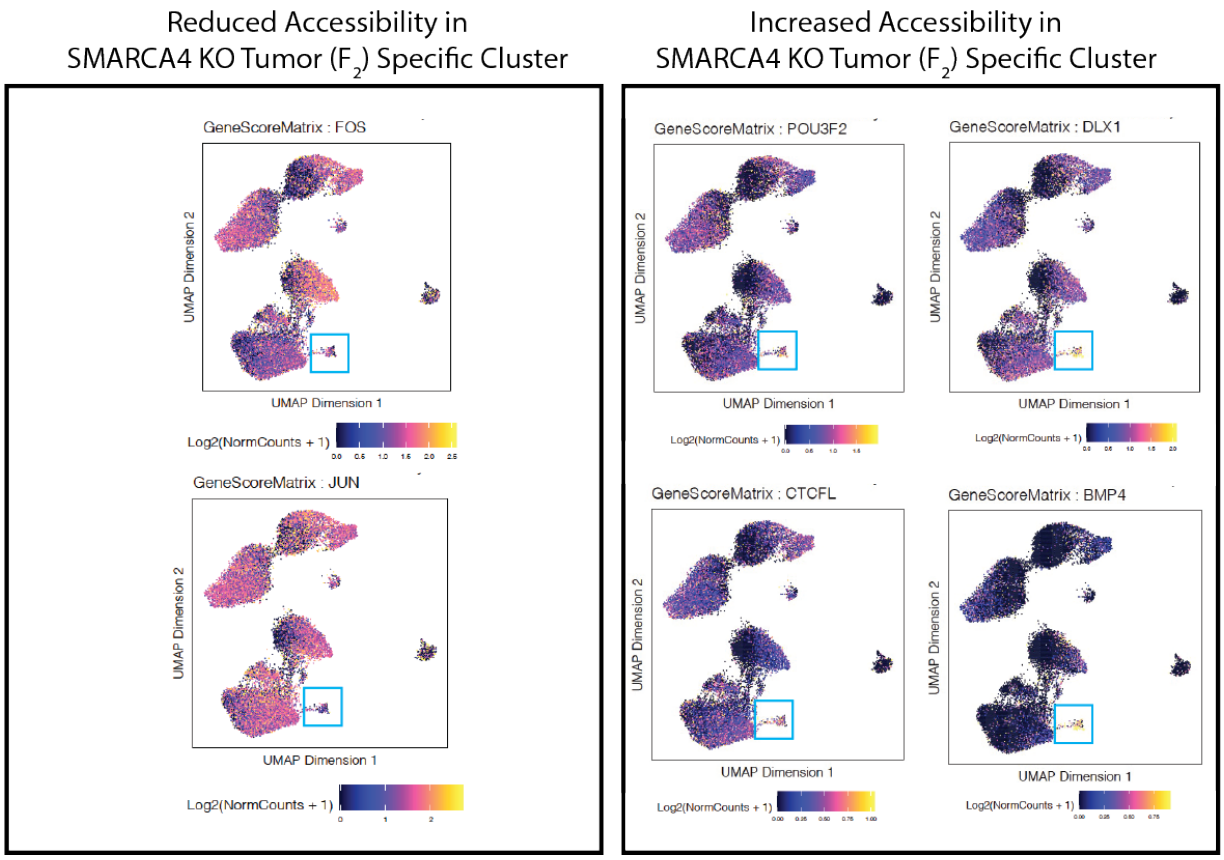


Figure 3.34. Integration of HEC 116 Plasmid Derived scATAC-seq datasets leads to the retainment of a unique SMARCA4 KO cluster of serially passaged cells with alterations to chromatin accessibility nearby genes associated with phenotype switching. UMAP plots depicting chromatin accessibility changes linked to genes implicated in phenotype switching: *JUN, FOS, CTCFL, DLX1, POU3F2,* and *BMP4* in SMARCA4 KO cells subjected to *in vivo* selective pressure. Colored boxes highlight the unique cluster of interest.

Using chromVAR within the ArchR platform, we then measured chromatin accessibility changes at known transcription factor (TF) binding sites, primarily focusing on the transcription factor deviation scores of the cluster consisting only of SMARCA4 deficient serially passaged tumor cells (37). Gene expression is a dynamic process that typically involves coordination of TF expression with accessible chromatin that in turn, permits binding of TFs to defined motifs. Our cluster of interest, in line with our

expectations showed dynamic changes and enrichment in accessibility at DNA-binding motifs for CTCFL (mean z-score of 6) and lesser accessibility of PAX8, FOSL1, and JUNB motifs (mean z scores of -1.5, -10 and -12, respectively) **[Figure 3.35]**.

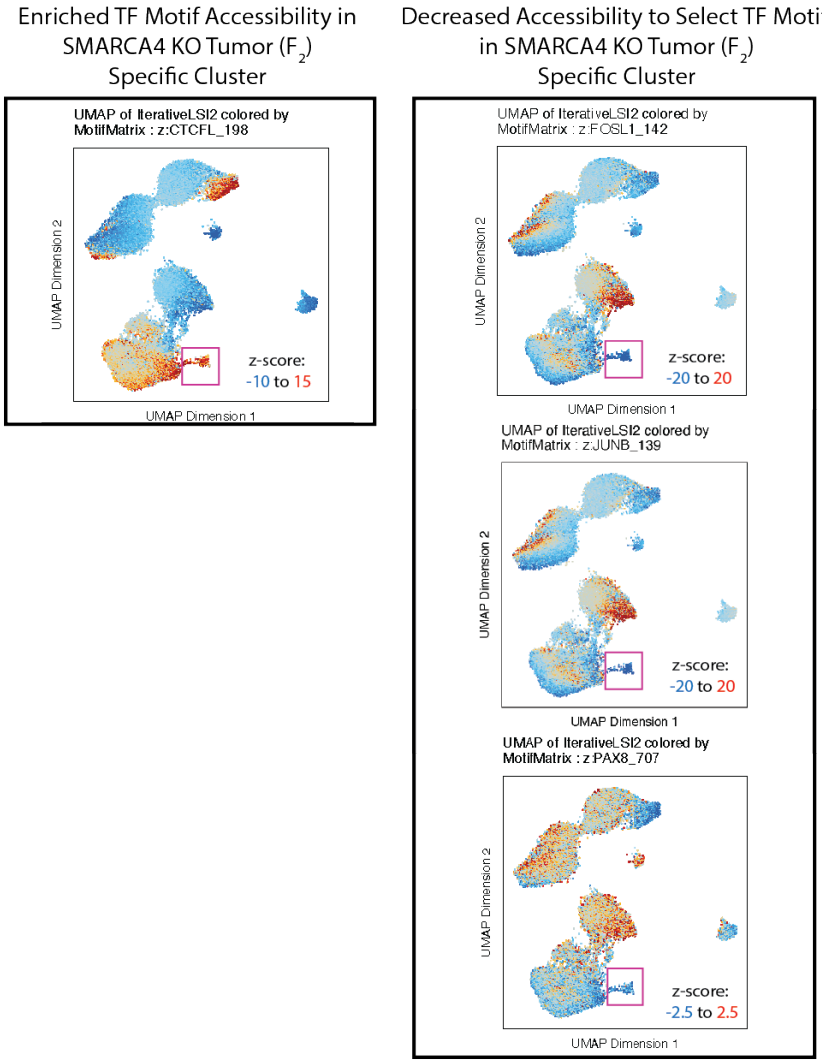


Figure 3.35. Transcription factor activities across integrated HEC 116 Plasmid Derived scATAC-seq datasets. UMAP plots highlighting deviation z-scores for a motif and demonstrating varying degrees of chromatin accessibility at motifs coupled with *PAX8, CTCFL,* and AP-1 (*JUNB* and *FOSL1*) transcription factor activity.

Finally, we performed pseudotemporal analyses within ArchR to try and answer whether dedifferentiation of HEC 116 Plasmid Derived SMARCA4 KO follows a continuum (38). We generated a graph trajectory that originated from Cluster 9

proceeded through Cluster 10 and ended with Cluster 3, as these groups of cells all possessed a high number of serially passaged tumor cells lacking SMARCA4 expression but showed varying degrees of chromatin accessibility at marker genes associated with the undifferentiated regions of DDEC **[Figure 3.36]**. Interestingly with the F2 generation samples we examined by scATAC seq, the state of cells characterized by decreased accessibility near numerous epithelial markers and AP-1 transcription factors **[Figure 3.36A & Figure 3.36B]** and that have increased chromatin accessibility at known oncogenic effectors of transcription, appears quite abruptly **[Figure 3.36C]**. Alternatively put, what we have defined as the fully undifferentiated cells in our single cell sequencing studies, does not seem with our sample preparation and analysis conditions to evolve gradually from its more well-differentiated serially passaged predecessors.

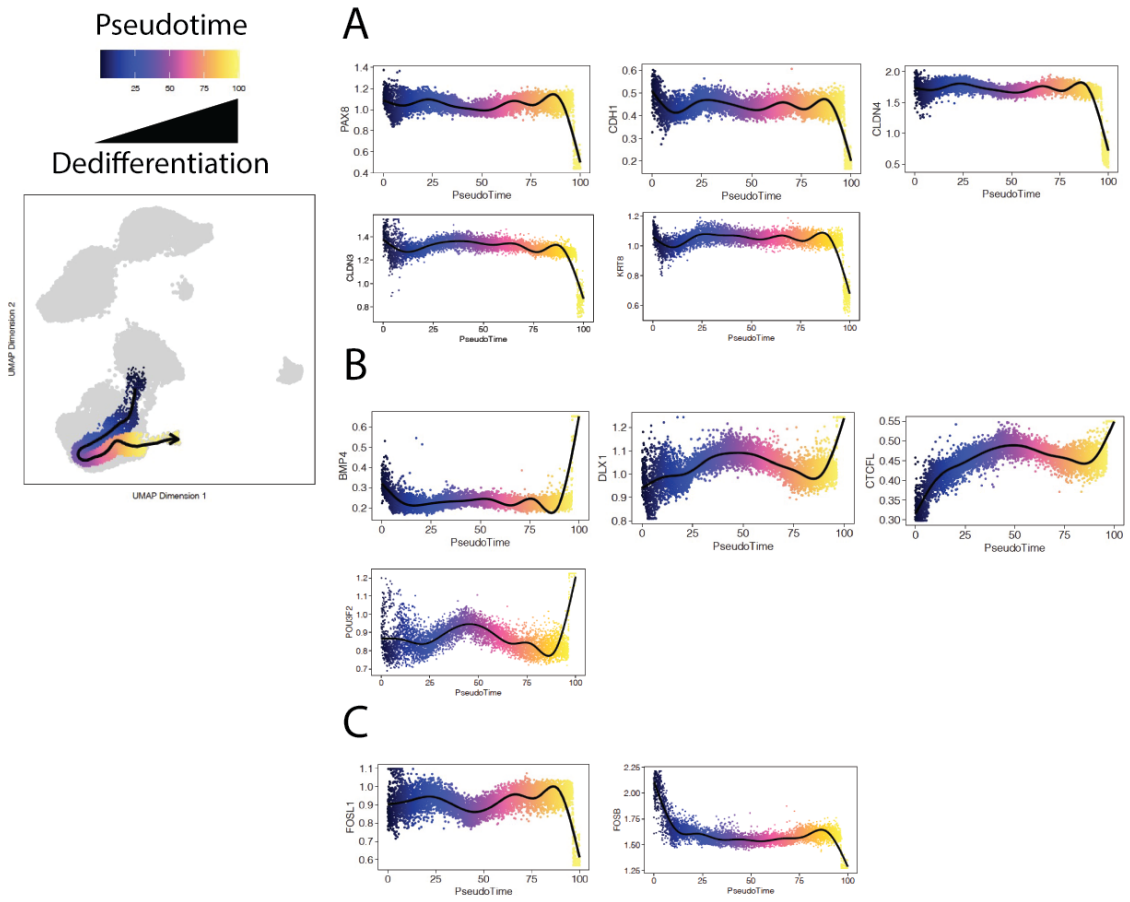


Figure 3.36. **Pseudotemporal analysis of integrated HEC 116 Plasmid Derived scATAC-seq datasets suggests an unlikely abrupt collapse in the endometrial epithelial EC cell chromatin landscape and sudden increase in the accessibility of drivers of oncogenic transformation.** UMAP plots showing chromatin accessibility patterns as a function of pseudotime and focused primarily on serially passaged SMARCA4 KO EC cells.

3.3. Discussion:

For the first time, using mismatch repair protein deficient endometrial cancer cell lines, we have shown the development of models of DDEC upon inactivation of the chromatin remodelling protein, SMARCA4. Our characterization of commercially available endometrial cancer cell lines yielded unsurprising results as HEC 116 and HEC 59 originate from intermediate-grade endometrial adenocarcinoma which is noted for its activation of distinct signalling pathways (39). It is well documented that endometrial

cancer development is achieved through disruptions in Wnt and Akt signalling and by targeted mutation profiling and phosphokinase arrays, we observed high levels of activation of Wnt and Akt/PI3K signaling pathway members paired with irregularities in genes such as *CTNNB1* and *PTEN* (40). A high degree of STAT3 activation, an observation we observed prominently in HEC 116 cells, is also increasingly witnessed in hormone dependent Type I endometrial cancers (41). Unique to HEC 59 was mutation to p53, which is seen less commonly amongst the endometrioid cancer type but can still be highly prevalent and when p53 protein is abnormally produced it is often associated with shorter patient survival (40, 42). ARID1A deficiency was seen in about 75% of the SMARCA4 deficient clinical cases profiled by our group and was also a feature of the EC cell lines chosen for SMARCA4 knockout (16). Taken together with their proven mismatch repair protein deficient genetic background and expression of PAX8, a proven positive marker of uterine adenocarcinomas regardless of type, our examination of HEC 116 and HEC 59 deemed these EC cells lines an appropriate starting point to test whether alterations in SMARCA4 expression would affect their features of well-differentiated endometrial cancer (43).

Introduction of frameshift mutations to *SMARCA4* by NHEJ following CRISPR/Cas9 gene editing, resulted in EC cells lacking SMARCA4 protein expression. Absence of SMARCA4 was confirmed in two ways at the protein level and with validation of the introduction of a frameshift mutation at the genetic level. For the most part, the various derivations behaved similarly *in vitro*, with cells lacking SMARCA4, possessing longer doubling times, forming fewer tumorspheres and being less capable of undergoing anchorage independent growth than their SMARCA4 wildtype counterparts. Deficiency

of the ATPase, SMARCA4 in primary mouse myoblasts, mouse adult fibroblasts, mammary epithelial cells, and breast cancer cells has been shown previously to reduce cellular proliferative capacity *in vitro* (44-47). In mouse adult fibroblasts reduction in SMARCA4 levels, led to most cells exhibiting abnormal mitotic division and even some manifesting evidence of mitotic catastrophe (44). While not the focus of this current study, it may be informative to examine the appearance of SMARCA4 deficient endometrial cancer cell nuclei for additional malformations such as aneuploidy and micronuclei. We demonstrated immediately upon SMARCA4 KO that EC cells enter a complex physiological state known as senescence as all our cell line models of DDEC could be characterized by changes in specific chromatin modification levels, high acid ß-galactosidase activity, and increased chemoresistance and secretion phenotypes [**Figure 3.37]**. A closer examination of SAHF could be accomplished and allow us to confirm that loss of SMARCA4 in EC does indeed leads to widespread chromatin alteration not just elevated levels of H3K9 trimethylation in SMARCA4 KO EC cells as we demonstrated. The main role of the SASP is to form a pool of cytokines, chemokines, and proteases that reinforces tumor suppression and evidence from both our transcriptomic and proteomic analyses implicate SASP as a major contributor to the state of SMARCA4 deficient cells *in vitro (48).* It would be of interest to validate the enhanced proinflammatory phenotype initially acquired upon SMARCA4 loss and observe if it changes over time by either targeted gene expression profiling or bulk RNA sequencing of tumor tissue from both our cell line models of DDEC and patient tissue **[Figure 3.38]**. Also *in vitro,* even though the full dedifferentiated phenotype of the patient samples was not achieved, evidence from qRT-PCR and multi-omics demonstrated some qualities of clinical DDEC were obtained

with SMARCA4 knockout alone. Loss of hormone receptor expression and downregulation of estrogen dependent signalling pathways are consistent with our previously published findings that absence of ER is a fundamental characteristic of DDEC **[Figure 3.38]** (49). We also revealed SMARCA4 loss in EC cells by qRT-PCR to be enough to dysregulate cell junction adhesions and ZEB1 likely mediates the substantial reduction in E-cadherin we observe at both the transcript and protein level **[Figure 3.37]**. Purely undifferentiated endometrial carcinomas have been reported to overexpress ZEB1 which in turn leads to downregulation of E-cadherin and potentially the start of the metastatic cascade by allowing for expression of mesenchymal markers (50). Our work also supports growing evidence first observed in mammary epithelial cells, that downregulation of SMARCA4 negatively impacts the expression levels of several extracellular matrix associated proteins (51). It should be remarked as well that our proteomic and transcriptomic datasets produced very similar findings not only in terms of proving the breakdown of protective epithelial barriers or ECM but also the acquisition of stemness upon SMARCA4 knockout **[Figure 3.37 & Figure 3.38]**. Stem cell functions and senescence are quickly proving to be overlapping signalling networks with such molecules as p21 playing a major role in maintenance of both processes (52, 53). Depletion of SMARCA4 across several tumor cell lines with wildtype p53, resulted in the induction of downstream transcriptional target, p21, whose expression levels were also found to be higher in p53 mutant HEC 59 SMARCA4 KO EC cells (54). Trimethylation of H3K9 thereby transcriptionally repressing targets of E2F and impacting the transition into S-phase, is reminiscent of iPSCs reprogramming, and similarly in cancer as well senescence is capable of reprogramming cells into a stem-cell like state (29, 52, 55). It

is likely that cellular senescence in our cell line models of DDEC is initiated not only by the loss of a known tumor suppressor, SMARCA4, but also in part by excessive mitogenic signals **[Figure 3.37]** (56). Performing phosphokinase arrays with the SMARCA4 intact and deficient HEC 116 Plasmid Derived cells both as adherent cells or spheres and unpassaged or serially passaged could reveal deeper insight into the signalling pathway adaptations arising in the absence of the chromatin remodeling subunit **[Figure 3.37]**. In mesenchymal stem cells (MSCs), knockdown of SMARCA4 has been shown to induce senescence through both an increase in heterochromatin in addition to activation of RB2/p130 and p53 pathways (57). In contrast, the histone acetyltransferase p300 in human fibroblast cells drives a senescent phenotype through the formation of active enhancer elements and a hyper-acetylated chromatin state and in data not shown H3K27ac levels by immunostaining have been found to be higher in HEC 116 Plasmid Derived SMARCA4 KO cells than in SMARCA4 WT EC cells (58). While our findings with our cell line models of DDEC agree with aspects of previous work done in a variety of contexts, further experimentation will be required to completely implicate the molecular players involved in the acquisition of senescence by SMARCA4 KO EC cells and the entire interplay between stemness and senescence **[Figure 3.37 & Figure 3.38]**. This is especially since HEC 116 models of DDEC did not exhibit upregulation of p21 and HEC 59 SMARCA4 deficient cells did not appear to have increased H3K9me3 levels. Other notable differences between the three models derived by CRISPR gene editing also emerged. Perhaps unsurprisingly, HEC 59 SMARCA4 deficient cells exhibited unique properties from the HEC 116 models regardless of the CRISPR delivery method applied. Targeted DNA sequencing demonstrated that the mutational landscape of parental HEC

59 EC cells differs from HEC 116 and qRT-PCR results, their appearance under microscopic magnification and propensity to form secondary and tertiary spheres suggest they begin more mesenchymal in nature.

We demonstrated that *in vivo* passaging is required for the abrupt emergence of a DDEC phenotype, suggesting that selective pressures from the microenvironment and/or the acquisition of genomic or epigenomic alterations are requisite for cellular dedifferentiation. Little characterization of these endometrial cancer cell lines has been carried out in terms of tumor formation in a subcutaneous setting. Indeed, to our knowledge, this is the first time the histology or growth rates of tumors from either HEC 116 or HEC 59 endometrial cancer cells have been documented. While CRISPR gene editing is increasingly criticized for its inappropriate modeling of phenomena in cancer biology, introduction of frameshift mutations to SMARCA4 is identical to what takes place naturally in DDEC patients, making the application of this methodology in our system incredibly suitable (59). The lengthy CRISPR workflow together with the clonal selection process, inevitable off-target effects, and utilization of cell lines with highly unstable genetic backgrounds are all likely contributors to the variable results we observe not only *in vitro* but *in vivo* and help explain the differences that emerge not only upon comparing the RNP Derived model to the Plasmid Derived HEC 116 cell line model but likely the behavior of HEC 116 models of DDEC to the HEC 59 cell line model too. It should be noted though that cell line derived models of DDEC, particularly the HEC 116 Plasmid Derived SMARCA4 KO cells, have consistently undergone dedifferentiation on multiple separate occasions of serial passaging and are dependably marked by the same changes in histology and marker expression that consistently characterize clinical disease (22, 49,

60). While the influence of growth factors, hormones, and infiltrating mouse stroma should not be overlooked in likely expediating the emergence of dedifferentiation, there is growing evidence that non stem cell like cancer cells that exhibit senescent-like properties are critical in supporting the tumorogenic potential of cancer stem-like cells (61). Possibly sorting SMARCA4 deficient cells based on markers of senescence and testing tumor formation in the absence of senescent cells could shed light as to their overall contribution to the mixed phenotype of DDEC **[Figure 3.38]**.

Our single cell sequencing studies also demonstrated the immense impact, selective pressure had on the abrupt emergence of undifferentiated regions during SMARCA4 deficient tumor formation. While a more substantial proportion of cells lack expression of markers of gynecological epithelium upon serial passaging regardless of the SMARCA4 status of the HEC 116 EC cells, only a small portion or 2% of SMARCA4 deficient tumor cells, tip into a more complete dedifferentiated state. Both at the level of gene expression and chromatin accessibility, dysregulation of AP-1 transcription factor family member expression was evident **[Figure 3.37].** Remarkably in SCCOHT, it has been reported that SMARCA4 associates with AP-1 to drive epithelial like differentiation (62). Moreover, not only in this SMARCA4 deficient neoplasm but in other contexts as well, SMARCA4 is required for access to be granted to AP-1 motifs upstream of epithelial genes (62-65). As our study supports previous reports of SWI/SNF and AP-1 associations, SMARCA4 reexpression in our cell line model of DDEC followed by multi-omic data analysis and other functional studies will further cement this biological connection also potentially driving differentiation in endometrial cancer **[Figure 3.38]**. Just as AP-1 transcription factors Jun and Fos can have important functions in cell-fate

decisions, we found within our single cell sequencing studies specific to our serially passaged SMARCA4 KO cells, several other transcription factors implicated in phenotype switching in cancer that warrant validation as drivers of dedifferentiation. Aberrant expression of CTCFL (BORIS) and POU3F2 (BRN2) in melanoma have been shown recently to promote a switch towards a more invasive phenotype (66-68). Interestingly, in liver cancer stem cells, CTCFL is a major promoter of CSC-like properties and upon overexpression upregulates Oct4 expression through modulation of histone modifications (69). Increased BRN2 is associated with the dedifferentiation of melanocytes and has been correlated and later found to under certain contexts, repress MITF, a transcription factor that contributes to melanoma development (66, 67). MITF levels have been examined in aggressive epithelial ovarian cancer and were shown to promote metastasis but no examination in more undifferentiated gynecological cancers such as SCOOHT or DDEC has been completed to test whether phenotype switching through a melanoma-like BRN2/MITF axis can take place **[Figure 3.37]** (70). Examination of the levels of these transcription factors within patient DDEC tumors would both illuminate and eliminate some of the drivers of dedifferentiation that arose from this preliminary analysis of our DDEC-like cell line models **[Figure 3.38]**.

Several downsides to our initial experimental design in relation to the single cell sequencing experiments happened to be revealed in retrospect. While our results highlighted the differences between SMARCA4 KO cells *in vitro* versus *in* vivo, we might have failed to capture many cells during the act of transitioning or dedifferentiating. This could be attributed to both technical and bioinformatics-based issues. Repetition of these single cell sequencing experiments with more time points across the serial passaging

workflow or incorporation of fluorescence-activated cell sorting (FACS) selection for proven markers of EMT or dedifferentiation may allow for the interrogation of those cells specifically exhibiting plasticity. Furthermore, as we better understand the models of DDEC at a molecular level, we may continue optimizing culture conditions that foster the growth of subsets of cells of interest. DDEC also serves as a perfect model for the latest iteration of 10X Genomics Single Cell Multiome ATAC + Gene Expression kits that can capture gene expression and chromatin accessibility changes at the onset of the experiment rather than just at the analysis stage. The Visium Spatial Gene Expression for FFPE assay would also allow for archived patient samples to be examined and gene expression information to be linked to its original location within tumor samples, adding valuable insight to what has already been garnered with the cell line based model of disease thus far (71).

Now in possession of culturable primary DDEC cells, it would also be extremely useful to compare the DDEC cell line-based models to the responses of the patient derived lines to the same *in vitro* functional experimentation. Work with the patient-derived cell lines, should result in further validation of our current experimental model, enlightening us on the shared properties between our derived models and the disease, and perhaps may implicate the exact microenvironmental factors necessary for complete dedifferentiation. Nevertheless, the HEC 116 Plasmid Derived cell line model of DDEC especially, serves as an incredibly valuable tool to continue studying cellular dedifferentiation in the broader context of cancer. While there is still is a lack of literature centred on the transition state/wobble point of cancer cells and the deciding cell fate decisions that push them towards acquiring plasticity, the development of this model

system has provided many starting points for future investigation. These cell line models of DDEC should continue to hold promise especially if used with patient models of DDEC, allowing for even more uncovering of the molecular mechanisms underlying dedifferentiation.

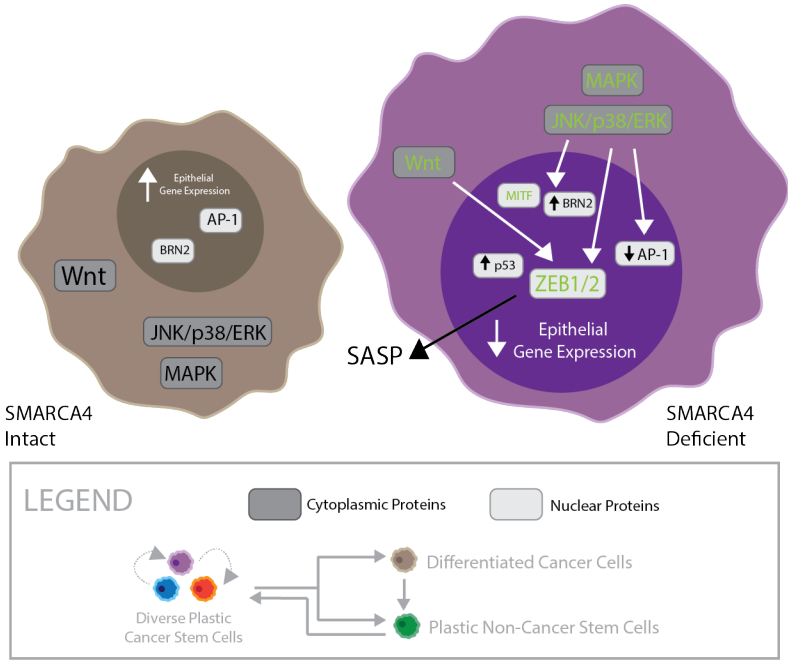


Figure 3.37. Proposed network of interactions in endometrial cancer cells that may be altered by loss of chromatin remodeling subunit, SMARCA4. SMARCA4 both indirectly and directly regulates inflammatory, mitogenic and transcription factors which in turn likely influence surrounding SMARCA4 deficient EC cells. Results obtained within this chapter highlight some integral signalling pathways and regulators of cellular plasticity shown in green that warrant future investigation.

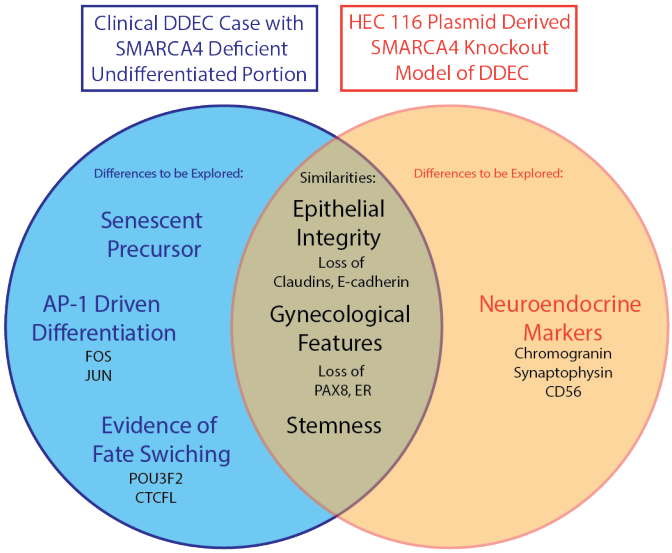


Figure 3.38. Simplified summary and comparison of the features of clinical DDEC against cell-line models of DDEC derived by CRISPR gene editing. While a substantial degree of overlap in both general histology and molecular signatures was observed between SMARCA4 deficient patient tissue and upon knockout of SMARCA4 in MMR-deficient EC cells, there are several novel discoveries that could be more thoroughly assessed with additional experimentation.

3.4. Methods:

3.4.1. Cell Culture:

HEC 116 and HEC 59 cells were obtained from JCRB and cultured in minimal essential media (MEM) supplemented with 10% fetal bovine serum (FBS). Cells were passaged using 0.25% w/v trypsin (Life Technologies). All cells were cultured at 37°C with 5% CO_2 supplementation. Cells were authenticated at the Sick Kids Research Institute, Toronto, Ontario Canada and tested for mycoplasma in house using the PCR-based universal mycoplasma detection kit (ATCC).

3.4.2. Phospho-Protein Array Analysis:

HEC 116 and HEC 59 endometrial cancer cells were subjected to overnight serum starvation, lysed. Phosphorylation levels for select human proteins in signal transduction pathways were analyzed using a commercially available phospho-protein array (R&D Systems, Minneapolis, MN, USA). The Proteome Profiler Human Phospho-Kinase Array Kit (ARY003B) was used following the manufacturer's protocol. Cell lysates (400 µg) were added to wells containing array membranes and incubated at 4°C overnight. Briefly, the levels of phosphorylation of 43 kinases were assessed with phospho-specific antibodies and chemiluminescent detection. Proteins were detected through exposure to film. ImageJ was used to determine the average pixel density signal of pairs of duplicate spots that represent each phosphorylated kinase protein. Negative control spots were used as background values and subtracted.

3.4.3. Targeted Gene Panel Sequencing Analysis and Validations:

As per the PureLink Genomic DNA Isolation Kit instructions (Thermo Fisher) genomic DNA (gDNA) was extracted. An Illumina custom TruSeq amplicon panel was designed within Illumina's Design Studio to include the following 26 recurrently mutated genes in gynecological carcinomas: POLE, TP53, PP2R1A, FBXW7, SPOP, ARID1A, CTNNB1, KRAS, EP300, PTEN, PIK3CA, PIK3R1, PIK3R2, ARID5B, CMSD3, GRLF1, ZFHX3, CTCF, ABCC9, CHD4, MAP3K4, CCND1, FGFR2, RPL22, and TSPYL2. Custom amplicon libraries were generated using 250 ng DNA and following the TruSeq Library Preparation protocol as in (72). Prior to pooling at equal concentrations, normalization was achieved by quantifying the individual libraries using the Qubit fluorometer. Pooled libraries were quantitated for amplifiable libraries with the FAST

qPCR SYPR quantification kit (Kapa Biosystems). The Illumina MiSeq was used to sequence the Pooled TruSeq libraries using 300 cycle V2 kits. Analysis was completed with the MiSeq Reporter and somatic variant caller software 3.2.3.0. Frameshift, nonsense or indel mutations were identified only upon passing a quality filter with at least 10% variant allele frequency and were further validated by direct Sanger sequencing using primer sets targeting the regions surrounding the mutations and manual inspection of bam files with Integrated Genome Viewer (IGV). The corresponding genomic sequences were assessed to normal tissue to ascertain the somatic/germline status of discovered mutations.

3.4.4. Immunofluorescence:

Cells adherent to coverslips (Fisher Scientific) were fixed in 4% paraformaldehyde (PFA) (Chem Cruz) in 1x PBS for 10 minutes, permeabilized in 0.2% Triton X-100 (Fisher Scientific) in PBS for 5 min and then stained with primary antibodies diluted in 1x PBS. After incubation of the primary antibodies for 3 hours at room temperature the cells were washed 3x with 1x PBS. Cells were then incubated for 1 hour at room temperature with the secondary antibodies at a dilution of 1:500 (goat anti mouse Alexa 488 and goat anti rabbit Alexa 594 (Cell Signaling Technology)) that were diluted in 1x PBS. After another three rinses with 1x PBS, the coverslips were incubated with a 1:5000 dilution of DAPI before being mounted onto microscope slides (Fisher Scientific) with mounting media (Vectamount). Images were captured using a Zeiss LSM 710 Meta Confocal Microscope (Carl Zeiss, Germany) with the pinhole diameter set to 1 airy unit for all channels and the exposure gains kept constant for acquisition of all samples.

Primary antibody dilutions are as follows -

Primary Antibody	Dilution
Anti-Brg-1 G7 [sc-17796] (Santa Cruz Biotechnology, Inc.)	1:150
Anti-PAX8 [10336-1-AP] (Proteintech)	1:150

3.4.5. Transfections for CRISPR Gene Editing:

3.4.5A. <u>Plasmid Based System:</u>

One-step cloning was accomplished of a custom gRNA (SMARCA4 target sequence = CGCCCGTGATGCCACCGC) into an all-in-one CRISPR/Cas9 LacZ plasmid as described in (18). Following the manufacturer's instructions, GeneIn transfection reagent (GlobalStem, Rockville, Maryland, USA) was used to deliver the all-in-one CRISPR/Cas9 Lacz plasmid to HEC 116 endometrial cancer cells. Flow cytometry (University of Alberta, Faculty of Medicine and Dentistry, Flow Cytometry Facility) was utilized to enrich for CRISPR transfected cells and achieved by sorting for cells expressing mCherry. Single clones were either generated by flow cytometry plating a single cell per well into a 96 well plate (VWR) or manual plating of 0.5 cells/well into a 96 well plate upon filtration through a cell strainer (Fisher Scientific).

3.4.5B. <u>RNP Based System:</u>

cRNA targeting SMARCA4 (sequence = GCGGTGGCATCACGGGCG) and tracrRNA duplexes (1 μM) were formed by heating at 95°C and gradual cool down to room temperature. RNP complexes were formed by combining the 1 μM guide RNA oligos with 1 μM Alt-R *S.pyogenes* Cas9 endonucleases (IDT) in Gibco Opti-MEM media (ThermoFisher Scientific) for 5 minutes at room temperature. Transfection complexes

containing the RNP complex and Liptofectamine RNAiMAX transfection reagent (ThermoFisher Scientific) were diluted in Opti-MEM media and incubated at room temperature for 20 minutes. HEC 116 endometrial cancer cells and HEC 59 endometrial cancer cells were added to transfection complexes in the wells of a 24-well tissue culture plate to achieve a final concentration of cells of 40000 cells/well and final concentration of RNP of 10 nM. Flow cytometry (University of Alberta, Faculty of Medicine and Dentistry, Flow Cytometry Facility) was utilized to enrich for CRISPR transfected cells positive for tracrRNA-ATTO^{TM}550 fluorescence. Single clones were either generated by flow cytometry plating a single cell per well into a 96 well plate or manually plating of 0.5 cells/well into a 96 well plate upon filtration through a cell strainer.

3.4.6. Genomic DNA Isolation for CRISPR Gene Editing:

As per the PureLink Genomic DNA Isolation Kit instructions (Thermo Fisher) genomic DNA (gDNA) was extracted. Quantification of gDNA by spectrophotometry was achieved using the Epoch Microplate Spectrophotometer (BioTek).

3.4.7. Sanger Sequencing of CRISPR Gene Edited Single Clones:

Single cell-derived clones analyzed were validated by Sanger sequencing over the guided nuclease target region. AmpliTaq polymerase (Thermo Fisher) was used to generate PCR products that were cloned using the TOPO TA Cloning Kit (Thermo Fisher). Several colonies for each clone were Sanger sequenced using the M13R primer which binds the pCR-4-TOPO backbone (University of Alberta, Molecular Biology Service Unit) to ensure all target alleles were detected. *Escherichia coli (E.coli)* colonies were miniprepped using the High Speed Plasmid Mini Kit (Frogga Bio).

3.4.8. Immunohistochemistry:

For mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) the primary antibodies and staining methods were the same as that reported previously (73, 74). Cell lines were pelleted, fixed overnight in 4% PFA (Chem Cruz) prior to being suspended in 1% agarose solution. Formalin-fixed, paraffin-embedded tissue and agarose-embedded cell sections were subjected to deparaffinization in xylenes, hydration with an ethanol series and antigen retrieval at pH 9 (Dako). Peroxidase and serum-free protein blocking for 5 and 10 minutes, respectively, was also performed. Primary antibodies were applied for 30 minutes at 25°C or overnight at 4°C. Slides were washed in TBS + 0.1% Tween (TBST) and Envision HRP anti-rabbit or anti-mouse secondary antibody was applied for 1 hour at room temperature. Slides were again washed in TBST before the brown signal was developed using DAB substrate (Dako). Counterstaining was achieved with Mayer's haematoxylin (Vector Laboratories). Tissue was dehydrated using a reverse ethanol series prior to application of coverslips with mounting medium (Vectamount). The entire cross-sectional area of the slides was scanned by an Aperio Digitial Pathology slide scanner. Image analysis software (Image Scope; Leica Biosystems) was used to convert the whole slide scans into TIFF image formats.

Primary antibody dilutions are as follows -

Primary Antibody	Dilution
Anti-ARID1A [HPA005456] (Sigma)	1:200
Anti-BRG1 [EPNCIR111A] (AbCam)	1:200
Anti-p53 [DO-7] (Invitrogen)	1:100
Anti-PAX8 BC12 [ACI 438] (Biocare Medical)	1:100
E-cadherin G10 [sc-8426] (Santa Cruz Biotechnology, Inc.)	1:100

3.4.9. RNA Extraction:

Both the PerfectPure RNA Cultured Cell Kit (5-Prime) and Rneasy Mini Kit (Qiagen) were used to isolate total RNA from cultured cells. On-column DNase treatment was performed with final elution volumes equaling 40 µl. Purified RNA was quantified using the Epoch Microplate Spectrophotometer (BioTek).

3.5.10. cDNA Synthesis & Real Time PCR:

The high capacity cDNA reverse transcription kit (Applied Biosystems) was utilized to generate cDNA from 2 µg purified total RNA. A 1:1 mixture of random hexamers to oligodTs (Thermo Fisher Scientific) was used to prime the reverse transcription reactions. Separate controls lacking either RNA or reverse transcriptase were included. Real time PCR was performed on 1 µl of cDNA using TaqMan Gene Expression Master Mix and 6-carboxyfluorescein (FAM) labeled TaqMan gene expression human primer/probe sets as per manufacturers' protocols (Thermo Fisher Scientific). A CFX96 Touch Real-Time PCR Detection System was used with the following thermocycling conditions: Activation 95 °C

for 10 min; Melting 95 °C for 15 s; Annealing/extension 60 °C for 1 min. Returning to step 2 for a total of 40 cycles.

Primer/Probe sets that were utilized are listed below:

Primary/Probe Set	Dilution
E-cadherin	Hs_01023894_m1
N-cadherin	Hs_00983056_m1
Sox2	Hs_01053049_m1
Oct4	Hs_04260367_gH
ER	Hs_00174860_m1

mRNA expression of SMARCA4 deficient samples was compared to SMARCA4 intact cells using the delta CT method.

3.4.11. Growth Curves by Trypan Blue Counting:

7500 cells were plated in two 12-well plates. Every 24 hours, over a period of 12 days, two wells were washed with PBS, the cells trypsinized and pelleted by centrifugation. The duplicate cell pellets were resuspended in 1 mL media to obtain a single cell suspension. An aliquot of 10 µL was removed and added to 10 µL of 0.4% trypan blue solution (MP) to obtain a 1:2 dilution. The number of live cells was determined using a hemocytometer and a light microscope. Manual counting was performed in triplicate for each well of cells. Doubling times for each cell line were calculated from the growth curves using the exponential growth equation found in GraphPad Prism.

3.4.12. Sphere Formation:

Cells were trypsinized with 0.25% (w/v) trypsin (Life Technologies) and neutralized in fresh media. Single cell suspensions were obtained by filtering cells though 40 µm filters (Supplier) prior to counting viable cells with trypan blue (MP). Concentrations of cells to be plated were achieved through dilution in sphere formation media composed of

MEM, 1x B27 (Life Technologies), 20 ng/mL epidermal growth factor (EGF) (Life Technologies) and 10 ng/mL fibroblast growth factor (FGF) (Life Technologies). 100 µL of diluted cells per well were plated onto Ultra-Low Attachment Surface 96-well plates (Corning). Spheres were allowed to grow over a two-week period before being counted manually under a light microscope. Images of the spheres were obtained using the EVOS FL Cell Imaging System (Thermo Fisher Scientific).

3.4.13. Soft-Agar Colony Formation Assays:

Anchorage-independent growth was assessed by soft agar colony formation experiments. Six-well culture dishes (VWR) were coated in 1:1 ratio of 1% agarose in 2X MEM medium (Sigma-Aldrich). Cells were plated at a density of 1000 cells in 2X MEM medium containing 0.7% agarose (Invitrogen) (1:1). Colonies were allowed to form over a two-week period with media changed every three days. Colonies were washed with PBS prior to being stained with 0.5 % crystal violet solution in methanol. The number of colonies formed were counted by light microscopy. Images of the colonies were obtained using the EVOS FL Cell Imaging System (Thermo Fisher Scientific).

3.4.14. Bulk RNA Sequencing:

RNA was extracted from HEC 116 Plasmid Derived SMARCA4 KO and WT cells that were subjected to serum starvation as described in section 3.4.18 using the Qiagen RNeasy kit and quantified using Qubit (Thermo Fisher Scientific). The Genome Quebec Innovation Center was sent the RNA samples where quality was further assessed by Bioanalyser. NEB mRNA stranded library preparation was carried out followed by sequencing via Illumina NovaSeq. Reads were aligned to the GRCh38 human reference genome using STAR and post sequencing quality check was performed with samtools

and fastqc. Read quantification was performed with FeatureCounts using GrCh38.87 annotations. edgeR was used to determine expression values for paired knockout and wildtype samples, using the exact test and a FDR cutoff of 0.05 was set, determining statistically significant differences. The camera package for R was used to compare data to the hallmark gene sets from Molecular Signatures Database, which was used for gene set enrichment analysis.

3.4.15. Mass Spectrometry of Conditioned Media:

Cells were plated into T150 flasks (VWR) and cultured to sub-confluency. At 80-90% confluency, complete media was removed and cells were washed in 10 mL pre-warmed PBS. To further minimize contamination from serum, serum-free media was added after the PBS rinses and the cells incubated for 1 hour at 37°C. Basal media, 20 mL, was added to the cells for 24 hours. Conditioned media was collected and centrifuged at 4°C at 450g for 5 minutes. Supernatants were stored at -80°C until concentrated. Thawed conditioned media was added to 3 kDa molecular weight cutoff (MWCO) ultra-centrifugal units (Millipore) and spun in a swinging bucket rotor (ThermoLegend XTR) at 4000g at 8°C for 45 minutes until all media that was collected was concentrated. Excess salts and media were removed by the addition of 10 mL of distilled water to the centrifugal unit and further centrifugation at 4000g at 8°C for 15 minutes until the remaining volume of conditioned media was approximately 500 µL. The concentrated conditioned media was stored at -80°C until processed for mass spectrometry. Samples were prepared as described in (75, 76). Frozen concentrated media was lyophilized overnight prior to resuspension of the freeze-dried pellet in urea containing lysis buffer (8 M Urea, 2% sodium dodecyl sulfate (SDS), 10 mM dithiothreitol

(DTT), 5 mM Ammonium Bicarbonate). Conditioned media was quantified using a 660 nm Protein assay reagent kit with ionic detergent compatibility reagent (Pierce™). 100 mM DTT was added to 10-50 µg of conditioned media samples to a final concentration of 10 mM and incubated at RT for 30 minutes. Samples were also alkylated in 100 mM iodoacetamide for 30 minutes at RT in the dark. Protein was precipitated using methanol-chloroform extraction. 50 mM ammonium bicarbonate was added to dry protein pellets prior to sonication with a probe sonicator (Fisher Scientific). Protein digestion was achieved by incubating every 50 µg of protein sample with 1 µg of Trypsin/LysC (Promega) overnight at 37°C. Further digestion was accomplished by adding 1 µg of Trypsin/LysC to every 100 µg of sample protein for an additional 4 hours. Protein digestion was carried out on a ThermoMixer C (Eppendorf) at 400 and 1400 rpm for the overnight and4 hour incubation, respectively. Acidification of the samples was completed through the addition of 10% formic acid (FA) to 1/10th the final volume of the digest. Undigested insoluble material was pelleted by centrifugation at 14000g for 1 minute. A Q Exactive Plus mass spectrometer (Thermo Scientific) connected to a Waters ACQUITY M-Class UPLC was used to analyze the digests. Solvent A consisted of water/0.1% FA while Solvent B was made up of acetonitrile/0.1% FA. 1 µg of sample peptides was loaded onto an ACQUITY UPLC M-Class Symmetry C18 Trap Column and trapped for 6 min at a flow rate of 5 µl/min at 99% Solvent A/ 1% Solvent B. Separation of peptides was achieved on an ACQUITY UPLC M-Class Peptide BEH C18 Column operating at a flow rate of 300 nL/min at 35°C and using a non-linear gradient consisting of 1-7% Solvent B over 1 min, 7–23% Solvent B over 179 min and 23–35% Solvent B over 60 min. MS acquisition instrument settings were the same as though utilized in (75, 76). Files from

the mass spectrometer were searched in MaxQuant against the Human Uniprot database with missed cleavages set to 3 and cysteine carbamidomethylation set as a fixed modification. Oxidation (M), N-terminal acetylation (protein), and deamidation (NQ) were set as variable amino acid modifications (max. number of modifications per peptide = 5). LFQ min. ratio count was set to 1 and all other settings were left as default settings. Protein and peptide FDR was left at 0.01 (1%) and the decoy database was set to revert. The 'match between runs' feature was utilized to maximize proteome coverage and label-free quantification. Search results were loaded into Perseus or R, and proteins labeled as 'only identified by site', 'matched to reverse' or 'potential contaminant' were removed as previously reported in (75, 76). Protein identifications with LFQ values in ≥2 biological replicates were retained for downstream analysis, and missing values were imputed using a width of 0.3 and down shift of 1.8. Gene set enrichment analysis (GSEA) were performed in GSEA v3.0 (Broad Institute) with a minimum gene set size of 10 and permutation type set to gene_set. MSigDB v6.2 collections used were: canonical pathways, hallmark, KEGG gene sets, and C2 curated sets.

3.4.16. ß-Galactosidase Assays:

15000 cells were plated onto coverslips (Fisher Scientific) in 6-well plates. At 80-90% confluency, complete media was removed, and cells were washed in 10 mL pre-warmed PBS. To further minimize contamination from serum, serum-free media was added after the PBS rinses and the cells incubated for 1 hour at 37°C. Basal media, 20 mL, was added to the cells for 24 hours. Cells that were not serum starved served as negative controls. Plates were rinsed in PBS prior to fixation in 1 mL Fixative Solution (Cell Signalling Technology) for 10-15 minutes at room temperature. Following fixation,

cells were washed in PBS twice prior to the addition of 1 mL ß-Galactosidase Staining Solution (Cell Signalling Technology) to each well. Plates were sealed with parafilm and kept at 37°C in a dry incubator overnight. ß-Galactosidase Staining Solution was removed and cells stored in 70% glycerol solution until the coverslips were mounted onto glass slides (Fisher Scientific). Images of cells were obtained with the Zeiss AxioSkop microscope (University of Alberta, Cross Cancer Institute, Imaging Facility). Percent senescent cells were calculated by dividing the number of positively-stained blue cells by the number of total cells in the field of view. As the cells were auto-fluorescent in the FITC+ channel, an accurate number of total cells could be determined for six fields of view examined.

3.4.17. Protein Extraction:

Protein was extracted from adherent cells directly on plates using radioimmunoprecipitation assay (RIPA) Lysis and Extraction buffer (Thermo Fisher Scientific) with added Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Lysed cells were incubated at 4°C prior to centrifugation at 13000 rpm for 20 minutes at 4°C to pellet insoluble cell debris. Supernatants containing protein were quantified using the DC Protein Assay (Bio-Rad) and measured on a FLUOstar Omega plate reader (BMG LABTECH; Offenburg, Germany). Protein was also extracted from flash-frozen tumors that were ground by mortar and pestle and resuspended in RIPA Lysis and Extraction buffer (Thermo Fisher Scientific). Following sonication, lysed tumor cells were incubated, centrifuged and quantified in a similar manner as the lysates from adherent cells.

3.4.18. Western Blotting:

4X Laemmli sample buffer (BioRad) with 5% (v/v) 2-Mercaptoethanol (Sigma-Aldrich) were added to protein samples prior to being boiled for 5 minutes. 7.5% SDS-PAGE gels were utilized and then transferred onto nitrocellulose membranes (Bio-Rad) at 100 V for 90 minutes. Membranes were blocked with 5% milk in TBST for one hour at room temperature, followed by primary antibody incubation overnight at 4°C. Membranes were washed in TBS + 0.1% Tween before incubation with horseradish-peroxidase (HRP)-conjugated secondary antibodies at a concentration of 1:3000 (BioRad) for 1 hour at room temperature. Excess secondary antibody was washed from the membrane prior to utilizing Clarity Western ECL Substrate (BioRad) for detection of chemiluminescent signal. Film (Fujifilm) was used to image the western blots which were subsequently quantified by densitometry using ImageJ.

Primary antibody dilutions are as follows -

Primary Antibody	Dilution
Anti-p21 [EPR3993] (AbCam)	1:500
Anti-H3K9me3 [D4W1U] (Cell Signalling Technology)	1:1000
Anti-SMARCA2 [A301-015A] (Bethyl Laboratories, Inc.)	1:100
Anti-Beta Actin (Santa Cruz Biotechnology, Inc.)	1:10000
Anti-Beta Tubulin (Licor)	1:7000

3.4.19. Histone Mark Immunofluorescence:

Cells adherent to coverslips were fixed in 4% PFA in 1x PBS for 10 minutes, permeabilized in 0.5% Triton X-100 in PBS for 5 min and then stained with primary antibodies diluted in 1x PBS. After incubation of the primary antibodies for 30 minutes at room temperature the cells were washed 1x with 0.1% Triton X-100 in PBS for 1min then rinsed 3x with 1x PBS. Then the cells were incubated for 30 min at room temperature with the secondary antibodies that were diluted in 1x PBS. After another washing step of 0.1% Triton X-100 in PBS for 1 min and three rinses with 1x PBS the coverslips were mounted onto microscope slides with in-house made PVA mounting media containing 1 µg/ml DAPI. Primary antibodies: αH3K9me3 Active Motif 3916 (1:1000); Secondary antibodies: Goat anti rabbit Alexa 488 (1:500). Images were captured using a Zeiss LSM 710 Meta Confocal Microscope (Carl Zeiss, Germany) with the pinhole diameter set to 1 airy unit for all channels and the exposure gains kept constant for acquisition of all samples. Imaris (v9.2.1) was used to analyze confocal images.

3.4.20. Xenograft Formation, Tumor Dissociation & Serial Passaging:

3 million cells were mixed in 100 microlitres MEM and Matrigel (Corning) in a one to one ratio and were injected subcutaneously over both the left and right flank of 7-8 week old female NSG mice. Tumors of 10 mm in size were halved with a portion fixed and embedded in paraffin for histology and immunohistochemical staining (University of Alberta Lab Medicine and Pathology Core Facility). The remainder of the tumors were either flash frozen and stored a -80°C for future RNA or protein extraction or dissociated according to the MACS Human Tumor Dissociation Kit (Miltenyi Biotec) with the only modification being the elimination of the final strainer wash. The gentleMACS Dissociator

(Miltenyi Biotec) program utilized was for the dissociation of tough tumors named 37C_h_TDK_3. Dissociated tumor cells were then reinjected as described above.

3.4.21. In vivo Studies:

Animal experiments were carried out in accordance with the guidelines laid out by the Canadian Council on Animal Care and were approved by the University of Alberta Animal Policy and Welfare Committee. HEC 116 Plasmid Derived SMARCA4 KO and WT PDX tumor sections approximately 2 mm in diameter, were expanded from tumors generated by serial passaging and collected at the third generation. A single section was implanted in subcutaneously into 6-8 week-old female NOD.Cg-PrkdcscidIl2rg (NSG) mice. Fourteen days post-implantation, mice were randomized into treatment groups: either vehicle (PBS) or carboplatin (Cayman Chemicals). Treatment (60 mg/kg by intravenous (IV)) was continued weekly until tumors reached a final diameter of 10 mm. Measurements of tumor size were carried out twice a week as well. Digital calipers were utilized to measure the length and width of tumors to calculate tumor area over time. Mice were euthanized at experimental endpoint when tumor size equaled approximately 60 mm^2. Tumors were subjected to weighing post excision and split either to be flash-frozen and stored at -80°C or fixed in formalin for eventual paraffin embedding and sectioning.

3.4.22. Single Cell RNA Sequencing Using the 10X Platform:

Multiple F2 generation serially passaged tumors were dissociated as outlined in section 3.4.20. Prior to storage at -80°C, HEC 116 Plasmid Derived SMARCA4 KO and WT tumor cells were depleted of mouse and dead cells using the MACS Mouse Cell Depletion Kit and MACS Dead Cell Removal Kit (Miltenyi Biotec), respectively.

3.4.22A. Library Construction:

Single cell suspensions cells of either unpassaged or serially passaged tumor cells were prepared in 0.04% PBS-BSA with greater than 95% viability to a concentration of 1,000 cells/mL. Cell suspensions were then used to generate the gel bead-in emulsions, reverse transcription, cDNA amplification, library preparation and sample indexing following the latest available manufacturer's protocol, using the 10X Chromium Controller (PN-1000202), the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3 (PN-1000075), the Chromium Next GEM Chip B Single Cell Kit (PN-1000073) and the Single Index Chromium i7 Multiplex Kit, (PN-120262).

3.4.22B. Single Cell Sequencing Using the 10X Platform:

Samples were pooled in a molar concentration such that when loaded into Illumina NovaSeq S4, it produced paired-end and single-indexing sequences at an average depth of ~25,000 reads per cell.

3.4.22C. Data Analysis:

Illumina sequencing outputs were used to generate demultiplexed FASTQ files. Then the sequences were aligned against the human reference genome (10X Genomics, hg19) using the standard 'cellranger count' pipeline on CellRanger software version 3.1.0 (10X Genomics). Downstream analyses were performed with Seurat (v3.9.9.9038) using cells with more than 250 genes and less than 20% mitochondrial reads. Regression for cell-cycle effects was also executed. Non-linear dimensional reduction was performed using the UMAP method at a resolution of 0.5.

3.4.23. Single Cell ATAC Sequencing Using the 10X Platform:

Multiple F2 generation serially passaged tumors were dissociated as outlined in section 3.4.20. Prior to storage at -80°C, HEC 116 Plasmid Derived SMARCA4 KO and WT tumor cells were depleted of mouse and dead cells using the MACS Mouse Cell Depletion Kit and MACS Dead Cell Removal Kit (Miltenyi Biotec), respectively.

3.4.23A. Nuclei Isolation:

Nuclei suspensions were isolated, washed and counted according to the Demonstrated Protocol: Nuclei Isolation for Single Cell ATAC Sequencing (10X Genomics). 500,000 cells either unpassaged or serially passaged tumor cells were pelleted and lysed on ice for a maximum of 3 minutes with Lysis Buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20, 0.1% Nonidet P40 Substitute, 0.01% digitonin and 1% BSA). Upon the conclusion of the optimized lysis incubation time, Wash Buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl, 0.1% Tween-20 and 1% BSA) was added and the nuclei pelleted at 500 g for 5 min at 4°C. Nuclei were then resuspended in Diluted Nuclei Buffer (10X Genomics) at greater than approximately 5000 nuclei/microlitre and the resulting nuclei concentration confirmed with a Countess Counter (Invitrogen).

3.4.23B. Library Construction:

scATAC-seq libraries were prepared in accordance with the Chromium Single Cell ATAC Reagent Kits User Guide (10X Genomics) by the University of Alberta, Faculty of Medicine and Dentistry, High Content Core Facility. Nuclei suspensions were incubated with transposase mix to fragment DNA and simultaneously, add the adapter sequences to the ends of the DNA fragments. These

nuclei were then used to generate the gel bead-in emulsions, nuclei barcoding and the Illumina® P5 sequence Read 1 (Read 1N) sequence. Finally, the Illumina® P7 and a sample index were added during library construction via PCR. All the steps were performed following the latest available manufacturer's protocol, using the 10X Chromium Controller (PN-1000202), the Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1 (PN- 1000176), the Chromium Next GEM Chip H Single Cell Kit (PN- 1000162) and the Single Index Plate N Set A(PN-3000427).

3.4.23C. Single Cell Sequencing Using the 10X Platform:

Samples were pooled in a molar concentration such that when loaded into Illumina NovaSeq S4, it produced paired-end and single-indexing sequences at an average depth of ~37,500 reads per nuclei.

3.4.23D. Data Analysis:

Illumina sequencing outputs were used to generate demultiplexed FASTQ files. Then the sequences were aligned against the human reference genome (10X Genomics, hg19) using the standard 'cellranger count' pipeline on CellRanger software version 3.1.0 (10X Genomics). Downstream analyses were performed with ArchR (v1.0.1) following the online manual: https://www.archrproject.com/bookdown/index.html.

3.5. References

1. Tessier-Cloutier B, Coatham, M., Carey, M., Nelson, GC., Hamilton, S., Lum, A., Soslow, RA., Stewart, CJR., Postovit, LM., Koebel, M., Lee, C-H. SWI/SNF-deficiency defines highly aggressive undifferentiated endometrial carcinoma. J Pathol Clin Res. 2020.
2. Gaj T, Sirk SJ, Shui SL, Liu J. Genome-Editing Technologies: Principles and Applications. Cold Spring Harbor perspectives in biology. 2016;8(12).
3. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020;5(1):1.

4. Rouet P, Smih F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci U S A. 1994;91(13):6064-8.
5. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol. 2018;36(8):765-71.
6. O'Driscoll M, Jeggo, PA. The role of double-strand break repair—insights from human genetics. Nat Rev Genet. 2006;7(1):45-54.
7. Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol. 2017;18(8):495-506.
8. Kim H, Kim, J-S. A guide to genome engineering with programmable nucleases. Nature Reviews Genetics. 2014;15:321-34.
9. Jiang W, Bikard, D., Cox, D., Zhang, F., Marraffini, LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature Biotechnology. 2013;31:233-9.
10. Cong L, Ran, FA., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, PD., Wu, Z., Jiang, W., Marraffini, LA., Zhang, F. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819-23.
11. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.
12. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823-6.
13. Van Campenhout C, Cabochette, P., Veillard, A-C., Laczik, M., Zelisko-Schmidt, A., Sabatel, C., Dhainaut, M., Vanhollebeke, B., Gueydan, C., Kruys, V. Guidelines for optimized gene knockout using CRISPR/Cas9. BioTechniques. 2019;66(6).
14. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet. 2013;45(10):1113-20.
15. Karnezis AN, Wang, Y., Ramos, P., Hendricks, W.Pd., Oliva, E., D'Angelo, E., Prat, J., Nucci, M.R., Nielsen, T.O., Chow, C., Leung, S., Kommoss, F., Kommoss, S., Silva, A., Ronnett, B.M., Rabban, J.T., Bowtell, D.D., Weissman, B.E., Trent, J.M., Gilks, C.B., Huntsman, D.G. Dual Loss of the SWI/SNF Complex ATPases SMARCA4/BRG1 and SMARCA2/BRM Is Highly Sensitive and Specific for Small Cell Carcinoma of the Ovary, Hypercalcaemic Type. J Pathol. 2016;238(3):389-400.
16. Coatham M, Li X, Karnezis AN, Hoang LN, Tessier-Cloutier B, Meng B, et al. Concurrent ARID1A and ARID1B inactivation in endometrial and ovarian dedifferentiated carcinomas. Mod Pathol. 2016;29(12):1586-93.
17. Brunner AH, Riss P, Heinze G, Meltzow E, Brustmann H. Immunoexpression of PAX 8 in endometrial cancer: relation to high-grade carcinoma and p53. Int J Gynecol Pathol. 2011;30(6):569-75.
18. Findlay SD, Vincent KM, Berman JR, Postovit LM. A Digital PCR-Based Method for Efficient and Highly Specific Screening of Genome Edited Cells. PloS one. 2016;11(4):e0153901.
19. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

20. Hamburger A, Salmon, SE. Primary bioassay of human tumor stem cells. Science. 1977;197:461-3.
21. Horibata S, Vo TV, Subramanian V, Thompson PR, Coonrod SA. Utilization of the Soft Agar Colony Formation Assay to Identify Inhibitors of Tumorigenicity in Breast Cancer Cells. J Vis Exp. 2015(99):e52727.
22. Onder S, Taskin OC, Sen F, Topuz S, Kucucuk S, Sozen H, et al. High expression of SALL4 and fascin, and loss of E-cadherin expression in undifferentiated/dedifferentiated carcinomas of the endometrium: An immunohistochemical and clinicopathologic study. Medicine (Baltimore). 2017;96(10):e6248.
23. Meng E, Mitra A, Tripathi K, Finan MA, Scalici J, McClellan S, et al. ALDH1A1 maintains ovarian cancer stem cell-like properties by altered regulation of cell cycle checkpoint and DNA repair network signaling. PloS one. 2014;9(9):e107142.
24. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget. 2016;7(10):11018-32.
25. Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. PLoS Biol. 2020;18(1):e3000599.
26. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol. 2013;75:685-705.
27. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130(2):223-33.
28. Paluvai H, Di Giorgio E, Brancolini C. The Histone Code of Senescence. Cells-Basel. 2020;9(2).
29. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell. 2003;113(6):703-16.
30. Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, et al. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell. 2006;126(3):503-14.
31. Miller WR. Effects of serial passage on the endocrine response and steroid metabolism of a rat mammary carcinoma. Br J Cancer. 1980;42(2):326-30.
32. Leibovici J. Serial passage of tumors in mice in the study of tumor progression and testing of antineoplastic drugs. Cancer Res. 1984;44(5):1981-4.
33. Morton JP, Timpson P, Karim SA, Ridgway RA, Athineos D, Doyle B, et al. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci U S A. 2010;107(1):246-51.
34. Herpel E, Rieker RJ, Dienemann H, Muley T, Meister M, Hartmann A, et al. SMARCA4 and SMARCA2 deficiency in non-small cell lung cancer: immunohistochemical survey of 316 consecutive specimens. Ann Diagn Pathol. 2017;26:47-51.
35. Wilson BG, Helming KC, Wang X, Kim Y, Vazquez F, Jagani Z, et al. Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. Mol Cell Biol. 2014;34(6):1136-44.

36. Hayford CE, Tyson DR, Robbins CJ, 3rd, Frick PL, Quaranta V, Harris LA. An in vitro model of tumor heterogeneity resolves genetic, epigenetic, and stochastic sources of cell state variability. PLoS Biol. 2021;19(6):e3000797.
37. Schep AN, Wu B, Buenrostro JD, Greenleaf WJ. chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data. Nat Methods. 2017;14(10):975-8.
38. Granja J, Corces, MR., Pierce, SE., Bagdatli, T., Choudhry, H., Change HY, Greenleaf, WJ. . ArchR is a scalable software package for integrative single-cell chromatin accessibility analysis. Nature Genetics. 2021;53:403-11.
39. Kuramoto H, Nishida M, Morisawa T, Hamano M, Hata H, Kato Y, et al. Establishment and characterization of human endometrial cancer cell lines. Ann N Y Acad Sci. 1991;622:402-21.
40. Markowska A, Pawalowska M, Lubin J, Markowska J. Signalling pathways in endometrial cancer. Contemp Oncol (Pozn). 2014;18(3):143-8.
41. Tierney B, McCann, GA., Naidu, S., Rath, KS., Saini, U., Wanner, R., Kuppusamy, P., Suarez, A., Goodfellow, PJ., Cohn, DE., Selvendiran, K. Aberrantly activated pSTAT3-Ser727 in human endometrial cancer is suppressed by HO-3867, a novel STAT3 inhibitor. Gynecologic Oncology. 2014;135(1):133-41.
42. Kohlberger P, Gitsch, G., Loesch, A., Tempfer, C., Kaider, A., Reinthaller, A., Kainz, C., Breitenecker, G. p53 protein overexpression in early stage endometrial cancer Gynecol Oncol. 1996;62:213-7.
43. Yemelyanova A, Gown AM, Wu LS, Holmes BJ, Ronnett BM, Vang R. PAX8 expression in uterine adenocarcinomas and mesonephric proliferations. Int J Gynecol Pathol. 2014;33(5):492-9.
44. Bourgo RJ, Siddiqui H, Fox S, Solomon D, Sansam CG, Yaniv M, et al. SWI/SNF deficiency results in aberrant chromatin organization, mitotic failure, and diminished proliferative capacity. Mol Biol Cell. 2009;20(14):3192-9.
45. Cohet N, Stewart KM, Mudhasani R, Asirvatham AJ, Mallappa C, Imbalzano KM, et al. SWI/SNF chromatin remodeling enzyme ATPases promote cell proliferation in normal mammary epithelial cells. J Cell Physiol. 2010;223(3):667-78.
46. Padilla-Benavides T, Nasipak BT, Imbalzano AN. Brg1 Controls the Expression of Pax7 to Promote Viability and Proliferation of Mouse Primary Myoblasts. J Cell Physiol. 2015;230(12):2990-7.
47. Wu Q, Madany P, Akech J, Dobson JR, Douthwright S, Browne G, et al. The SWI/SNF ATPases Are Required for Triple Negative Breast Cancer Cell Proliferation. J Cell Physiol. 2015;230(11):2683-94.
48. Hoare M, Narita, M. The Power Behind the Throne: Senescence and the Hallmarks of Cancer. Annual review of cancer biology. 2018;2:175-94.
49. Hoang LN, Lee YS, Karnezis AN, Tessier-Cloutier B, Almandani N, Coatham M, et al. Immunophenotypic features of dedifferentiated endometrial carcinoma - insights from BRG1/INI1-deficient tumours. Histopathology. 2016;69(4):560-9.
50. Romero-Perez L, Lopez-Garcia, MA., Diaz-Martin, J., Biscuola, M., Castilla, MA., Tafe, LJ., Garg, K., Oliva, E., Matias-Guiu, X., Soslow, RA., Palacios, J. . ZEB1 overexpression associated with E-cadherin and microRNA-200 downregulation is characteristic of undifferentiated endometrial carcinoma. Mod Pathol. 2013;26(11).

51. Barutcu AR, Lajoie BR, Fritz AJ, McCord RP, Nickerson JA, van Wijnen AJ, et al. SMARCA4 regulates gene expression and higher-order chromatin structure in proliferating mammary epithelial cells. Genome Res. 2016;26(9):1188-201.
52. Milanovic M, Fan DNY, Belenki D, Dabritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature. 2018;553(7686):96-100.
53. Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature. 2008;453(7193):306-13.
54. Naidu S, Love, IM., Imbalzano, AN., Grossman, SR., Androphy, EJ. The SWI/SNF chromatin remodeling subunit BRG1 is a critical regulator of p53 necessary for proliferation of malignant cells. Oncogene. 2009;28:2492-501.
55. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature. 2000;406(6796):593-9.
56. Kumari R, Jat P. Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype. Front Cell Dev Biol. 2021;9:645593.
57. Alessio N, Squillaro, T., Cipollaro, M., Bagella, L., Giordano, A., Galderisi, U. The BRG1 ATPase of chromatin remodeling complexes is involved in modulation of mesenchymal stem cell senescence through RB-P53 pathways. Oncogene. 2010;29(40):5452-63.
58. Sen P, Lan Y, Li CY, Sidoli S, Donahue G, Dou Z, et al. Histone Acetyltransferase p300 Induces De Novo Super-Enhancers to Drive Cellular Senescence. Mol Cell. 2019;73(4):684-98 e8.
59. Jin J, Xu Y, Huo L, Ma L, Scott AW, Pizzi MP, et al. An improved strategy for CRISPR/Cas9 gene knockout and subsequent wildtype and mutant gene rescue. PloS one. 2020;15(2):e0228910.
60. Tessier-Cloutier B, Soslow RA, Stewart CJR, Kobel M, Lee CH. Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas. Histopathology. 2018;73(2):299-305.
61. Ouchi R, Okabe S, Migita T, Nakano I, Seimiya H. Senescence from glioma stem cell differentiation promotes tumor growth. Biochem Biophys Res Commun. 2016;470(2):275-81.
62. Orlando K, Douglas, AK, Abudu, A., Wang, Y., Tessier-Cloutier, B., Su, W., Peters, A., Sherman, LS., Moore, R. Re-expression of SMARCA4/BRG1 in small cell carcinoma of ovary, hypercalcemic type (SCCOHT) promotes an epithelial-like gene signature through an AP-1-dependent mechanism. eLife. 2020.
63. Vierbuchen T, Ling E, Cowley CJ, Couch CH, Wang X, Harmin DA, et al. AP-1 Transcription Factors and the BAF Complex Mediate Signal-Dependent Enhancer Selection. Mol Cell. 2017;68(6):1067-82 e12.
64. Michel B, D'Avino, AR., Cassel, SH., Mashtalir, N., McKenzie, ZM., McBride, MJ., Valencia, AM., Zhou, Q., Bocker, M., Soares, LMM., Pan, J., Remillard, DI., Lareau, CA., Zullow, HJ., Fortoul, N., Gray, NS., Bradner, JE., Chan, HM., Kadoch, C. A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation. Nature Cell Biology. 2018;20:1410-20.
65. Xue Y, Meehan, B., Fu, Z., Wang, XQD., Fiset, PO., Rieker, R., Levins, C., Kong, T., Zhu, X., Morin, G., Skerritt, L., Herpel, E., Venneti, S., Martinez, D., Judkins, AR., Jung, S., Camilleri-Broet, S., Gonzalez, AV., Guiot, M-C., Lockwood, WW., Spicer, JD.,

Agaimy, A., Pastor, WA., Dostie, J., Rak, J., Foulkes, WD., Huang, S. SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer. Nature communications. 2019;10(557).
66. Fane ME, Chhabra Y, Smith AG, Sturm RA. BRN2, a POUerful driver of melanoma phenotype switching and metastasis. Pigment Cell Melanoma Res. 2019;32(1):9-24.
67. Pinner S, Jordan P, Sharrock K, Bazley L, Collinson L, Marais R, et al. Intravital imaging reveals transient changes in pigment production and Brn2 expression during metastatic melanoma dissemination. Cancer Res. 2009;69(20):7969-77.
68. Janssen SM, Moscona R, Elchebly M, Papadakis AI, Redpath M, Wang H, et al. BORIS/CTCFL promotes a switch from a proliferative towards an invasive phenotype in melanoma cells. Cell Death Discov. 2020;6:1.
69. Liu Q, Chen K, Liu Z, Huang Y, Zhao R, Wei L, et al. BORIS up-regulates OCT4 via histone methylation to promote cancer stem cell-like properties in human liver cancer cells. Cancer letters. 2017;403:165-74.
70. Kitami K, Yoshihara M, Koya Y, Sugiyama M, Iyoshi S, Uno K, et al. Microphthalmia-Associated Transcription Factor-Dependent Melanoma Cell Adhesion Molecule Activation Promotes Peritoneal Metastasis of Ovarian Cancer. Int J Mol Sci. 2020;21(24).
71. Rao A, Barkley, D., Franca, GS., Yanai, I. Exploring tissue architecture using spatial transcriptomics. Nature. 2021;596:211-20.
72. Kobel M, Meng, B., Hoang, L.N., Almadani, N., Li, X., Soslow, R.A., Gilks, C.B., Lee, C.H. . Molecular Analysis of Mixed Endometrial Carcinomas Shows Clonality in Most Cases. Am J Surg Pathol. 2016;40(2):166-80.
73. Hoang L, Han, G., McConechy, M., Lau, S., Chow, C., Blake Gilks, C., Huntsman, DG., Kobel, M., Lee, CH. Immunohistochemical characterization of prototypical endometrial clear cell carcinoma-diagnostic utility of HNF-1beta and oestrogen receptor. Histopathology. 2014;64(6):585-96.
74. Hoang LN, McConechy MK, Meng B, McIntyre JB, Ewanowich C, Gilks CB, et al. Targeted mutation analysis of endometrial clear cell carcinoma. Histopathology. 2015;66(5):664-74.
75. Dieters-Castator DZ, Rambau PF, Kelemen LE, Siegers GM, Lajoie GA, Postovit LM, et al. Proteomics-Derived Biomarker Panel Improves Diagnostic Precision to Classify Endometrioid and High-grade Serous Ovarian Carcinoma. Clin Cancer Res. 2019;25(14):4309-19.
76. Dutta I, Dieters-Castator D, Papatzimas JW, Medina A, Schueler J, Derksen DJ, et al. ADAM protease inhibition overcomes resistance of breast cancer stem-like cells to gammadelta T cell immunotherapy. Cancer letters. 2021;496:156-68.

4
Treatment of cell line models of DDEC with a synthetic lethality based approach

4.1. Introduction

Currently accounting for only 2% of endometrial carcinomas overall likely due to under recognition, DDEC has continuously demonstrated itself to be clinically aggressive (1). Despite the unusually poor prognosis of this subtype of endometrial cancer, less toxic therapeutic more effective options have not been implemented and vulnerabilities unique to DDEC have not been revealed and targeted. Advances in sequencing technologies and analysis of numerous human tumors resulted in the discovery of many clinically successful genetically targeted cancer therapies (2-6). For example, drugs that target EGFR mutations in NSCLC and amplification of ERBB2 in breast cancer have led to increased progression free-survival in many patients (6-17). Despite noted successes, partial responses to targeted therapy are an issue in select patient populations that only combination regimens may begin to address (6). Furthermore, while many oncogenes that arise because of genetic alterations have been discovered by DNA sequencing, few are considered druggable (18-20). In recent years, the impact of harnessing the immune system to treat cancer has surpassed the enthusiasm for targeted cancer therapy (6). The success in terms of clinically meaningful responses in for example, NSCLC patients, to checkpoint inhibitors has been enough to dampen interest for targeted therapies, but it is unclear whether immunotherapies will produce the long-term responses desired by cancer patients (6, 21-23). To keep improving targeted cancer treatments for people, strategies that attempt to overcome barriers in targeting lost tumor suppressor genes and driver genes untouched by genetic alterations known as unmarked oncogenes will need to be explored further (6). Synthetic lethality (SL) together with functional genomic

screening could be effective in identifying the next generation of effective cancer drugs alone or in combination. SL occurs when the loss of two genes leads to cell death but inactivation of either gene has little impact on the viability of cells (6). SL can also be achieved if one gene is inactivated either through deletion or mutation and the other is inhibited pharmacologically (6). SL has quickly emerged as an approach to identifying therapies that could kill cancer cells with a driver mutation that is absent in normal cells, thus sparing non-transformed cells from cell death (6). RNA interference (RNAi) technology allowed for the systemic identification of synthetic lethal gene pairs in human cancer cell lines (24-29). One of the most clinically successful applications of SL in the context of tumor suppressor gene loss is the use of PARP inhibitors in BRCA-mutant ovarian cancers (30-34). Screening using small interfering RNA (siRNA) has led to the discovery that chromatin remodeling complex members SMARCA4, SMARCA2, ARID1A and ARID1B under certain genetic contexts act as synthetic lethal pairs (24-27). In NSCLC, a SMARCA4 deficient neoplasm, several synthetic lethal interactions have been uncovered besides SMARCA2 including, EZH2 and Aurora kinase A (24, 26, 35-37). Unfortunately, these interactions are not druggable with any FDA approved inhibitors (37). Recent work by Xue *et al*., using a kinome-based RNAi-screen, demonstrated that inhibition of cyclin-dependent kinases 4/6 (CDK4/6) in NSCLC and SCCOHT is a vulnerability in these SMARCA4 deficient carcinomas (37, 38). In this context, CDK4/6 seem to be essential for neoplastic growth, such that CDK4/6 inhibition can reduce tumor progression (37). In NSCLC and SCCOHT with SMARCA4 loss, cyclin D1 expression is downregulated hinting at cell cycle dysregulation (37, 38). These two studies are promising in that they suggest SMARCA4-deficient NSCLC and SCCOHT patients may

benefit from treatment strategies incorporating CDK4/6 inhibitors that are already clinically approved to treat other carcinomas (39). It remains to be seen what additional compensatory mechanisms are activated in the absence of SMARCA4 and whether this druggable vulnerability in some SMARCA4-deficient neoplasms is shared in other tumor types lacking SWI/SNF subunits. SMARCA4 loss is not directly targetable in DDEC therefore in this chapter we employed an unbiased functional genetic screen to discover the molecular vulnerabilities present in these neoplasms. One of the isogenic cell line pairs derived in Chapter 3 wherein the DDEC phenotype can be recapitulated upon knockout of SMARCA4 served as a well-controlled system to search for druggable synthetic lethal interaction partners using RNAi-based technology. We revealed several molecular vulnerabilities in SMARCA4 deficient DDEC that were validated and effectively targeted by clinically approved inhibitors in other models of DDEC. We unveil some of the possible paradigms underlying these synthetic lethal interactions in our DDEC models. Finally, pre-clinical animal studies were carried out to begin translating the efficacy of SL treatment of DDEC-like tumors *in vivo*. This body of work provides for the first time, the possibility of rationalized and targeted therapy for SMARCA4-deficient DDEC patients and provides further insight into the signalling pathways that may be critical to the growth of dedifferentiated cancers.

4.2. Results

4.2.1. Cell line models of DDEC derived by CRISPR knockout of SMARCA4 exhibit variable responses to current standard of care chemotherapeutics for the treatment of endometrial cancer.

Examination of advanced stage SMARCA4 deficient DDEC patients has revealed that these neoplasms are refractory to conventional chemotherapy regimens with disease recurrence occurring within 4 months of completion or even during treatment (40). We examined then, the viability of three different cell line models of DDEC in the presence of increasing concentrations of carboplatin, one of two commonly administered chemotherapeutic options for patients with aggressive endometrial carcinomas (41) **[Figure 4.1].** Similar half maximal inhibitory concentration (IC_{50}) values regardless of SMARCA4 status were observed for Plasmid Derived HEC 116 (SMARCA4 KO and WT IC_{50}= 11 μM) and RNP Derived HEC 59 EC cells (SMARCA4 KO IC_{50}= 9 μM, SMARCA4 WT IC_{50}= 15 μM) when treated with carboplatin **[Figure 4.1A & Figure 4.1C & Supplemental Table 4.1A]**. In contrast, SMARCA4 deficient HEC 116 EC cells that were RNP Derived (IC_{50}= 12 μM) were more susceptible to carboplatin treatment than SMARCA4 intact HEC 116 cells (RNP Derived IC_{50}= 19 μM) as reflected by IC_{50} values **[Figure 4.1B & Supplemental Table 4.1A]**. Application of carboplatin to HEC 116 plasmid derived cultured tumor cells that had undergone several rounds of serial passaging, did not result in altered carboplatin susceptibility from their unpassaged counterparts **[Figure 4.2]**. Cultured CDX tumor cells that did not possess SMARCA4 (IC_{50}= 9 μM) were more sensitive to carboplatin treatment than SMARCA4 proficient

tumor cells (IC_{50}= 13 μM) as tested by viability assays **[Figure 4.2 & Supplemental Table 4.1B]**.

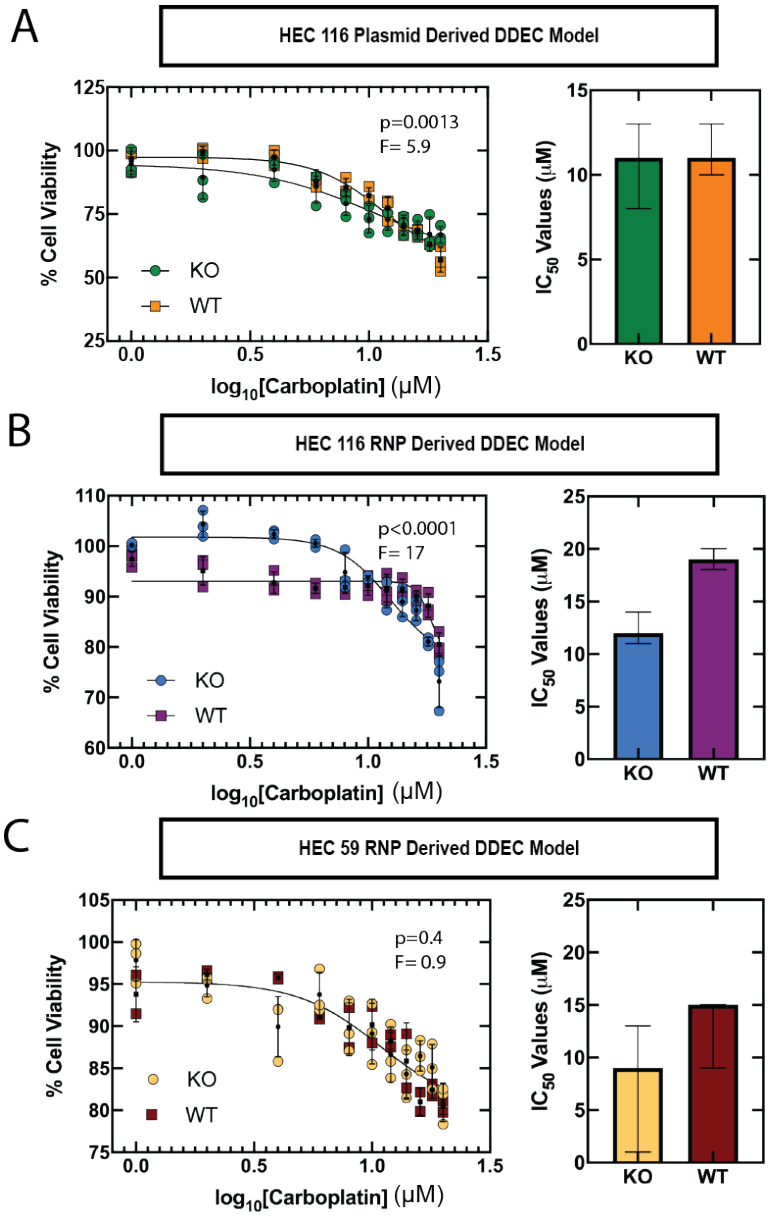


Figure 4.1. Models of DDEC generated by CRISPR gene editing to HEC 116 EC cells are sensitive to carboplatin treatment. Cell viability assays with **A)** HEC 116 Plasmid Derived **B)** HEC 116 RNP Derived and **C)** HEC 59 RNP Derived EC cell lines. SMARCA4 KO and WT cell lines were treated with increasing concentrations of carboplatin (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

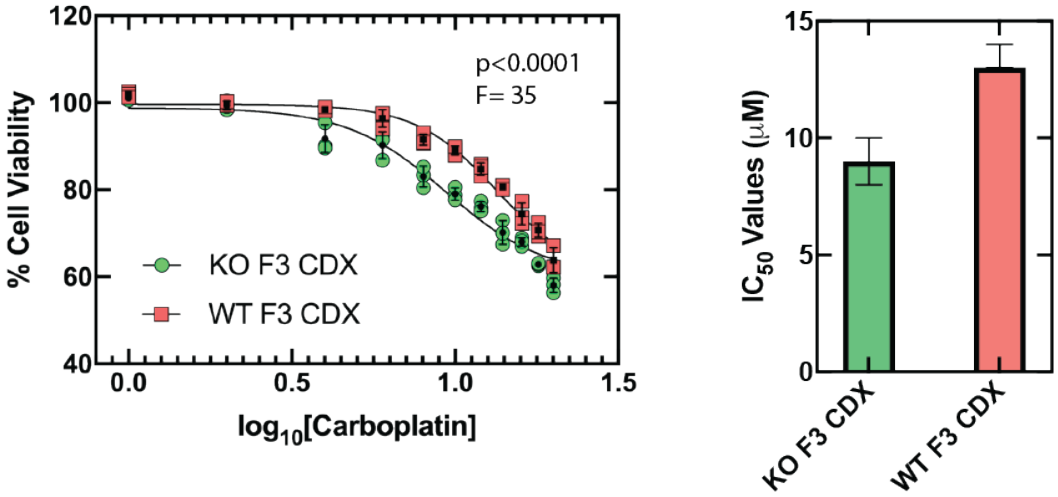


Figure 4.2. Serially passaged tumor cells from a model of DDEC generated by CRISPR gene editing are sensitive to carboplatin treatment. Cell viability assay with third generation HEC 116 Plasmid Derived SMARCA4 knockout and wildtype EC cell lines that had been cultured *in vitro*. These tumor-derived cell lines were treated with increasing concentrations of carboplatin (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

Longer term colony forming experiments were carried out to examine the response of cell line models of DDEC to paclitaxel, another frequent chemotherapeutic regimen for endometrial cancer patients with fast-spreading disease (41). In contrast with the results seen for the HEC 116 Plasmid Derived model of DDEC when treated with carboplatin, SMARCA4 KO cells were significantly less susceptible to paclitaxel treatment than SMARCA4 intact EC cells **[Figure 4.3]**. This difference in sensitivity between SMARCA4 deficient and SMARCA4 wildtype cells to increasing concentrations of paclitaxel was not evident though when comparing HEC 116 plasmid derived cultured tumor cells that had

undergone several rounds of serial passaging **[Figure 4.4].** Similar susceptibility to paclitaxel application was observed regardless of the SMARCA4 status of the cultured tumor cells comprising this model of DDEC **[Figure 4.4]**.

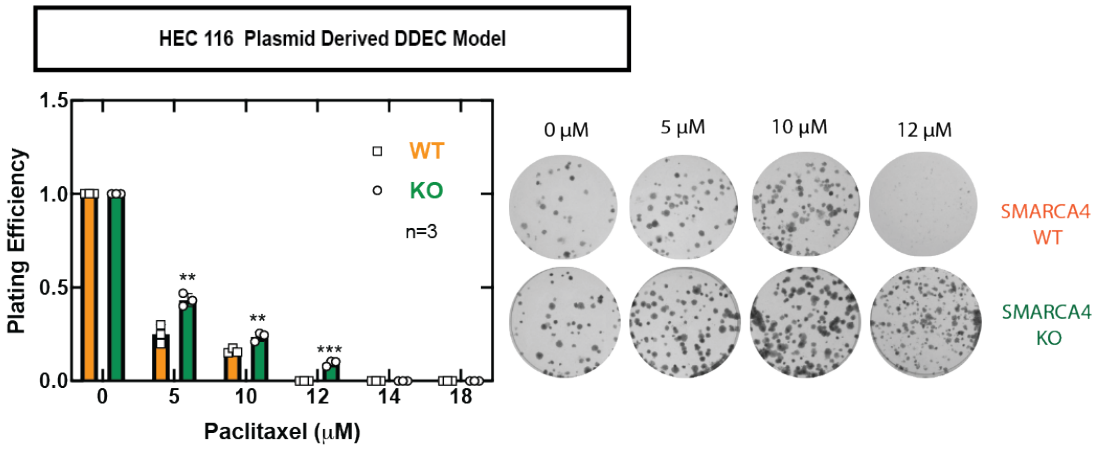


Figure 4.3. HEC 116 Plasmid Derived SMARCA4 knockout cells are more resistant to paclitaxel than HEC 116 Plasmid Derived SMARCA4 wildtype cells. Clonogenic assay with the HEC 116 Plasmid Derived cell line model of DDEC. The cell lines were cultured in the presence or absence of increasing concentrations of paclitaxel (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of three independent biological replicates depicted as white-colored shapes. Multiple t-tests confirmed the presence of any significant difference (*** = $p < 0.001$. ** = $p < 0.01$. * = $p < 0.05$).

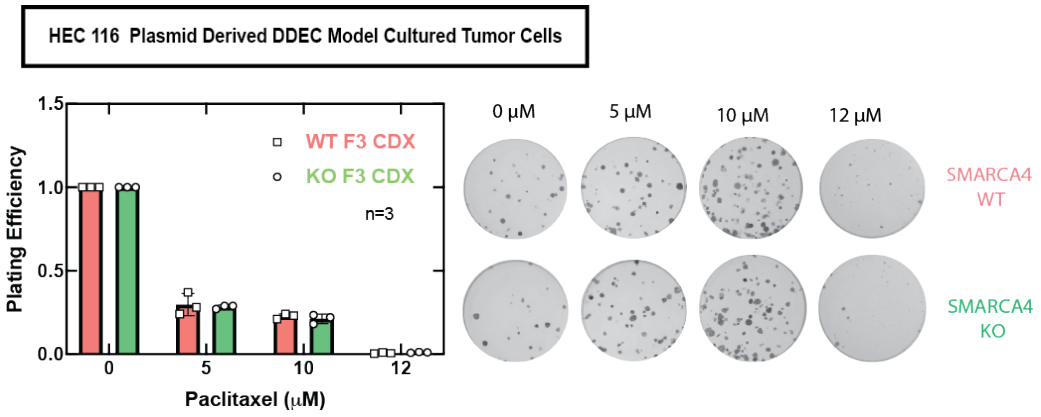


Figure 4.4. Serially passaged tumor cells from a model of DDEC generated by CRISPR gene editing are sensitive to paclitaxel treatment. Clonogenic assay with third generation HEC 116 Plasmid Derived SMARCA4 KO and WT EC cell lines that had been cultured *in vitro*. These tumor-derived cell lines were cultured in the presence or absence of increasing concentrations of paclitaxel (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of three independent biological replicates depicted as white-colored shapes.

4.2.2. RNA interference based synthetic lethality screen reveals negative gene interacting partners of SMARCA4 in DDEC.

In vitro, having only observed slight differences in response to carboplatin between cell lines models of DDEC regardless of SMARCA4 status and resistance to paclitaxel treatment with HEC 116 Plasmid Derived SMARCA4 KO cells, a genome wide short hairpin RNA (shRNA)-based screen was carried out on the SMARCA4 KO and WT HEC 116 Plasmid Derived pair of cells to examine if SL may result in personalized DDEC therapy. This isogenic pair of cells represents an excellent model of DDEC wherein SMARCA4 has been lost due to the introduction of a frameshift mutation. The incomplete gene suppression by RNAi is a more realistic mimic of chemical inhibition than full knockout. Hence, a lentiviral library containing 90,000 unique viral hairpins and representing 18,000 genes was utilized to assess thousands of digenic interactions across the two genetic backgrounds **[Figure 4.5]** (42). Microarrays were used upon infection of the cell line pairs, to ascertain what gene knockdowns caused lethality through examination of loss of associated barcodes **[Figure 4.5]** (42). We were most interested in those shRNAs lost in SMARCA4 KO HEC 116 Plasmid Derived EC cells as those interactions would be suspected to be synthetically lethal with SMARCA4 deficiency **[Figure 4.5]**.

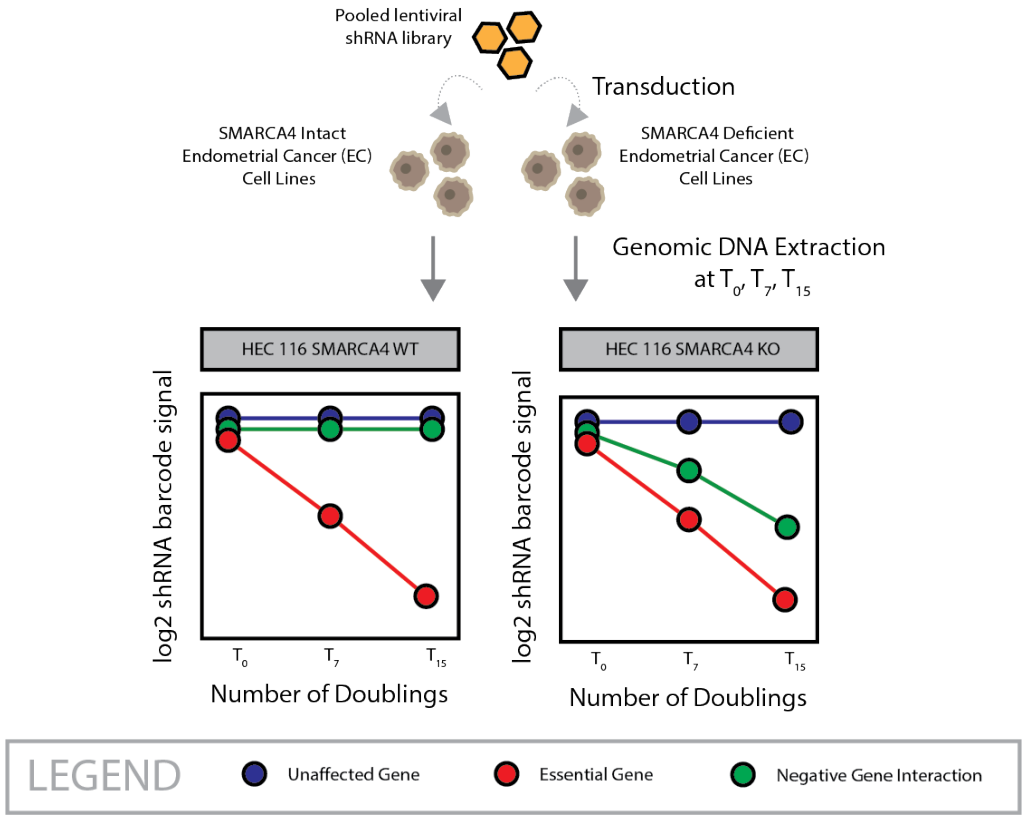
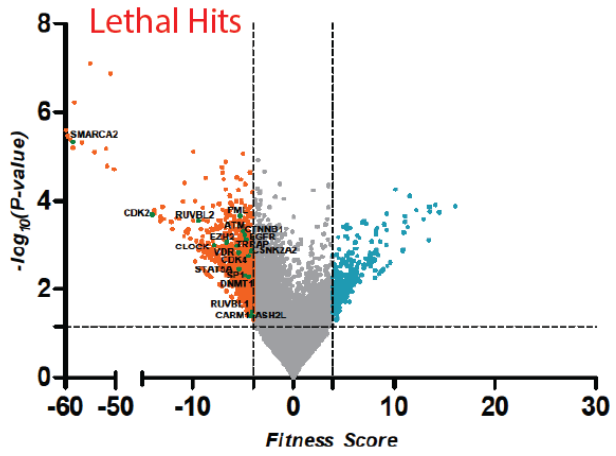


Figure 4.5. Schematic outlining the steps of the shRNA pooled screening pipeline. Quantification of the abundance of each shRNA was achieved through amplification of hairpin sequences from genomic DNA (42).

Top hits were identified by computing Difference Cumulative Change (DCC) scores based on the trend at which the hairpins dropped at different time points in the SMARCA4 deficient cells (42). Two hairpin scores per gene were utilized to increase the validity of the SL hits reported and reduce possible off-target effects (42). Using a metric that assesses the quality of genome-scale lethality screens by the degree of recall of a reference set of essential genes, our screen possessed acceptable performance scores with F-measures of 0.7 **[Supplemental Figure 4.1]** (43). Over 850 statistically significant hits were identified **[Figure 4.6A]**. A ranking of potential synthetical lethal interacting partners based on already known associations to SMARCA4 in published literature revealed several potential candidates associated with chromatin, DNA repair or cell cycle **[Figure 4.6B]**.

A



B

	Fitness Score
SMARCA2	-58
RUVBL2	-9
CTNNB1	-5
SP1	-5
EZH2	-7
RUVBL1	-4
TRRAP	-6
DNMT1	-4
CARM1	-4
CLOCK	-8
ASH2L	-4
PML	-5
ATM	-5
CDK2	-14
STAT5A	-5
EGFR	-5
VDR	-5
CSNK2A2	-4
CDK4	-5

Figure 4.6. shRNA based screen uncovers synthetic lethal interactions in cell line model of DDEC. A) Volcano plot in which significance is plotted against the DCC fitness score. Genes that met a significance threshold of $p<0.05$ and a fitness score below -2 were color-coded orange to indicate relative dropout. **B)** List of SMARCA4 associated synthetic lethal interactions in HEC 116 EC cells.

To validate the results of the SL screen, we tested multiple clinically approved inhibitors to several possible negative genetic interaction candidates (*CTNNB1*, *EZH2*, *SMARCA2*, *ATM* and *CARM1*) and found consistent with the results of the shRNA screen, greater suppression of HEC 116 Plasmid Derived SMARCA4 KO cell growth than SMARCA4 WT cell growth, when CDK4 or EGFR was targeted **[Supplementary Figures 4.2 & 4.3]**. Molecular function and KEGG pathway enrichment analyses also confirmed several kinase family members to be enriched and ErbB signalling to be significantly upregulated **[Supplementary Tables 4.2A & 4.2B]**.

4.2.3. Synthetic lethal interactions between either CDK4 and SMARCA4 or EGFR and SMARCA4 are targetable by small molecule inhibitors in all CRISPR derived cell line models of DDEC.

Both CDK4 and EGFR are well studied members of signalling pathways highly associated with disease progression in a variety of epithelial cancer types (44, 45). We modeled the SL interaction we observed between CDK4 or EGFR and SMARCA4 by chemical genetics, subjecting both HEC 116 and HEC 59 models of DDEC to increasing concentrations of CDK4 inhibitors (palbociclib and ribociclib), or EGFR inhibitors (gefitinib and erlotonib). Consistently, regardless of the CRISPR methodology used to derive the DDEC model, HEC 116 cells lacking SMARCA4 expression were more susceptible to application of CDK4 and EGFR inhibitors than their wildtype counterparts **[Figure 4.7 - Figure 4.10].** Using cell viability as a readout, IC_{50} values were determined for several clinically relevant CDK4 and EGFR inhibitors, respectively **[Figure 4.7 - Figure 4.10 & Supplemental Table 4.3A & 4.3B].** Clonogenic assays assessing colony formation in the presence of these highly selective CDK4 and EGFR inhibitors further demonstrated

that SMARCA4 KO HEC 116 cells whether plasmid or RNP derived are significantly more sensitive than HEC 116 cells with SMARCA4 intact **[Figure 4.11 - Figure 4.14]**.

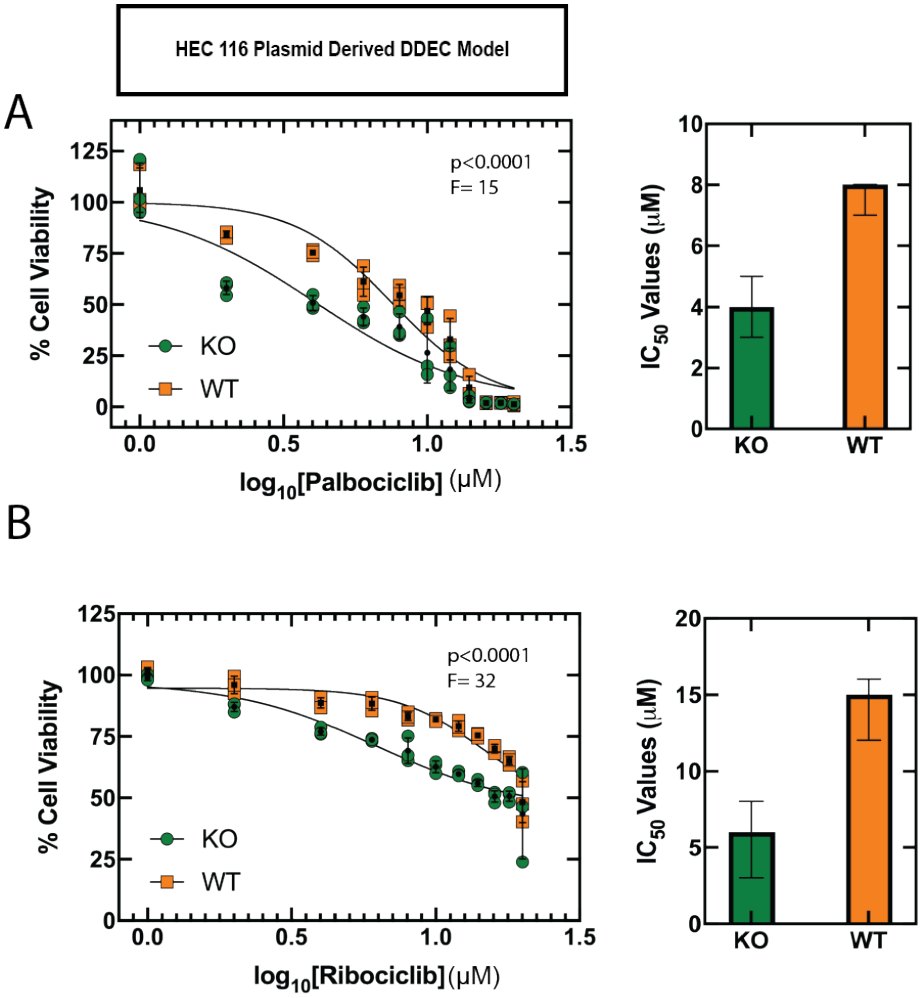


Figure 4.7. Application of CDK4 inhibitors preferentially suppresses SMARCA4 deficient HEC 116 Plasmid Derived EC cells. The viability of HEC 116 endometrial cancer cells lacking SMARCA4 expression is reduced in the presence of inhibitors against CDK4 activity such as **A)** palbociclib (SMARCA4 KO IC_{50}=4 μM vs. SMARCA4 WT IC_{50}=8 μM) and **B)** ribociclib (SMARCA4 KO IC_{50}=6 μM vs. SMARCA4 WT IC_{50}=15 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

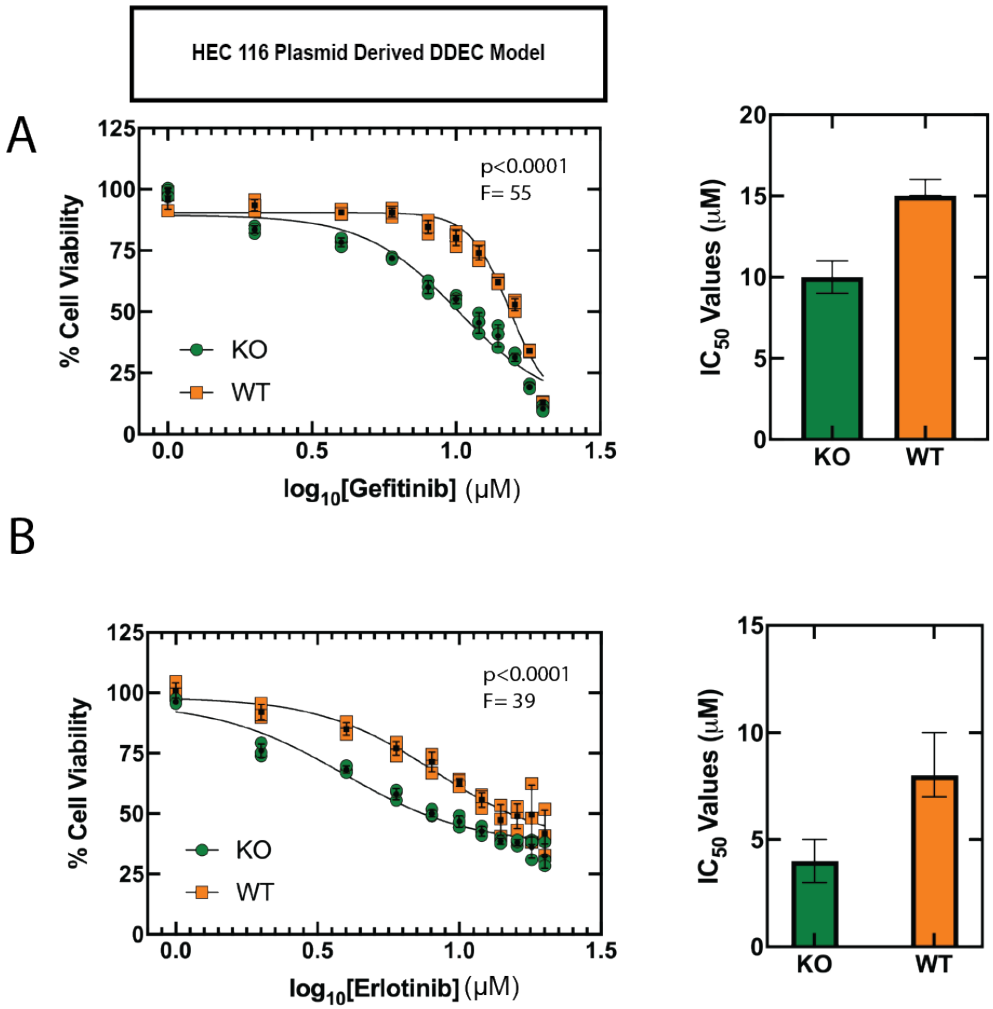


Figure 4.8. Application of EGFR inhibitors preferentially suppresses SMARCA4 deficient HEC 116 Plasmid Derived EC cells. The viability of HEC 116 endometrial cancer cells lacking SMARCA4 expression is reduced in the presence of inhibitors against EGFR activity such as **A)** gefitinib (SMARCA4 KO IC_{50}=10 μM vs. SMARCA4 WT IC_{50}=15 μM) and **B)** erlotinib (SMARCA4 KO IC_{50}=4 μM vs. SMARCA4 WT IC_{50}=8 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

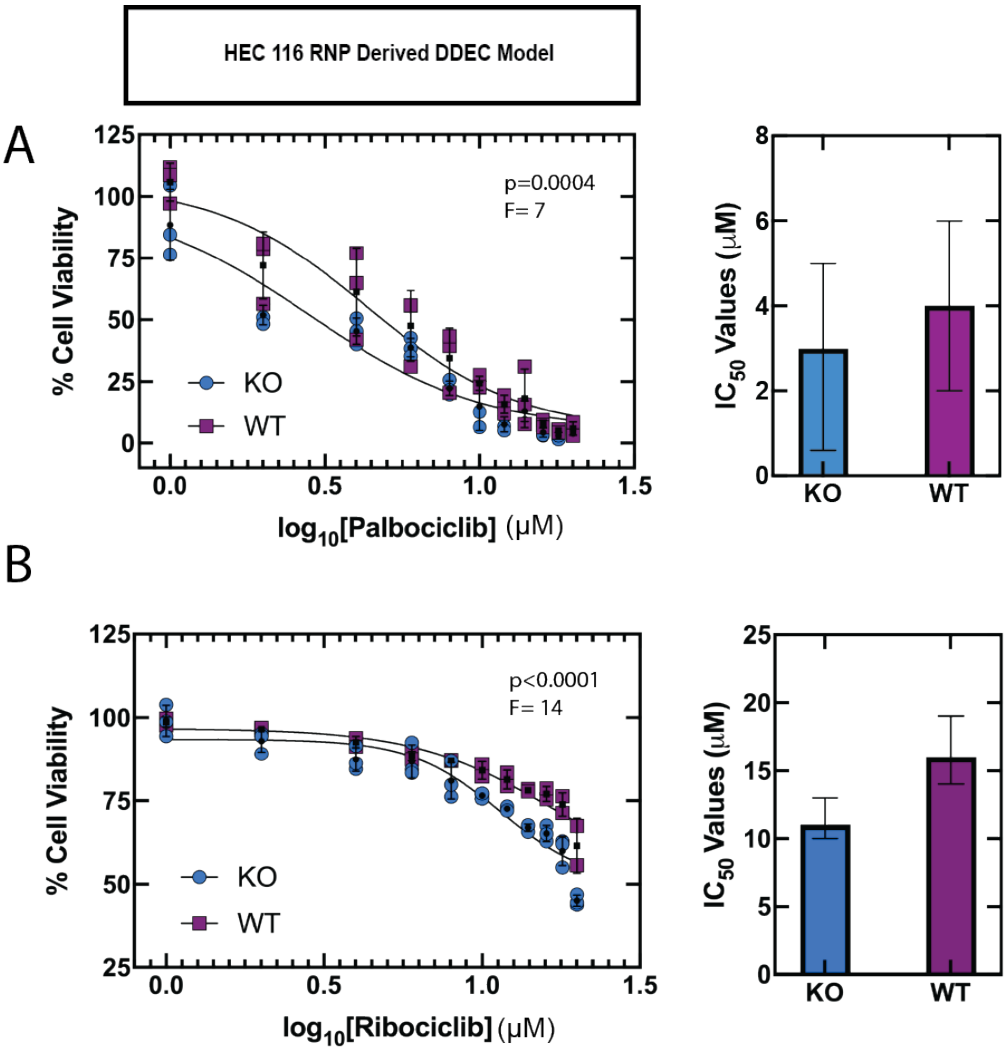


Figure 4.9. Application of CDK4 inhibitors preferentially suppresses SMARCA4 deficient HEC 116 RNP Derived EC cells. The viability of HEC 116 endometrial cancer cells lacking SMARCA4 expression is reduced in the presence of inhibitors against CDK4 activity such as **A)** palbociclib (SMARCA4 KO IC_{50}=3 μM vs. SMARCA4 WT IC_{50}=4 μM) and **B)** ribociclib (SMARCA4 KO IC_{50}=11 μM vs. SMARCA4 WT IC_{50}=16 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

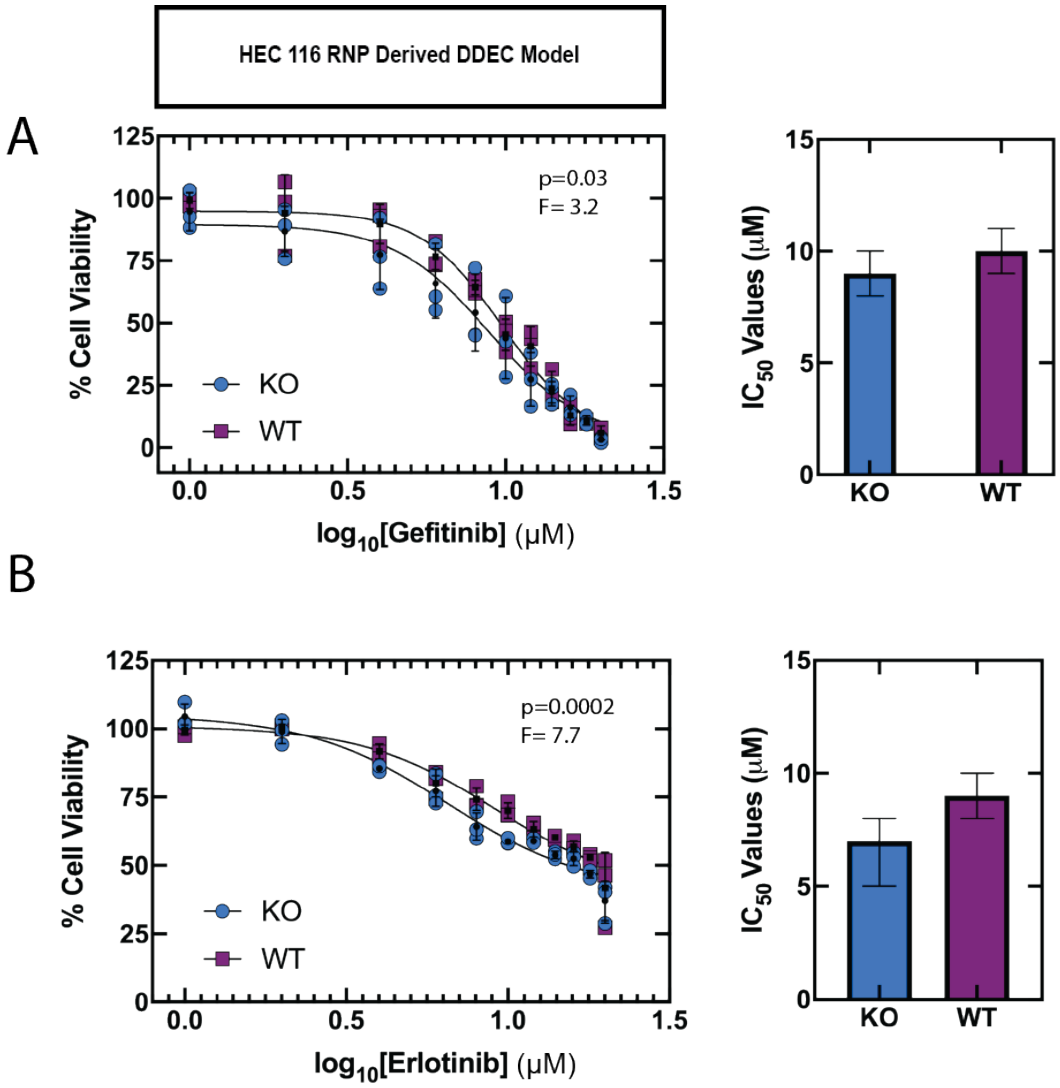


Figure 4.10. Application of EGFR inhibitors preferentially suppresses SMARCA4 deficient HEC 116 RNP Derived EC cells. The viability of HEC 116 endometrial cancer cells lacking SMARCA4 expression is reduced in the presence of inhibitors against EGFR activity such as **A)** gefitinib (SMARCA4 KO IC_{50}=7 μM vs. SMARCA4 WT IC_{50}=9 μM) and **B)** erlotinib (SMARCA4 KO IC_{50}=9 μM vs. SMARCA4 WT IC_{50}=10 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

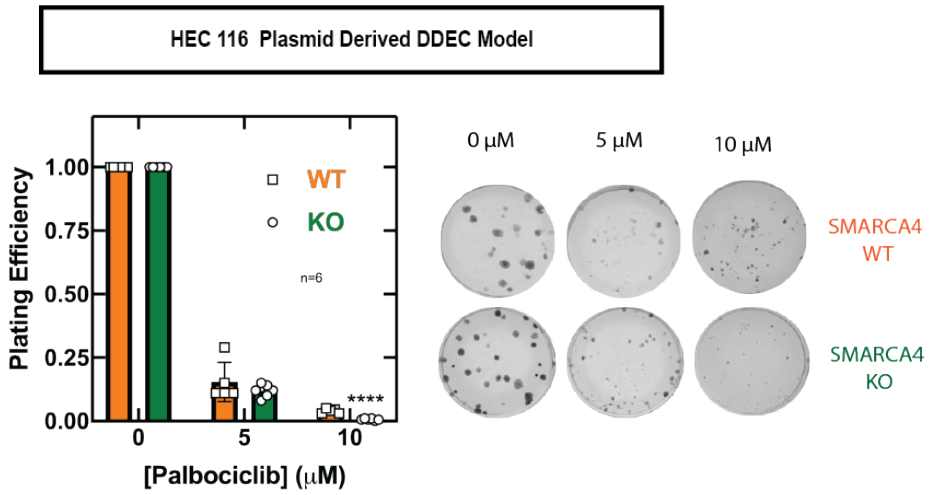


Figure 4.11. SMARCA4 knockout HEC 116 Plasmid Derived EC cells are more vulnerable to inhibition of CDK4 activity by palbociclib. Clonogenic assay with the HEC 116 Plasmid Derived cell line model of DDEC. The cell lines were cultured in the presence or absence of increasing concentrations of palbociclib (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of six independent biological replicates depicted as white-colored shapes. Multiple t-tests confirmed the presence of any significant difference (**** = $p < 0.0001$).

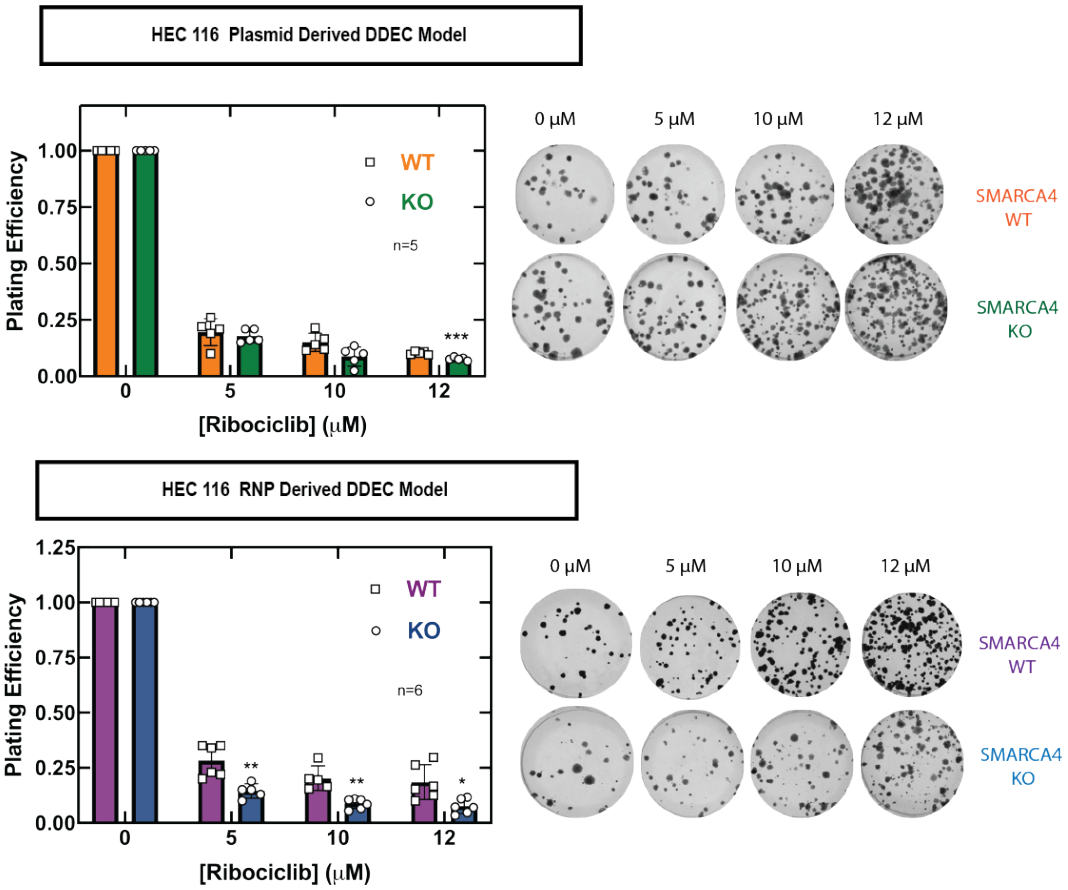


Figure 4.12. SMARCA4 knockout HEC 116 Plasmid and RNP Derived EC cells are more vulnerable to inhibition of CDK4 activity by ribociclib. Clonogenic assays with both HEC 116 CRISPR derivations of DDEC. The cell lines were cultured in the presence or absence of increasing concentrations of ribociclib (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of five or six independent biological replicates depicted as white-colored shapes. Multiple t-tests confirmed the presence of any significant difference (*** = $p < 0.001$. ** = $p < 0.01$. * = $p < 0.05$).

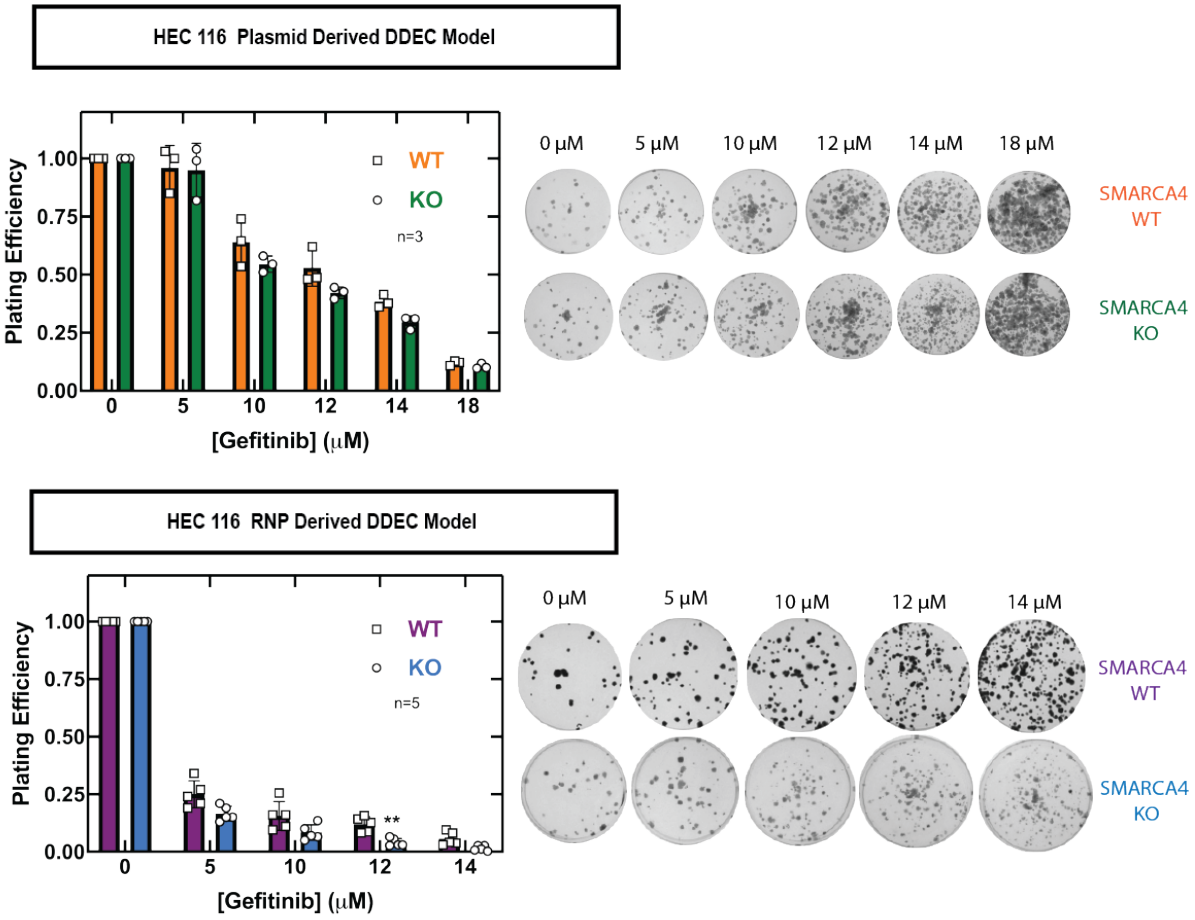


Figure 4.13. SMARCA4 knockout HEC 116 Plasmid and RNP Derived EC cells are slightly more vulnerable to inhibition of EGFR activity by gefitinib. Clonogenic assays with both HEC 116 CRISPR derivations of DDEC. The cell lines were cultured in the presence or absence of increasing concentrations of gefitinib (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of three to five independent biological replicates depicted as white-colored shapes. Multiple t-tests confirmed the presence of any significant difference (** = $p < 0.01$. * = $p < 0.05$).

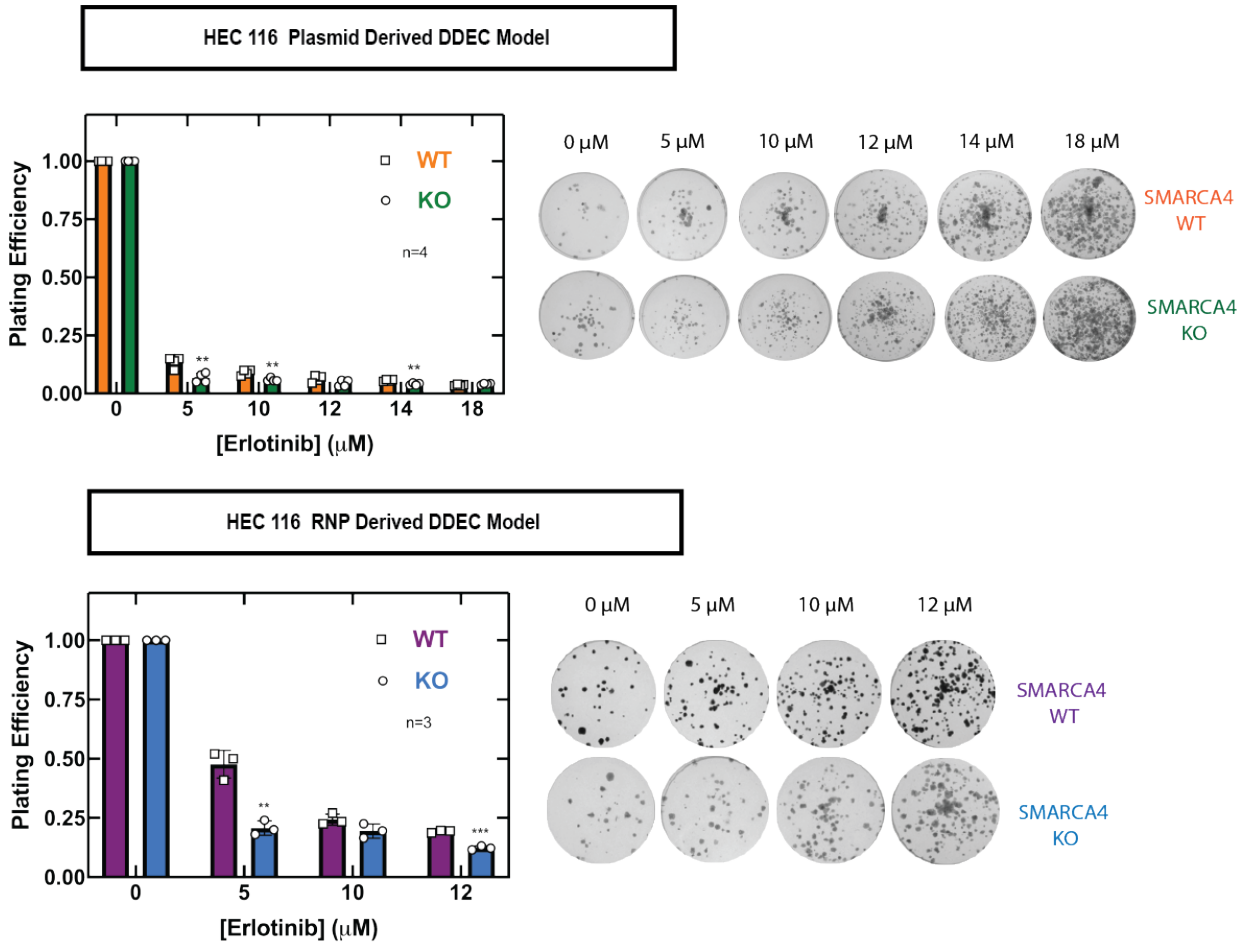


Figure 4.14. SMARCA4 knockout HEC 116 Plasmid and RNP Derived EC cells are more vulnerable to inhibition of EGFR activity by erlotinib. Clonogenic assays with both HEC 116 CRISPR derivations of DDEC. The cell lines were cultured in the presence or absence of increasing concentrations of erlotonib (0 to 18 μM) for 8 days prior to fixation and staining. Data is comprised of three to four independent biological replicates depicted as white-colored shapes. Multiple t-tests confirmed the presence of any significant difference (***= $p < 0.001$. ** = $p < 0.01$. * = $p < 0.05$).

To show that SL interaction between SMARCA4 and CDK4 or EGFR was not limited to HEC 116 EC cells, specifically the isogenic plasmid derived pair the pooled screen was performed in initially, we also examined the response of our HEC 59 RNP Derived model to both CDK and EGFR chemical inhibition. Surprisingly, HEC 59 SMARCA4 WT cells were more sensitive to the CDK4 inhibitor, ribociclib than their KO

counterparts but otherwise inhibition of CDK4 by palbociclib and inhibition of EGFR, suppressed SMARCA4 deficient HEC 59 cell growth more than HEC 59 SMARCA4 intact cell profileration **[Figure 4.15, Figure 4.16 & Supplemental Table 4.3C].**

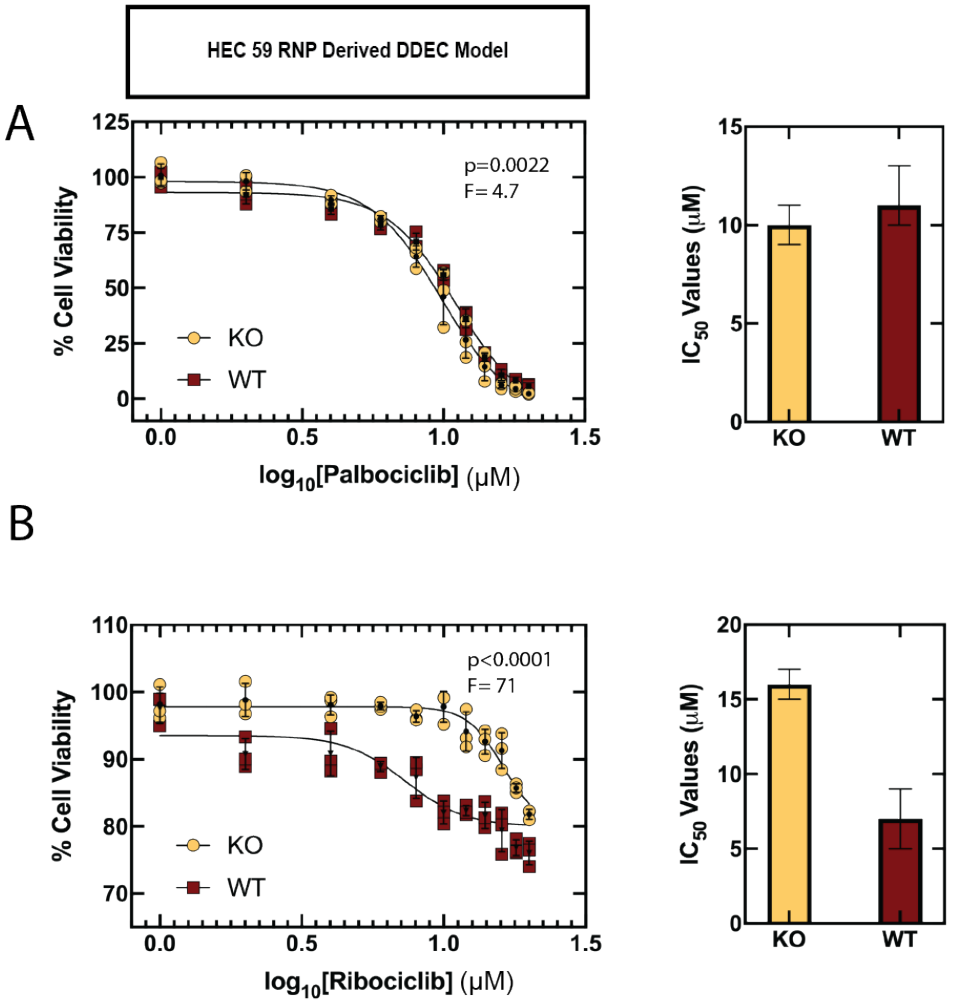


Figure 4.15. Only the application of CDK4 inhibitor palbociclib preferentially suppresses SMARCA4 deficient HEC 59 RNP Derived EC cells. The viability of HEC 59 EC cells lacking SMARCA4 expression is reduced in the presence of inhibitors against CDK4 activity such as **A)** palbociclib (SMARCA4 KO IC_{50}=10 μM vs. SMARCA4 WT IC_{50}=11 μM) but not **B)** ribociclib (SMARCA4 KO IC_{50}=16 μM vs. SMARCA4 WT IC_{50}=7 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

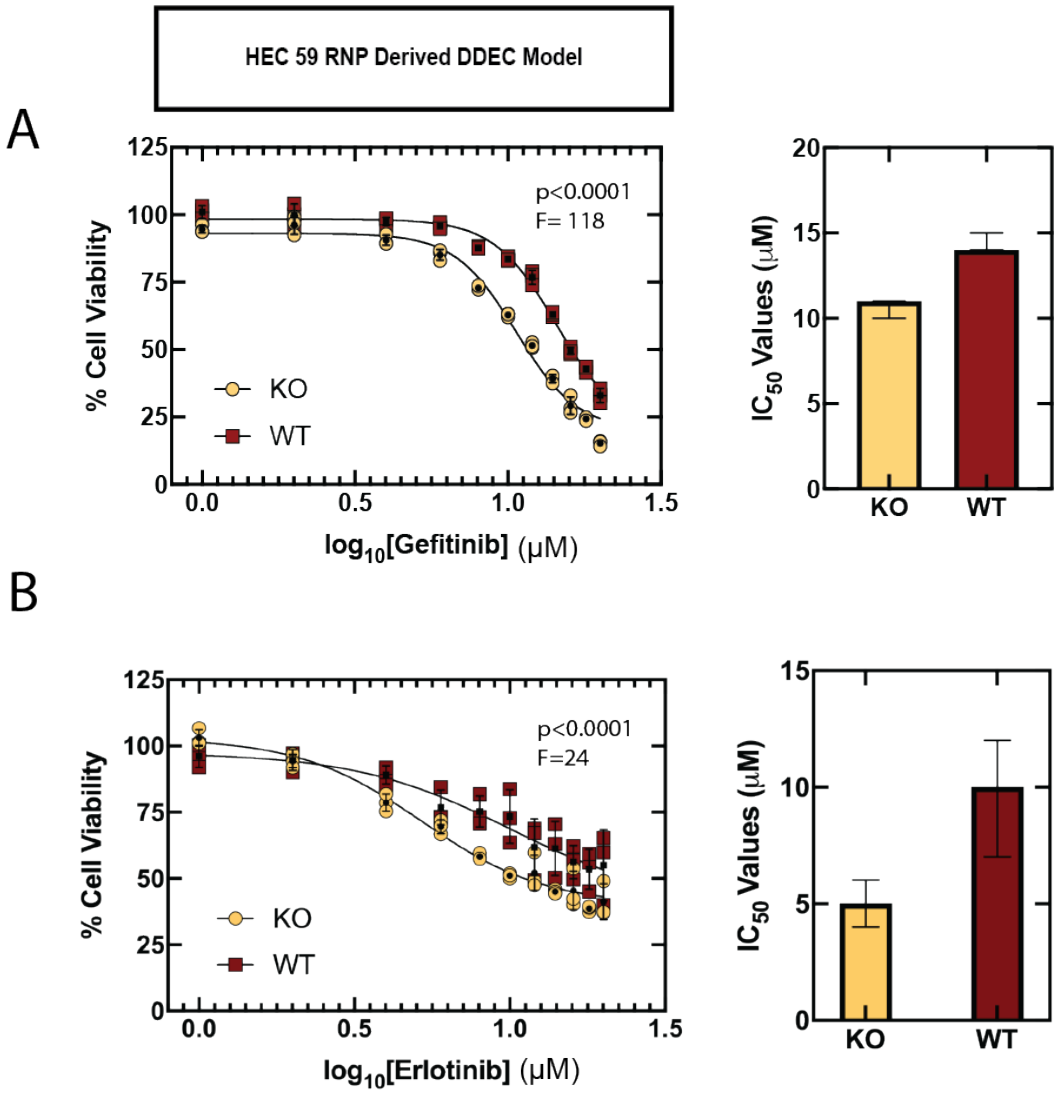
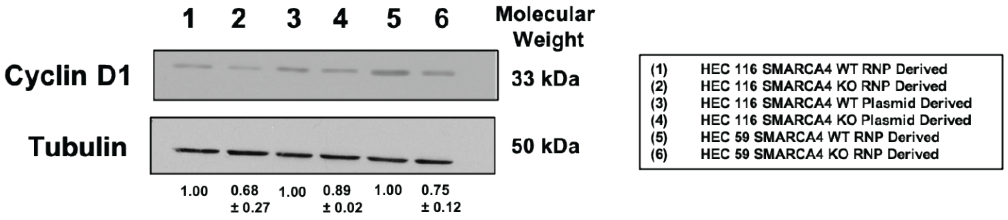


Figure 4.16. Application of EGFR inhibitors preferentially suppresses SMARCA4 deficient HEC 59 RNP Derived EC cells. The viability of HEC 59 endometrial cancer cells lacking SMARCA4 expression is reduced in the presence of inhibitors against EGFR activity such as **A)** gefitinib (SMARCA4 KO IC_{50}=11 μM vs. SMARCA4 WT IC_{50}=14 μM) and **B)** erlotinib (SMARCA4 KO IC_{50}=5 μM vs. SMARCA4 WT IC_{50}=10 μM). SMARCA4 KO and WT cell lines were treated with increasing concentrations of targeted drugs (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates represented as individual-colored shapes. Filled black circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

4.2.4. *Reduced cyclin D1 and increased phosphorylated EGFR levels in SMARCA4-deficient DDEC lead to sensitivity to CDK4 and EGFR inhibitors respectively.*

To find some of the molecular underpinnings driving drug sensitivity to inhibitors to CDK4 and EGFR in our cell line models of DDEC, we looked at the expression levels of different regulators of cell cycle progression as well as the phosphorylation state of EGFR. Previous studies examining other SMARCA4 deficient carcinomas such as NSCLC and SCCOHT, discovered cyclin D1 dysregulation to be linked with sensitivity of these cancer cells to CDK4/6 inhibitors (37, 38). In alignment with these earlier findings, we determined that cyclin D1 levels are lower in EC cells lacking SMARCA4 expression as compared to WT cells **[Figure 4.17A]**. Additionally, RNA and chromatin immunoprecipitation (ChIP)-sequencing experiments demonstrated that SMARCA4 KO in HEC 116 EC cells results in reduced *CDKN2A/B* transcript levels concomitant with H3K27 hypo-acetylation of the *INK4/ARF* locus **[Figure 4.17B]**.

A



B

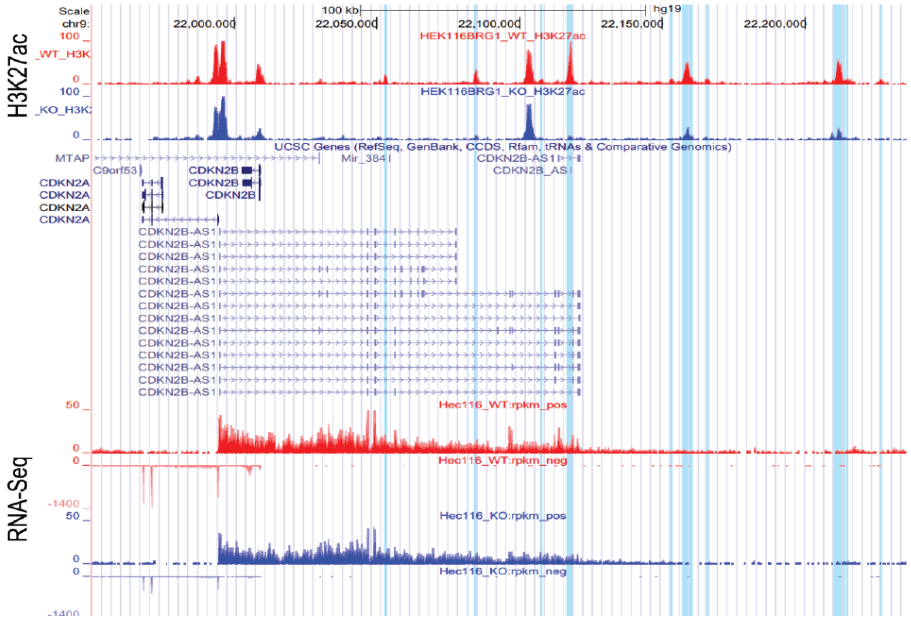


Figure 4.17. SMARCA4 KO in EC cells is associated with dysregulation of the p16/cyclin D1/Rb pathway. A) SMARCA4 loss reduces cyclin D1 expression in all line models of DDEC generated by CRISPR gene editing. Western blot represents three independent experiments with tubulin acting as a loading control. Densitometry allowed for the obtainment of ratios of band intensity relative to SMARCA4 WT samples (mean ± standard deviation). **B)** SMARCA4 KO dysregulates H3K27ac nearby *CDKN2A/B* affecting its transcription.

As the gene encoding *EGFR* is overexpressed in numerous carcinomas of epithelial origin, including the SMARCA4 deficient neoplasm, NSCLC, we investigated whether EGFR levels correlated with the response of our DDEC cell line models to erlotinib and gefitinib (44). No significant differences in EGFR protein levels were observed between SMARCA4 intact and deficient EC cells regardless of the cell line

tested **[Figure 4.18]**. Both HEC 116 Plasmid Derived and HEC 59 RNP Derived SMARCA4 WT EC cells had slightly elevated EGFR expression as observed by flow cytometry, but this trend was not evident with the HEC 116 RNP Derived DDEC derivation **[Figure 4.18]**. The phosphorylation status of EGFR particularly at Tyr1068 has also been posited to be biologically relevant in predicting the response of NSCLC patients with wildtype EGFR to gefitinib and erlotinib (46, 47). While all cell line models of DDEC exhibited some degree of sensitivity to EGFR inhibition by cell viability and clonogenic assays, only HEC 116 Plasmid Derived SMARCA4 KO EC cells had a higher ratio of phosphorylated EGFR expression to total EGFR expression when compared to wildtype counterparts **[Figure 4.19]**.

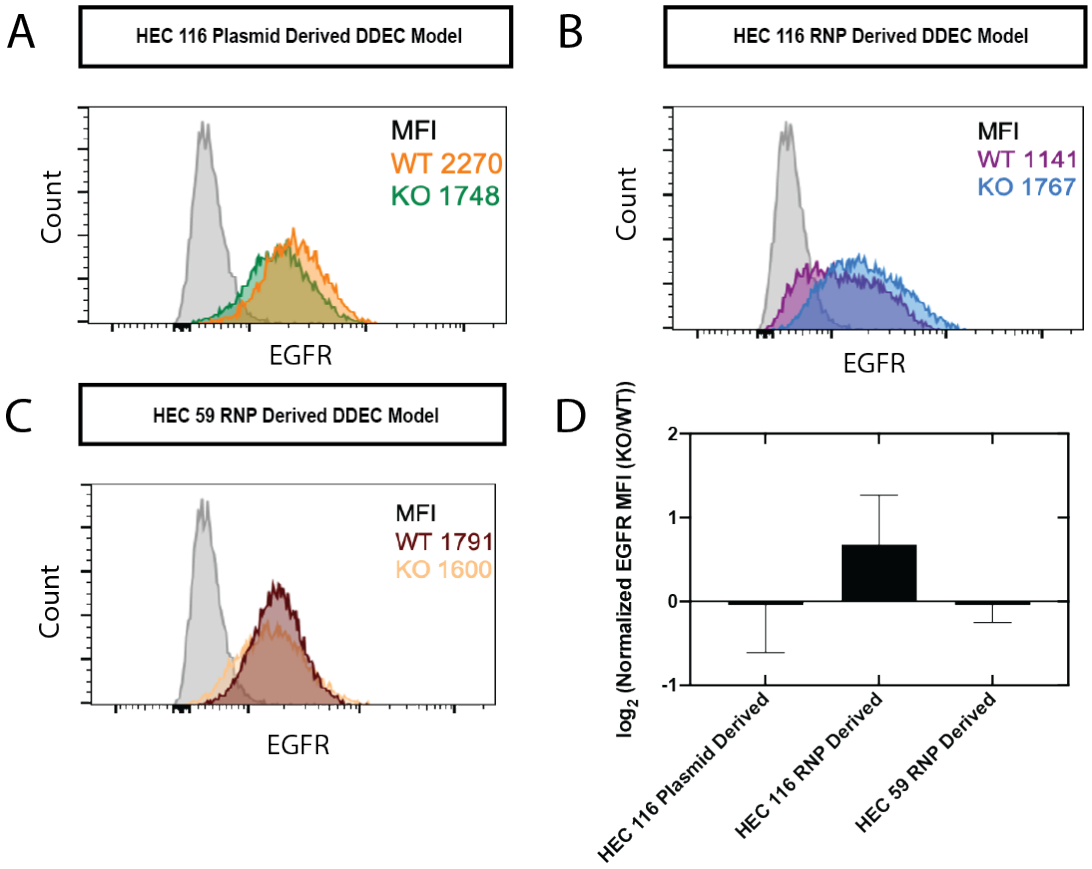


Figure 4.18. Absence of SMARCA4 expression does not significantly alter EGFR levels in EC cells. EGFR expression was detected by flow cytometry on the **A)** HEC 116 Plasmid Derived **B)** HEC 116 RNP Derived and **C)** HEC 59 RNP Derived cell line models of DDEC after staining with conjugated antibodies. **D)** Comparing SMARCA4 KO EC cells to SMARCA4 WT EC cells median fluorescence intensities (MFI) which were normalized to fluorescence minus one (FMO) controls (n=3).

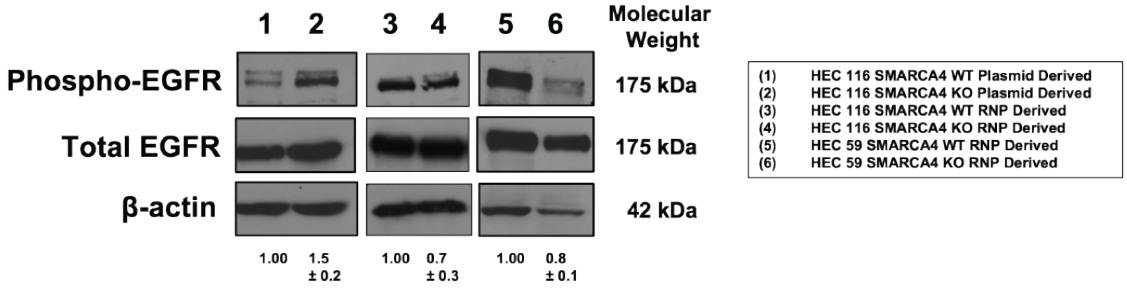
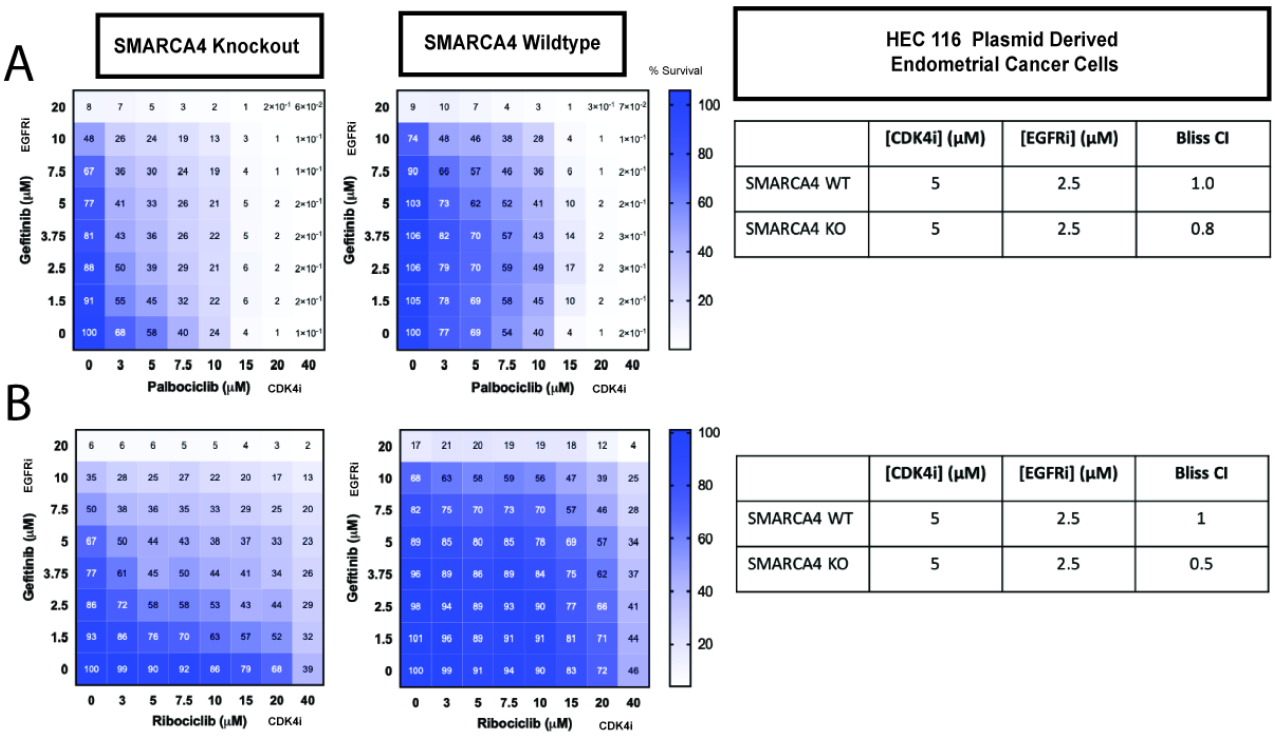


Figure 4.19. Response to EGFR inhibition in the absence of SMARCA4 is likely influenced through regulation of the receptor's tyrosine phosphorylation status. SMARCA4 loss leads to variable EGFR activation depending on the cell line model of DDEC. Western blot represents three independent experiments with β-Actin acting as a loading control. Densitometry allowed for the obtainment of ratios of band intensity relative to SMARCA4 WT samples (mean ± standard deviation).

4.2.5. Combination CDK4 and EGFR inhibition synergistically kills SMARCA4 deficient EC cells.

Many cancers tend to either fail to respond to single inhibitor therapy or acquire resistance after initially showing a response (48). Several studies and treatment paradigms have demonstrated that inhibiting two distinct pathways with two different drugs may compound the effects exhibited by the drugs on their own (49). We examined cell viability upon treatment of the HEC 116 cell line models of DDEC with combinations of CDK4 and EGFR inhibitors. Bliss combination indices (CI) were calculated for every combination tested. Synergy between CDK4 and EGFR inhibitors was observed in the plasmid derived SMARCA4 KO EC cells whereas these drugs were antagonistic in the SMARCA4 wildtype EC cells **[Figure 4.20 - Figure 4.21]**.

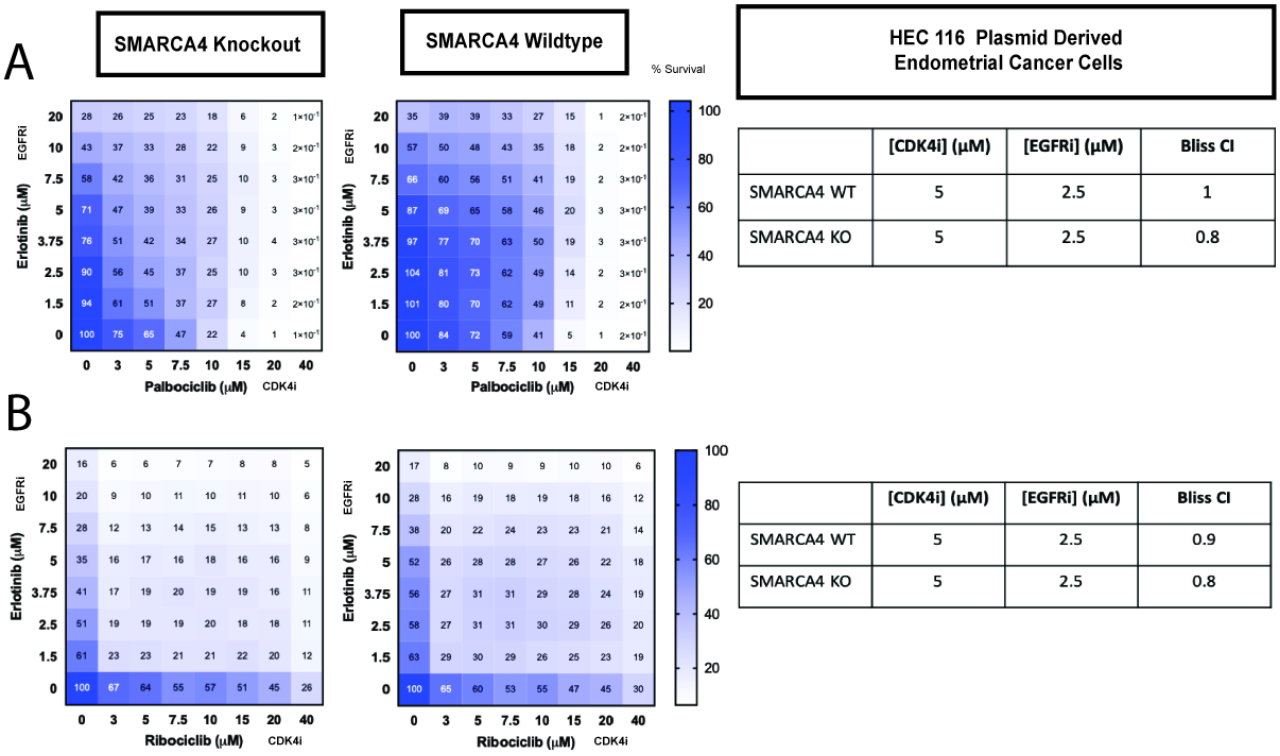


HEC 116 Plasmid Derived Endometrial Cancer Cells

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	1.0
SMARCA4 KO	5	2.5	0.8

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	1
SMARCA4 KO	5	2.5	0.5

Figure 4.20. Synergistic killing of HEC 116 Plasmid Derived SMARCA4 deficient cells by combination treatment with gefitinib and CDK4 inhibitors. Cell viability was assessed by luminescence (560 nm) using a microplate reader after SMARCA4 KO or WT HEC 116 Plasmid Derived cells were treated for 72 hours with increasing concentrations of Gefitinib (0 to 20 μM) and either **A)** palbociclib or **B)** ribociclib (0 to 40 μM). Percent survival as indicated in the color bar corresponds to percent cell survival when normalized to untreated cells. Tables of Bliss combination indices for Gefitinib with palbociclib or ribociclib wherein a Bliss CI of greater than 1 denotes antagonism, less than 1 indicates synergy and less than 0.7 implies strong synergy.



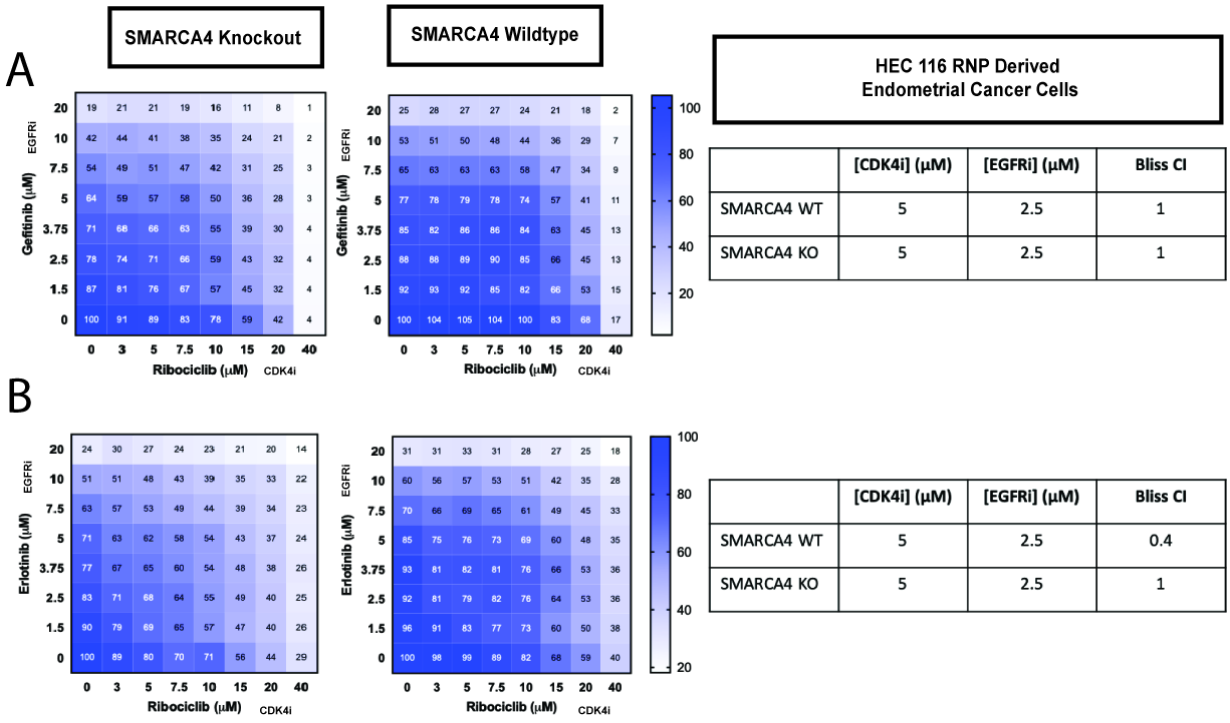
HEC 116 Plasmid Derived Endometrial Cancer Cells

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	1
SMARCA4 KO	5	2.5	0.8

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	0.9
SMARCA4 KO	5	2.5	0.8

Figure 4.21. Synergistic killing of HEC 116 Plasmid Derived SMARCA4 deficient cells by combination treatment with erlotinib and CDK4 inhibitors. Cell viability was assessed by luminescence (560 nm) using a microplate reader after SMARCA4 knockout or wildtype HEC 116 plasmid derived cells were treated for 72 hours with increasing concentrations of Erlotinib (0 to 20 μM) and either **A)** palbociclib or **B)** ribociclib (0 to 40 μM). Percent survival as indicated in the color bar corresponds to percent cell survival when normalized to untreated cells. Tables of Bliss combination indices for Erlotonib with palbociclib or ribociclib wherein a Bliss CI of greater than 1 denotes antagonism, less than 1 indicates synergy and less than 0.7 implies strong synergy.

Interestingly, when DDEC was modeled in HEC 116 EC cells using RNP based CRISPR gene editing methodology, less synergetic killing was observed for SMARCA4 deficient cells **[Figure 4.22]**. Combination therapy of palbociclib and gefitinib tested on serially passaged, cultured cells resulted in synergistic killing of HEC 116 third generation tumor cells regardless of SMARCA4 expression status **[Figure 4.23]**.

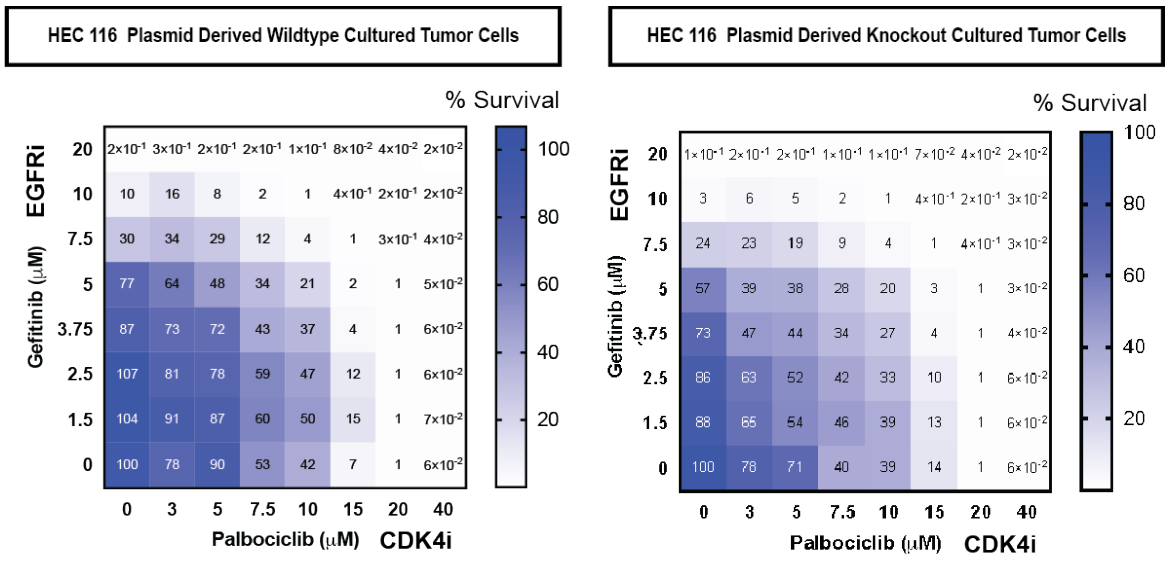


HEC 116 RNP Derived Endometrial Cancer Cells

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	1
SMARCA4 KO	5	2.5	1

	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT	5	2.5	0.4
SMARCA4 KO	5	2.5	1

Figure 4.22. Non synergistic killing of HEC 116 RNP SMARCA4 deficient cells by combination treatment with ribociclib and EGFR inhibitors. Cell viability was assessed by luminescence (560 nm) using a microplate reader after SMARCA4 knockout or wildtype HEC 116 plasmid derived cells were treated for 72 hours with increasing concentrations of ribociclib (0 to 40 μM) and either **A)** gefitinib or **B)** erlotinib (0 to 20 μM). Percent survival as indicated in the color bar corresponds to percent cell survival when normalized to untreated cells. Tables of Bliss combination indices for ribociclib with gefitinib or erlotinib wherein a Bliss CI of greater than 1 denotes antagonism, less than 1 indicates synergy and less than 0.7 implies strong synergy.



	[CDK4i] (μM)	[EGFRi] (μM)	Bliss CI
SMARCA4 WT F3 CDX	5	2.5	0.5
SMARCA4 KO F3 CDX	5	2.5	0.8

Figure 4.23. Synergistic killing of both serially passaged HEC 116 Plasmid Derived SMARCA4 knockout and wildtype EC cells by combination treatment with the CDK4 and EGFR inhibitors, palbociclib and gefitinib, respectively. Cell viability was assessed by luminescence (560 nm) using a microplate reader after third generation, *in vitro* cultured SMARCA4 knockout or wildtype HEC 116 plasmid derived cells were treated for 72 hours with increasing concentrations of gefitinib (0 to 20 μM) and palbociclib (0 to 40 μM). Percent survival as indicated in the color bar corresponds to percent cell survival when normalized to untreated cells. Tables of Bliss combination indices for ribociclib with gefitinib or erlotinib wherein a Bliss CI of greater than 1 denotes antagonism, less than 1 indicates synergy and less than 0.7 implies strong synergy.

4.2.6. Heterogeneity of DDEC tumors derived from gene edited cell lines convolutes efficacy of palbociclib and gefitinib treatment in vivo.

To test the efficacy of CDK4 and EGFR inhibition on DDEC tumors *in vivo*, on two separate occasions mice bearing either HEC 116 Plasmid Derived SMARCA4 knockout or wildtype CDX tumors were treated biweekly by oral gavage with palbociclib, gefitinib or a combination of palbociclib and gefitinib **[Supplemental Figure 4.4]**. Tumors were extracted at an experimental endpoint of 10 mm in diameter **[Figure 4.24].**

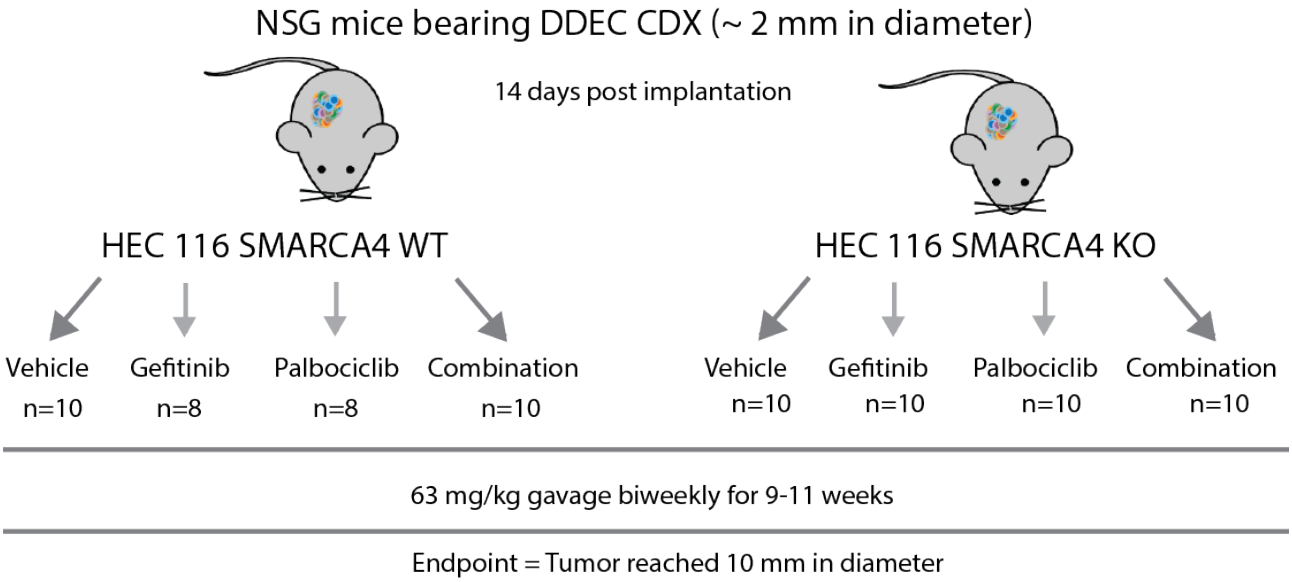


Figure 4.24. Experimental design to determine the effect CDK4 or EGFR inhibition alone or in combination has on SMARCA4 KO and WT EC CDX growth in immunodeficient mice. Briefly, NSG mice were implanted subcutaneously with either HEC 116 Plasmid Derived SMARCA4 deficient or intact tumor sections. 14 days post-implantation mice were randomized into experimental groups whereby oral gavage either vehicle, CDK4, EGFR or a combination of CDK4 and EGFR inhibitors were administered. Data is comprised of two independent rounds of *in vivo* experimentation.

Body weight of mice with HEC 116 Plasmid Derived SMARCA4 KO **[Figure 4.25A]** did not significantly alter over the course of any of the *in vivo* experiments. It should be noted that during the first thirty of eighty days when compared to untreated animals, mice

bearing SMARCA4 intact tumors treated with CDK4 and EGFR inhibitors exhibited substantial reduction in body weight ($p<0.001$ for palbociclib and gefitinib alone and $p<0.01$ when the two drugs were combined) **[Figure 4.25B]**. Additionally, at the endpoint of both the first and second rounds, final tumor weights regardless of SMARCA4 status did not significantly differ across the four treatment arms **[Figure 4.26]**. SMARCA4 KO tumor volume within the palbociclib and combination treatment groups was notably lower when compared to the final volumes of tumors exposed to vehicle **[Figure 4.26]**. Time to endpoint was longest for mice bearing HEC 116 Plasmid Derived SMARCA4 deficient cells when subjected to any treatment (median survival = 47 days versus median survival of 44 days for vehicle treated SMARCA4 KO mice) whereas HEC 116 Plasmid Derived SMARCA4 intact tumor bearing mice were conferred a survival advantage particularly when treated with palbociclib (median survival = 76 days) or the combination therapy (median survival = 63 days versus median survival of 50 days for vehicle treated SMARCA4 WT mice) **[Figure 4.27]**. Log-rank analysis of the Kaplan-Meier survival curves though did not demonstrate a significant difference in survival between the treated and untreated animals ($p=0.3$ for SMARCA4 deficient tumor bearing mice and $p=0.1$ SMARCA4 intact tumor bearing mice) **[Figure 4.27]**.

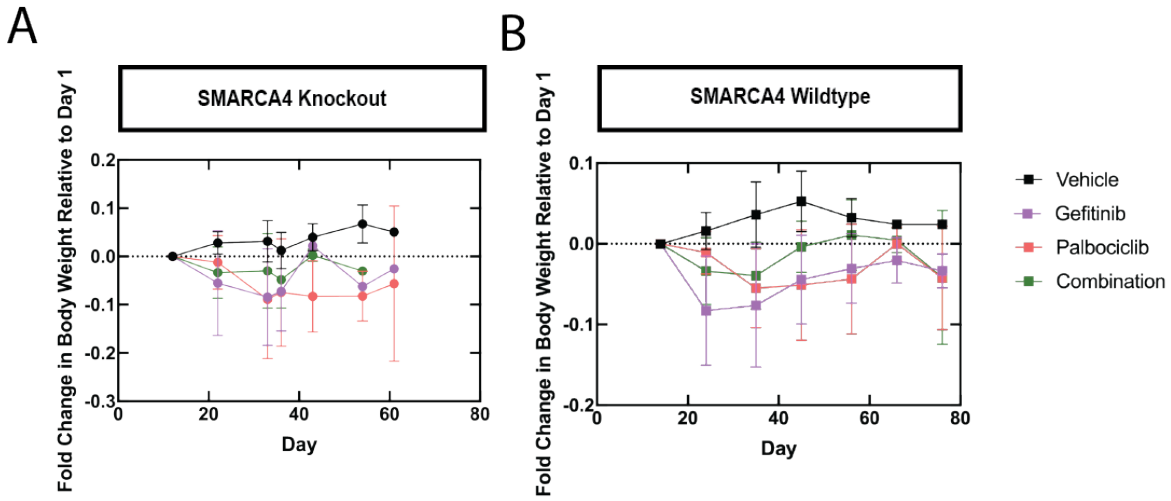


Figure 4.25. Palbociclib and gefitinib either alone or in combination do not overly affect the body weight of mice. Fold change in body weight relative to day 1 of mice bearing HEC 116 Plasmid Derived **A)** SMARCA4 KO or **B)** SMARCA4 WT tumors that were randomized into various treatment groups. Data consists of two independent rounds or biological replicates. Filled circles or squares with error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates, respectively. Multiple unpaired t-tests confirmed the absence of any significant difference in body weight except for SMARCA4 intact tumor bearing mice during the first thirty days of treatment with palbociclib, gefinitib or combination therapy.

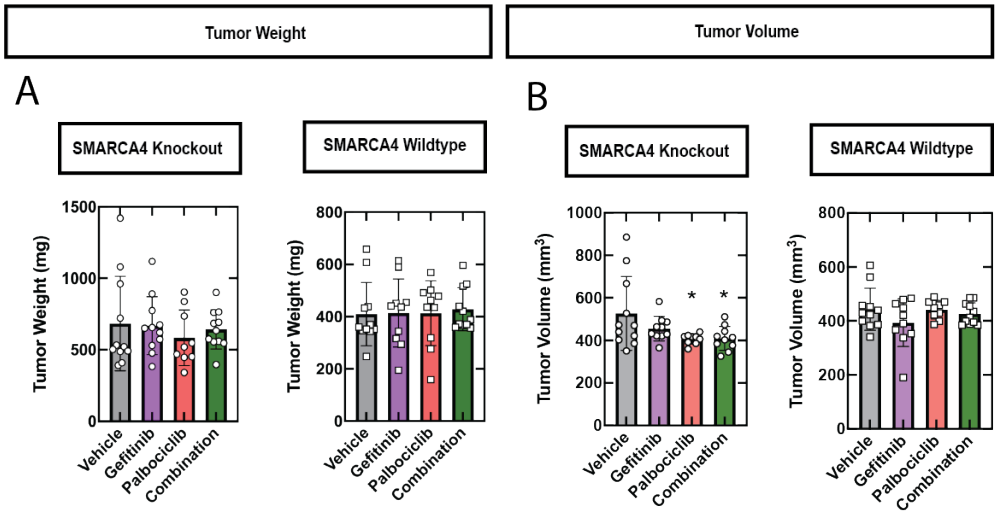


Figure 4.26. Tumor volume but not tumor weight significantly differs amongst SMARCA4 knockout tumor bearing animals treated with palbociclib alone or in combination. HEC 116 Plasmid Derived SMARCA4 KO and SMARCA4 WT **A)** tumor weight and **B)** tumor volume upon excision at experimental endpoint. Data is comprised of two independent rounds or biological replicates depicted as white-colored shapes. One-way analysis of variance (ANOVA) analysis revealed no significant difference between treatment groups except for SMARCA4 KO tumor volume upon treatment with palbociclib alone or in combination (* = $p < 0.05$).

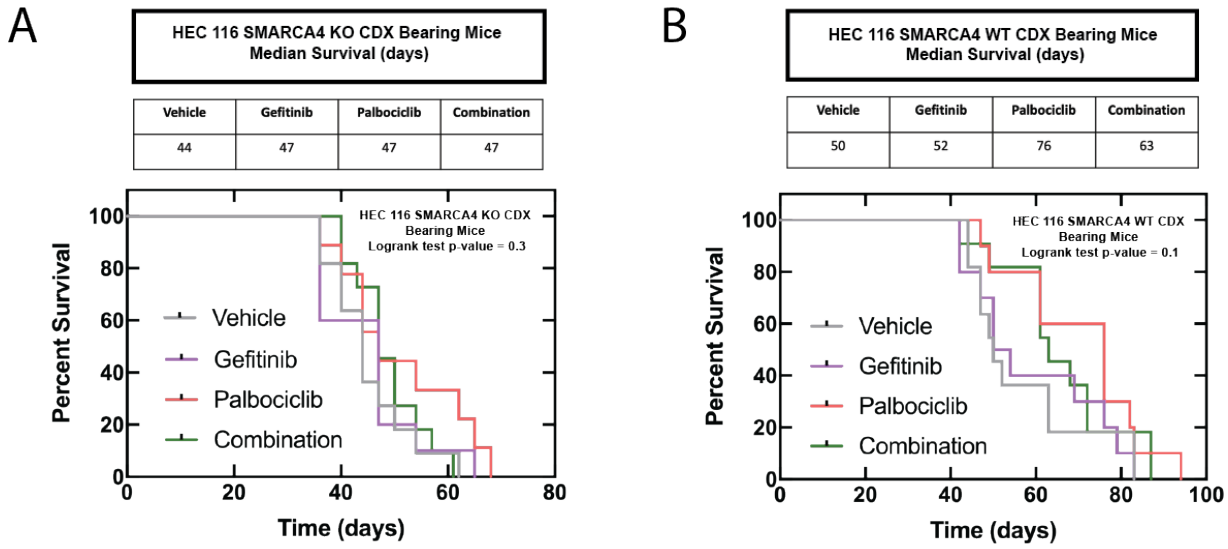


Figure 4.27. Insignificant increased survival of HEC 116 Plasmid Derived tumor bearing mice receiving synthetic lethality-based treatment. Kaplan-Meier survival curves compared using log-rank tests, of mice bearing HEC 116 Plasmid Derived **A)** SMARCA4 KO and **B)** SMARCA4 WT tumors and subjected to either vehicle, palbociclib, gefitinib or combination treatment (63 mg/kg). Mice were sacrificed when tumors reached 10 mm by caliper measurement.

Growth in HEC 116 Plasmid Derived EC tumor area over time was very similar across the treatment arms **[Figure 4.28]**. Closer examination of this dataset from our preclinical animal studies hinted at the possibility that it deviated considerably from normality therefore D'Agostino & Pearson tests were executed **[Supplemental Figure 4.5]**. Normality tests failed (alpha=0.05) for every treatment arm including those mice receiving only the vehicle **[Supplemental Figure 4.5]**. Inspection of the quantile-quantile (QQ) plots revealed extremely two-tailed data or in other words, most tumor area values were observed at extremes rather than centered around the mean or median of that particular days' measurements **[Supplemental Figure 4.5 & Figure 4.28]**.

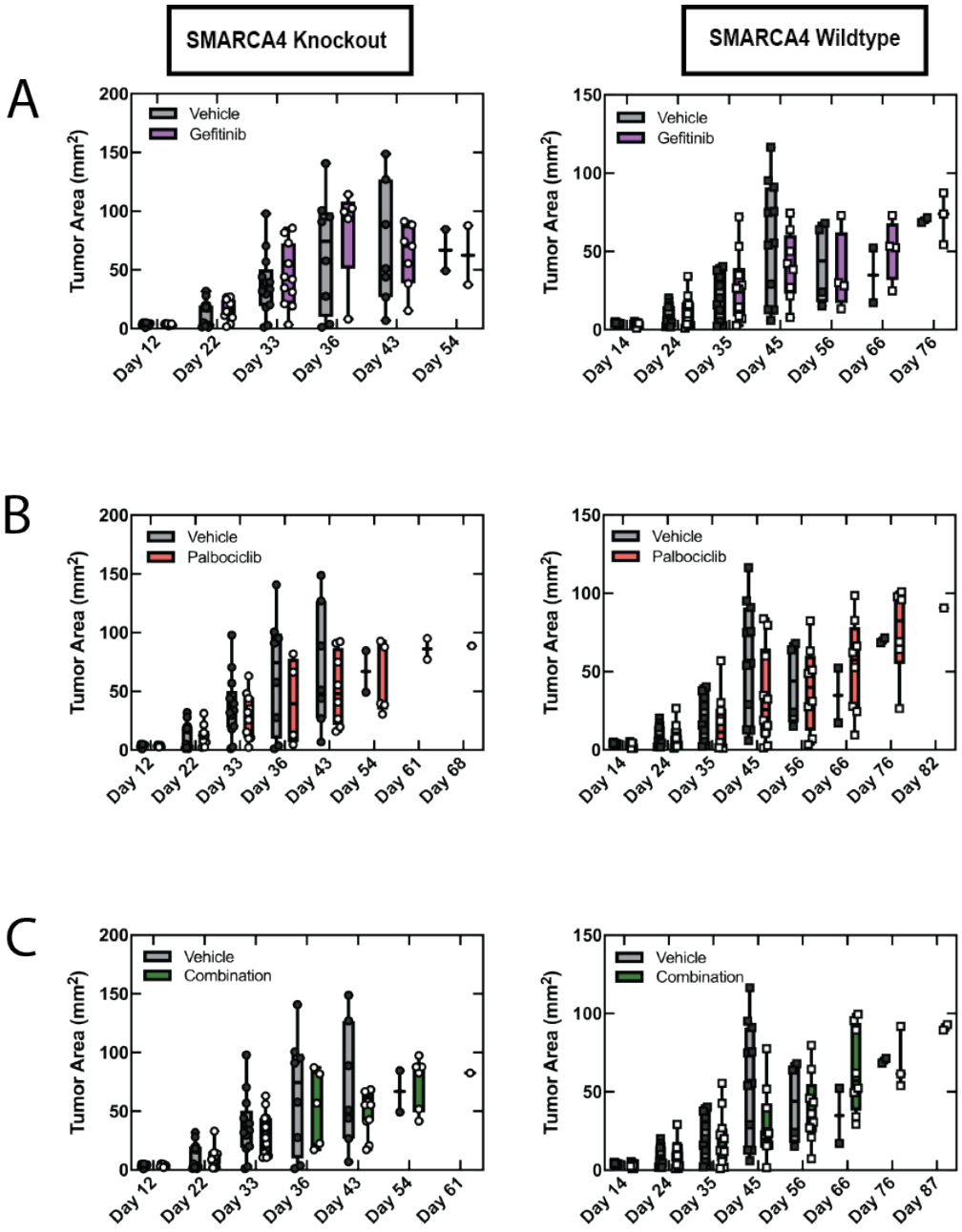


Figure 4.28. Effect of palbociclib, gefitinib and combination CDK4/EGFR inhibition on HEC 116 Plasmid Derived DDEC tumor growth. Tumor area of HEC 116 Plasmid Derived SMARCA4 KO and SMARCA4 WT tumors and subjected to either **A)** palbociclib, **B)** gefitinib or **C)** combination treatment over time. Data consists of two independent rounds or biological replicates represented as white-colored shapes for any of the tumors originating from the treatment groups and filled black shapes for tumors from the vehicle treated mice. Box plots shown in grey and color have whiskers that indicate variability outside the upper and lower quartiles. Statistical differences between the tumor growth curves on various days were calculated in GraphPad Prism using nonparametric conditions and Kolmogorov-Smirnov t-tests.

From a histological perspective, examination of SMARCA4 deficient tumors from the *in vivo* experimentation that were treated with palbociclib or combination therapy exhibited some increased regions of necrosis compared to gefitinib or vehicle treatment **[Figure 4.29]**. Similarly, SMARCA4 intact tumors were discovered to be somewhat more necrotic upon subjection to any synthetic lethality-based therapy **[Figure 4.29]**. While a similar trend in necrosis upon drug treatment was witnessed regardless of SMARCA4 status, tumors deficient in SMARCA4 were generally more necrotic than their wildtype counterparts **[Figure 4.29 & Table 4.1]**.

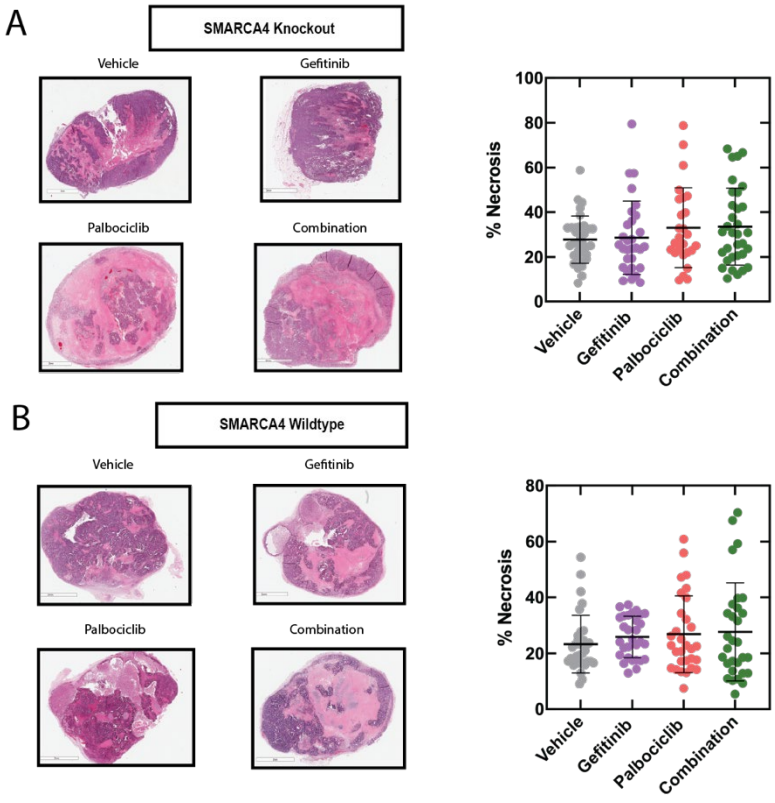


Figure 4.29. Effect of vehicle, palbociclib, gefitinib and combination treatment on HEC 116 Plasmid Derived SMARCA4 tumor necrosis. Representative H&E images of **A)** SMARCA4 KO and **B)** SMARCA4 WT tumors extracted from immunocompromised mice from the various treatment groups. Quantification of the average area of necrosis was accomplished by analyzing three tumor sections per mouse with ImageJ software. Error bars represent the mean and standard deviation of the SMARCA4 KO and WT biological replicates.

To ensure that palbociclib and gefitinib administered by oral gavage effectively penetrated the tumors, immunoblotting was performed on lysates from single tumors within each treatment group and round of *in vivo* study. We had previously confirmed by immunoblotting in unpassaged cells, reduction in phosphorylation levels of Rb and EGFR, which are molecular targets affected by application of palbociclib and gefitinib, respectively **[Supplemental Figure 4.6].** Activation of phospho-EGFR at tyrosine 1068 was compromised in the gefitinib and combination treated mice across both the first and second round and regardless of the SWI/SNF status of the tumor **[Figure 4.30]**. Confirmation of palbociclib reaching and acting upon our DDEC CDX tumors was assessed by examining the activation of status of a signalling factor downstream of CDK4, Rb. Palbociclib was less effective in inhibiting the activation of Rb particularly during the first round of *in vivo* experimentation and in the single samples we examined appears more successful in inhibiting the phosphorylation of Rb in SMARCA4 deficient DDEC tumors over SMARCA4 intact neoplasms **[Figure 4.30]**.

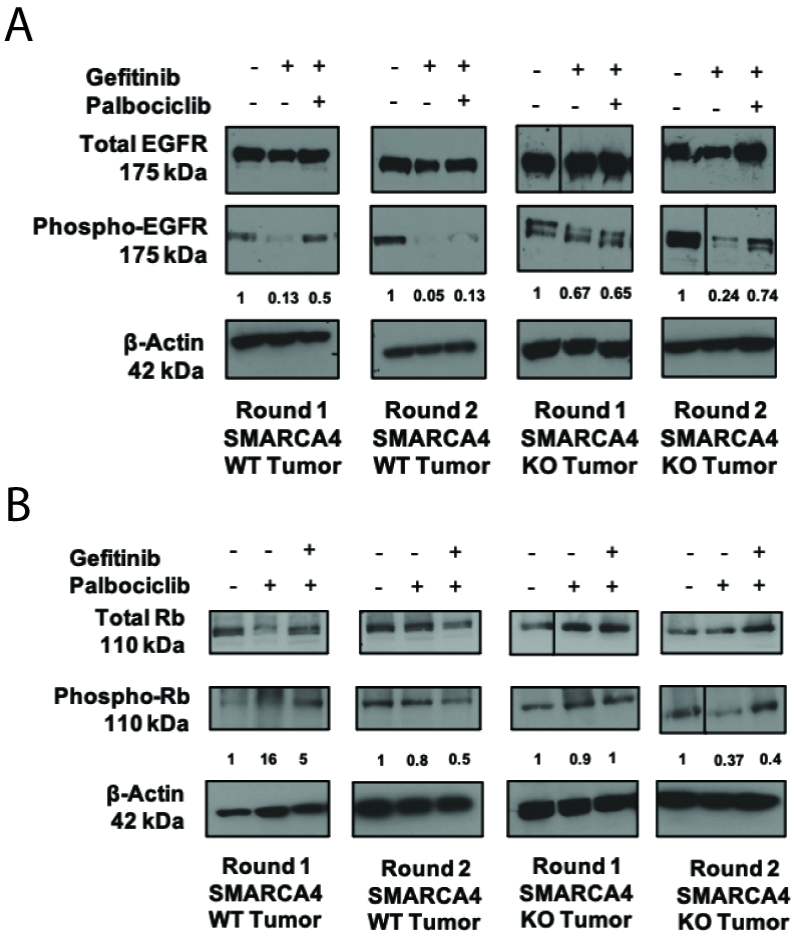


Figure 4.30. Administration of gefitinib and palbociclib alone or in combination by oral gavage to mice bearing cell line models of DDEC results in variable regulation of the phosphorylation status of downstream signalling factors. A) Drug treatment with gefitinib consistently led to reduced activation of EGFR. **B)** Drug treatment with palbociclib led to less Rb activation within tumors lacking SMARCA4. Western blot represents a single experiment with β-Actin acting as a loading control. Densitometry allowed for the obtainment of ratios of band intensity relative to untreated samples of same SMARCA4 status and from the same round of *in vivo* experimentation.

In summary, treated SMARCA4 wildtype CDX generally exhibited substantial pathway inhibition when growth rates were slow, and necrosis constituted a small portion of the neoplasm **[Table 4.1]**. The SMARCA4 knockout tumors, we have profiled thus far by immunoblotting do not exhibit the same predictable behavior as their wildtype counterparts, especially those tumors from mice receiving palbociclib alone or in combination **[Table 4.1]**. Consolidation of our preclinical animal study findings therefore highlights the heterogeneity of our DDEC-like cell line model particularly the CDX formed from SMARCA4 deficient EC cells **[Table 4.1]**.

Table 4.1 Summary of growth rate, degree of pathway inhibition and percent necrosis for select treated cell line derived DDEC neoplasms. Rates of tumor growth were determined by fitting data with simple linear regression fits in GraphPad Prism. Quantification of the average area of necrosis was accomplished by analyzing three tumor sections per mouse with ImageJ software. Densitometry allowed for the obtainment of ratios of band intensity relative to untreated samples of same SMARCA4 status and from the same round of *in vivo* experimentation (- indicates an intensity of 1, ▼ < 1, ▼▼ < 0.2, ▲ > 1 and ▲▲ > 5).

A

SMARCA4 Wildtype

	Band Intensity Ratio: EGFR	Band Intensity Ratio: Rb	Rate of Tumor Growth (Slope)	% Necrosis
Vehicle Treated (Round 1)	-	-	1.5 ± 0.6	18%
Vehicle Treated (Round 2)	-	-	1.6 ± 0.3	24%
Palbociclib Treated (Round 1)	-	▲▲	2.4 ± 0.1	36%
Palbociclib Treated (Round 2)	-	▼	2.4 ± 0.7	19%
Gefitinib Treated (Round 1)	▼▼	-	1.5 ± 0.2	28%
Gefitinib Treated (Round 2)	▼▼	-	1.2 ± 0.1	23%
Combination Treated (Round 1)	▼	▲	1.9 ± 0.3	17%
Combination Treated (Round 2)	▼▼	▼	1.2 ± 0.3	20%

B

SMARCA4 Knockout

	Band Intensity Ratio: EGFR	Band Intensity Ratio: Rb	Rate of Tumor Growth (Slope)	% Necrosis
Vehicle Treated (Round 1)	-	-	3.8 ± 0.6	32%
Vehicle Treated (Round 2)	-	-	2.7 ± 0.9	32%
Palbociclib Treated (Round 1)	-	-	1.6 ± 0.5	10%
Palbociclib Treated (Round 2)	-	▼	2.2 ± 0.6	62%
Gefitinib Treated (Round 1)	▼	-	4.2 ± 0.6	19%
Gefitinib Treated (Round 2)	▼▼	-	2.2 ± 0.5	28%
Combination Treated (Round 1)	▼	-	2 ± 0.4	43%
Combination Treated (Round 2)	▼	▼	2.1 ± 0.4	55%

4.3. Discussion

As demonstrated by cell viability and clonogenic assays, DDEC-like cell lines deficient in SMARCA4 were not preferentially targeted by the current therapeutics administered to endometrial cancer patients and in the case of paclitaxel treatment, SMARCA4 knockout EC cells appear more resistant to this drug compared to their paired SMARCA4 wildtype EC cells. It is becoming well established that chemotherapies are ineffective treatment modalities for advanced stage DDEC patients exhibiting metastases (40). Our development of an isogenic pair of cell lines that partially recapitulates DDEC upon knockout of SMARCA4, allowed us to perform a shRNA-based screen capable of identifying possible synthetic lethal interaction partners with this chromatin remodelling subunit in the context of endometrial cancer. RNAi screens utilizing shRNA are known to be limited by off-target effects and differences in knockdown efficiencies, which may lead to false positive results (50). Fortunately, our functional genetic screen yielded results that were not unique to only the model in which it was performed on, but the phenotype was observed across our multiple cellular models of DDEC. Repeating this shRNA-based screen with multiple established cultured patient samples will ultimately confirm the prevalence and significance of certain synthetic lethal partners to SMARCA4 in DDEC.

We demonstrated *in vitro,* using cell viability and clonogenic assays, that endometrial cancer cells with SMARCA4 KO are susceptible to chemical inhibition of CDK4 or EGFR. The p16-cyclin D1-CDK4/6/Rb pathway has been found to be regularly dysregulated in many cancers and is critical in the cell-cycle transition from G1 to S phase (51). EGFR, a receptor tyrosine kinase, plays an essential role during development, relaying cues from the extracellular environment pertaining to proliferation, survival,

migration, and differentiation to the cell, therefore dysregulation of EGFR signalling is often associated with cancer (52-54). Particularly apparent with the clonogenic assays, across the range of CDK4 and EGFR inhibitors we tested, the size of colonies rather than the colony number was substantially altered in the presence of drug treatment. This suggests that these drugs especially EGFR inhibitors act in a cytostatic rather than cytotoxic manner when applied to our cell line models of DDEC, a result that should be unsurprising considering it is a targeted therapy (55). While perhaps incapable of eliminating DDEC within a patient entirely, with improved early detection to avoid the emergence of metastatic disease followed by surgical resection, these inhibitors might impart some survival benefits.

While there are a growing number of reported synthetic lethal interaction partners for SMARCA4, we were drawn to the finding of DDEC being vulnerable to CDK4 inhibition within our unbiased shRNA screen, due to its proven clinical efficacy as a targeted therapy in breast cancer (56-59). This novel finding in DDEC correlates with previous findings uncovered for other SMARCA4 deficient neoplasms, namely SCCOHT and NSCLC, wherein CDK4 was revealed as a druggable target, synthetic lethal with SMARCA4, because of studies that also took a functional genetic screen approach (37, 38). We also revealed *in vitro* that sensitivity to CDK4 inhibition in the absence of SMARCA4 is associated with a dysregulation of cyclin D1 at both the transcript and protein level **[Figure 4.31]**. This is in accordance with what has been reported in SMARCA4 deficient SCCOHT and NSCLC, which are also susceptible to CDK4 inhibitors (37, 38). Disease specific survival for patients diagnosed with endometrial cancer remains lowest with DDEC, thus any treatment capable of both extending and improving quality of life is

desired. Due to the additional fact that DDEC is also rare, drugs already approved for use should speed up the translation of these findings into the clinic. To further confirm if CDK4 is a viable candidate for targeted therapy in DDEC, assessment of cyclin D1 levels by immunohistochemistry in many patient samples will be extremely informative to carry out.

Our work was also the first to establish that combining CDK4 and EGFR inhibition led to synergistic killing of SMARCA4 deficient endometrial cancer cells. There is emerging evidence in NSCLC and other cancer types that combining these cyclin-dependent kinase inhibitors and EGFR tyrosine kinase inhibitors may prevent or delay the acquisition of drug resistance (60-63). In these cases, the cell cycle-related proteins cyclin D1 and CDK4 are amplified and chemical inhibition of these factors, enhances the activity of anti-EGFR therapy through the suppression of Rb phosphorylation (60-63). Our findings as well as others underscore how in cancer the regulation of the cell cycle, may be a major factor wherein numerous genetic alterations converge **[Figure 4.31]**. In the future, the expression levels and phosphorylation status of CDK4 in addition to the mutation status of EGFR in our cell line models would be worth examining closer to fully understand the interaction network between CDK4 and EGFR in the context of endometrial cancer lacking SMARCA4 expression **[Figure 4.31]** (60-62). Additionally, while CRISPR gene editing has been a beneficial advancement in the field of cancer biology, our deeper understanding of how SMARCA4 loss impacts response to drug treatments may still be complicated by clonal selection. To date, most CRISPR gene editing methodologies require a handful of single progenitor cells to repopulate themselves which may select for certain genetic alterations as this single cell selection is a notable genetic bottleneck (64). The synergistic response to CDK4 and EGFR inhibition

was observed most prominently in HEC 116 Plasmid Derived EC cells not HEC 116 RNP Derived EC cells, with one of the main differences between the two cell line models of DDEC being the number of single clones that were mixed.

Lastly, we were the first to perform pre-clinical animal studies assessing the effectiveness of the CDK4 and EGFR inhibitors, palbociclib and gefitinib respectively, on mice implanted with serially passaged cell-derived xenografts of our HEC 116 Plasmid Derived DDEC-like model. SMARCA4 deficient cancers across tissue types have been reported to display abundant necrosis, a phenomenon we also observed with both clinical samples as well our DDEC-like cell line xenografts (65, 66). While it was initially surprising that no treatment regimen enhanced tumor necrosis significantly, CDK4 inhibition through the action of palbociclib has been shown to not substantially alter necrosis in breast cancer patients (67). A deeper investigation of treated tumors is presently being conducted by evaluating proliferation and apoptotic index by Ki67 immunostaining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), respectively. Across the tumor samples we examined by immunoblotting, administration of gefitinib alone or in combination orally, reached the tumor site and impaired activation of EGFR. Palbociclib was less effective at inhibiting Rb activation among the tumor samples we examined by immunoblotting during the first round of *in vivo* studies, but reduced phospho-Rb levels were seen when the identical drug treatment was carried out in the second round of experiments. As reported previously with the clinical samples, the portion of a DDEC tumor that undergoes dedifferentiation can vary substantially, ranging from 10-95% (66). The formation of necrotic regions is often a result of abnormal tumor capillary development and as tumors become larger, the interstitial

fluid pressure permanently occludes the small vessels and leads to blood stasis (68). Our histological observations of tumors formed from HEC 116 Plasmid Derived SMARCA4 deficient cells are consistent to what has been seen with patient tumors, thus the starting CDX sections implanted and expanded for the various rounds of *in vivo* studies likely display sufficient tumoral heterogeneity in addition to unpredictable and varying amounts of necrosis.

Reliably across repetitions, oral administration of gefitinib and palbociclib did not significantly increase median survival of mice or slow tumor growth. The majority of orally administered targeted therapies can be affected by food and the acidity of the stomach (69). While some minor fluctuations in body weight were observed, it could be assumed that the food intake of mice was adequate and thereby inhibitor uptake or bioavailability was sufficient (70). It is also well-documented that patient-derived xenograft (PDX) tumors undergo increasing molecular instability with serial transplantation, with acquired genomic alterations mainly associated with features of an aggressive phenotype such as proliferation (71, 72). Our DDEC-like cell line models due to their mismatch repair protein deficiency possess an even higher degree of genomic instability, which suggests that both SMARCA4 WT and SMARCA KO cells may have a propensity to accumulate mutations during the highly selective pressure of serial passaging (73, 74). Paired genomic and transcriptomic analysis of HEC 116 Plasmid Derived SMARCA4 KO and WT tumors comparing unpassaged cells to the third generation serially passaged tumor sections used for preclinical animal work will likely provide a great deal of insight into the mutations and gene expression changes acquired by our DDEC model *in vivo* that render our treatments less effective than they were *in vitro*. Ultimately the most suitable *in vivo*

model to test EGFR and CDK4 targeted therapy may be deriving tumor tissue with as little transplantation steps and directly as possible from primary DDEC tumors.

The results of this work also emphasize the limitations of modelling drug response *in vitro* on adherent cells and the importance of further validating promising therapies in a three-dimensional *in vivo* setting where factors such as hormones and growth factors can have a major influence on a drugs ability to act on tumor cells. *In vivo* with immunocompromised mice, only mouse stromal components such as extracellular matrix and cancer-associated fibroblasts can infiltrate and interact with our human HEC 116 Plasmid Derived tumors (75, 76). In lung cancer, crosstalk between tumor cells and stromal fibroblasts has been observed, wherein induction of resistance to tyrosine kinase inhibitors like gefitinib is developed and mediated through exposure to hepatocyte growth factor (HGF) and activation of its receptor, Met **[Figure 4.31]** (77, 78). To increase the efficacy of EGFR-targeted therapy, pre-treatment with HGF/Met inhibitors as has been proposed in lung cancer might be required to eliminate other epithelial cancers such as DDEC (78). Prolonged exposure to palbociclib has been shown to induce senescence in normal fibroblast cells and more recent studies examining melanoma have demonstrated that the impact of inhibition of CDK4 on normal cells needs to be considered when translating this targeted therapy into a clinical setting (79). The robust SASP induced by CDK4 treated fibroblasts promoted tumor growth in melanoma, undermining the therapeutic efficacy of palbociclib **[Figure 4.31]** (79). The conclusions found by examining lung carcinomas and melanoma remain to be explored in the context of SMARCA4 deficient cancers, either with co-culture experiments *in vitro* or possibly with immunocompetent mice and syngeneic murine models of DDEC *in vivo.*

Ongoing *in vivo* studies are addressing whether palbociclib is indeed an improvement on current standard of care, not only on our cell line model of DDEC but also patient models of DDEC very recently successfully cultured and expanded as CDX tissue (data not published). Overall, our findings in this chapter correlate with conclusions found for SMARCA4 deficient neoplasms, demonstrating that the best way to target DDEC with absent chromatin remodeling proteins, is likely with a SL approach (37, 38). As stated previously, any treatment option that might improve DDEC patient survival regardless of the SWI/SNF status of the tumor should be considered and combining CDK4 inhibition with emerging epigenetic or immune based therapies also warrants thorough and immediate exploration.

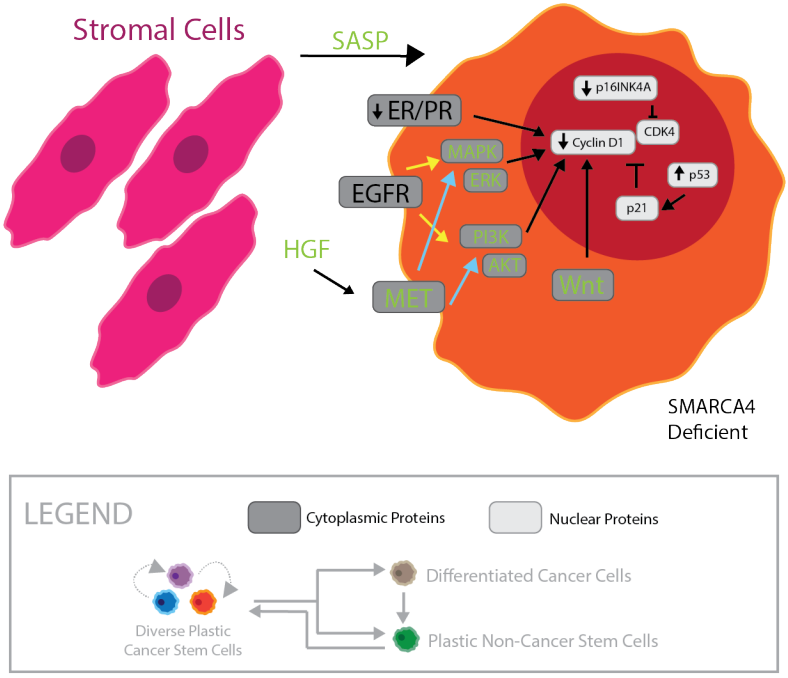


Figure 4.31. Proposed network of interactions in SMARCA4 deficient endometrial cancer cells that may explain their sensitivity *in vitro* and resistance *in vivo* to synthetic lethality-based therapies. Inflammatory and mitogenic factors from the surrounding tumor microenvironment likely influence surrounding SMARCA4 deficient EC cells. Results obtained within this chapter highlight some integral signalling pathways and secretory factors shown in green that warrant future investigation.

4.4. Methods

4.4.1. Cell Culture and Treatments:

HEC 116 and HEC 59 cells were obtained from JCRB and cultured in MEM medium supplemented with 10% fetal bovine serum (Sigma-Aldrich). Cells were passaged using 0.25% w/v trypsin (Life Technologies). All cells were cultured at 37°C with 5% CO_2 supplementation. Cells were authenticated at the Sick Kids Research Institute, Toronto, Ontario Canada and tested for mycoplasma in house using the PCR-based universal mycoplasma detection kit (ATCC). Paclitaxel and carboplatin (Sigma-Aldrich) were utilized in addition to palbociclib (Sigma-Aldrich), ribociclib (Cayman Chemical), erlotinib (Selleckchem) and gefitinib (Sigma-Aldrich) for *in vitro* studies. LC Laboratories produced the palbociclib and gefitinib for *in vivo* work.

4.4.2. shRNA Screening:

Pooled shRNA screening was accomplished as previously published (42). Briefly, Plasmid Derived HEC 116 SMARCA4 KO and WT EC cells were transduced with lentiviral particles containing a 90K shRNA library with over 200x hairpin representation. Post-transduction cells were passaged for at least 17 days with genomic DNA collected at three timepoints for analysis: T0, T7 and T15, PCR to detect the hairpins was accomplished by large-scale PCR on the genomic DNA. Thermocycling conditions were as follows: 98 °C for 3 min; 98 °C for 10 s; 55 °C for 15 sec; 72 °C for 15 sec; 72 °C for 5 min; ending and holding at 4 °C. PCR products were subjected to purification and XhoI (New England Biolabs) digestion. Half-hairpins were further purified before hybridization to a UT-GMAP 1.0 microarray (Affymetrix Inc).

4.4.3. Computational Scoring of Pooled shRNA Screen:

Computational scoring of the pooled shRNA screening was carried out as previously published (42). In short, at each time point, signal intensity for each hairpin was normalized and converted to log2 scale for both Plasmid Derived HEC 116 SMARCA4 KO and WT EC cells. The DCC between the Plasmid Derived HEC 116 SMARCA4 KO and WT EC cells were calculated for time points relative to the corresponding previous time point using the formula:

$$DCC = \sum_{t=1}^{T}(x_{t,k}^{KO} - x_{t-1,k}^{KO}) - \sum_{t=1}^{T}(x_{t,k}^{WT} - x_{t-1,k}^{WT})$$

The normalized signal intensity at time point *t* (0,…,T) and replicate *k* (1,…,K) was denoted by $x_{t,k}^{KO}$ for the HEC 116 Plasmid Derived SMARCA4 KO samples. $x_{t,k}^{WT}$ denotes the HEC 116 Plasmid Derived SMARCA4 WT samples, similarly. For each gene, DCC fitness scores were calculated by taking the two hairpin DCC values that were the most negative values for that particular gene.

$$DCC_g = arg_{h,h'}^{min}\left[DCC_{g,h} + DCC_{g,h'}\right]/2$$

Random shuffling of the DCC scores or a permutation test was performed subsequently and repeated until an empirical distribution of the DCC fitness scores over all the genes was accomplished. Significant p-values were estimated for each observed fitness score as the frequency of randomized, shuffled DCC with more negative scores.

$$p = \frac{1}{NL}\sum_{r=1}^{NL} I(DCC_r < DCC_g)$$

N represents the number of genes, L is the number of repeats required to construct an empirical distribution, DCC_r is the randomized shuffle with the more negative score and I() is a binary indicator that gives 1 for a true statement and 0 if otherwise.

4.4.4. Protein Extraction:

Protein was extracted from adherent cells directly on plates using RIPA Lysis and Extraction buffer (Thermo Fisher Scientific) with added Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Lysed cells were incubated at 4°C prior to centrifugation at 13000 rpm for 20 minutes at 4°C to pellet insoluble cell debris. Supernatants containing protein were quantified using the DC Protein Assay (Bio-Rad) and measured on a FLUOstar Omega plate reader (BMG LABTECH). Protein was also extracted from flash-frozen tumors that were ground by mortar and pestle and resuspended in RIPA Lysis and Extraction buffer (Thermo Fisher Scientific). Following sonication, lysed tumor cells were incubated, centrifuged and quantified in a similar manner as the lysates from adherent cells.

4.4.5. Western Blotting:

4X Laemmli sample buffer (BioRad) with 5% (v/v) 2-Mercaptoethanol (Sigma-Aldrich) were added to protein samples prior to being boiled for 5 minutes. 7.5% SDS-PAGE gels were utilized and then transferred onto nitrocellulose membranes (Bio-Rad) at 100 V for 90 minutes. Membranes were blocked with 5% milk in TBST for one hour at room temperature, followed by primary antibody incubation overnight at 4°C. Membranes were washed in TBS + 0.1% Tween before incubation with HRP-conjugated secondary antibodies at a concentration of 1:3000 (BioRad) for 1 hour at room temperature. Excess secondary antibody was washed from the membrane prior to utilizing Clarity Western

ECL Substrate (BioRad) for detection of chemiluminescent signal. Film (Fujifilm) was used to image the western blots which were subsequently quantified by densitometry using ImageJ (80).

Primary antibody dilutions are as follows -

Primary Antibody	Dilution
Anti-Cyclin D1 [EPR2241] C-terminal (AbCam)	1:5000
Anti-Phospho-EGF Receptor [Tyr1068] (Cell Signaling Technology)	1:5000
Anti-EGF Receptor total XP [D38B1] (Cell Signaling Technology)	1:1000
Anti-Rb phospho S807/811 [9308] (Cell Signaling Technology)	1:1000
Anti-Rb total 4H1 [9309] (Cell Signaling Technology)	1:2000
Anti-Beta Actin (Santa Cruz)	1:10000
Anti-Beta Tubulin (Licor)	1:7000

4.4.6. Flow Cytometry:

One million HEC 116 Plasmid Derived SMARCA4 KO or WT cells were stained with Zombie Aqua at RT for twenty minutes followed by washing and incubation with diluted fluorochrome-conjugated antibodies against EGFR in FACS buffer. Prior to antibody incubation, EC cells were blocked on ice for 30 minutes in FACS buffer containing Trustain FcX (BioLegend). In a volume of 20 µL, the antibody dilution of 1:200 of APC anti-human EGF receptor (Mitenyi Biotec) was added and cells were incubated for another 10-15 minutes on ice. Following washing, all cells were fixed in FACS buffer containing 2% formaldehyde, stored at 4°C, and samples were acquired within one week. Antibody-stained cells were analyzed using a FACS CANTO II (Becton Dickinson) and samples were resuspended in 300 µL before analysis. Acquired data was analyzed using

FlowJo software (Tree Star, v10) with fluorescence minus one (FMO) controls as well as forward and side scatter to set gates.

4.4.7. Luminescent Cell Viability Assays:

Cells (640 cells/well) were seeded into flat bottomed, opaque-wall 96 well microplates (Corning). At 48 hours after seeding, serial dilutions of carboplatin, CDK4/6 inhibitors or EGFR inhibitors were added to cells to final drug concentrations within the range of 2-20 μM for 72 hours until cell viability was measured as per the Cell Titer-Glo luminescent cell viability assays (Promega) manufacturer's protocol. Luminescence was measured at 560 nm using the FLUOstar Omega plate reader (BMG Labtech). Percent cell viability in the presence of the drugs was determined by normalizing to vehicle controls (dimethyl sulfoxide (DMSO)) after subtraction of background signal.

4.4.8. Clonogenic Growth Assays:

In six-well culture plates, cells were seeded at the following densities:
50 (untreated), 100 (5 μM), 200 (10 μM), 400 (12 μM), 800 (14 μM) and 1600 (18 μM) cells/well.

72 hours post-seeding, cells were treated with above-mentioned doses of either paclitaxel, palbociclib, ribociclib, erlotinib or gefitinib. Drug containing media was replaced every three days for a total growth period of 8 days. The resulting colonies were stained with 0.5 % crystal violet solution in methanol and washed with PBS to remove excess staining solution. Colonies of >50 cells were counted and plating efficiency determined by the following equation:

$$PE = \frac{\#\ colonies\ formed}{\#\ colonies\ seeded}$$

$$SF = \frac{\#\ colonies\ formed\ after\ treatment}{\#\ cells\ seeded} * PE$$

4.4.9. Combination Drug Treatments & Determination of Bliss Combination Indices:

To ascertain the effects of different drug combinations on cell viability, 640 cells/well were seeded into Corning flat bottomed, opaque-wall 96 well microplates (ThermoFisher Scientific) and incubated at 37°C for 48 hours before addition of inhibitors. A consistent DMSO percentage of 2% that was considered negligible to cell viability was maintained across the assay plates. Following addition of drugs, plates were incubated for another 72 hours prior to measurement of cellular ATP levels by Cell Titer-Glo luminescent cell viability assays (Promega). All experiments were performed in triplicate (4 technical replicates and 3 biological replicates). Cell viability was calculated relative to untreated cells. Synergy scores were determined using the Bliss model similar to the methodology employed in (81). Combinations of drugs that had 1) a Bliss combination index score greater than one were defined as antagonistic 2) a Bliss synergy score less than one exhibited synergistic behavior and 3) Bliss combination index scores less than 0.7 were considered as strongly synergistic in nature.

4.4.10. In vivo Studies:

Animal experiments were carried out in accordance with the guidelines laid out by the Canadian Council on Animal Care and were approved by the University of Alberta Animal Policy and Welfare Committee. HEC 116 Plasmid Derived SMARCA4 KO and WT PDX tumor sections approximately 2 mm in diameter, were expanded from tumors generated by serial passaging and collected at the third generation. A single section was implanted in subcutaneously into 6-8 week-old female NOD.Cg-PrkdcscidIl2rg (NSG)

mice. Ten days post-implantation, mice were randomized into treatment groups: either vehicle (0.3% methylcellulose (Wako), 0.1% Poly-80 (Sigma-Aldrich) at pH 4), palbociclib, gefitinib or combination therapy consisting of both palbociclib and gefitinib. Treatment (63 mg/kg by gavage) was continued biweekly until tumors reached a final diameter of 10 mm. Measurements of body weight and tumor size were carried out twice a week as well. Digital calipers were utilized to measure the length and width of tumors to calculate tumor area over time and tumor volume upon excision. Mice were euthanized at experimental endpoint when tumor size equaled approximately 60 mm^2. Tumors were subjected to weighing post excision and split either to be flash-frozen and stored at -80°C or fixed in formalin for eventual paraffin embedding and sectioning.

4.4.11. Examination of H&E stained Tumor Sections for Necrosis:

Post sectioning, tumors were stained with H&E and the entire cross-sectional area of the slide was scanned by an Aperio Digitial Pathology slide scanner. Image analysis software (Image Scope; Leica Biosystems) was used to convert the whole slide scans into TIFF image formats that could be further analysed further in ImageJ. Initially, thresholding against background pixel intensity compared to intact tumor tissue was carried out, followed by a second round of thresholding solely against necrotic regions. Areas of necrosis were reported as a percentage over the total tumor area.

4.4.12. Histone Mark Chromatin Immunoprecipitation Sequencing (ChIP-Seq) & RNA-Sequencing (RNA-Seq):

Nucleosome density chromatin immunoprecipitation sequencing and bulk RNA sequencing were carried out in collaboration with the authors of and as reported in (82, 83). Standard operating procedures for the generation of ChIP-Seq and RNA-seq

libraries are also available at http://www.epigenomes.ca/protocols-and-standards). Briefly, cells were lysed and digested by microccocal nuclease (MNase). Immunoprecipitation was performed using a validated antibodies against H3K27ac. DNA fragments were then stripped from histones, purified, and subjected to Illumina library construction. Libraries were sequenced on an Illumina HiSeq 2500 sequencing platform following the manufacturer's protocols. Sequence reads were aligned to hg_38_not alt using Burrows-Wheeler Aligner (BWA) (version 0.7.6) and converted to bam format by SAMtools (version 0.1.13) (84). Statistically significant enriched regions were identified by MACS2 using a corrected p-value of 0.01 and 0.05 for narrow and broad peak, respectively (85).

RNA-Seq libraries were sequenced on an Illumina HiSeq 2500 sequencing platform following the manufacturer's protocols. RNA-seq analysis was performed as previously described (86, 87). Briefly, adaptor sequences were stripped and reads were aligned to a transcriptome reference consisting of genomic sequences (GRCh38) supplemented by read-length-specific exon–exon junction sequences. An in-house RNA quality control and analysis pipeline was used to generate a report and calculate a normalization constant for computing reads per kilobase per million mapped reads (RPKM) values. Pairwise comparisons between different sample types were performed to identify differentially expressed genes using a custom DEfine matlab tool (cut-offs: RPKM >0.005; number of reads >25; FDR <0.015).

4.5. References

1. Murali R, Davidson B, Fadare O, Carlson JA, Crum CP, Gilks CB, et al. High-grade Endometrial Carcinomas: Morphologic and Immunohistochemical Features, Diagnostic Challenges and Recommendations. Int J Gynecol Pathol. 2019;38 Suppl 1:S40-S63.

2. Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, et al. Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. Cell. 2018;173(2):291-304 e6.
3. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47(D1):D941-D7.
4. International Cancer Genome C, Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, et al. International network of cancer genome projects. Nature. 2010;464(7291):993-8.
5. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet. 2013;45(10):1113-20.
6. Huang A, Garraway, L.A., Ashworth, A., Weber, B. Synthetic lethality as an engine for cancer drug target discovery. Nature Reviews Drug Discovery 2019;19:23-8.
7. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.
8. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol. 2010;11(2):121-8.
9. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010;362(25):2380-8.
10. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012;13(3):239-46.
11. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011;12(8):735-42.
12. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 2013;31(27):3327-34.
13. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(2):113-25.
14. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med. 2014;371(23):2167-77.
15. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368(25):2385-94.
16. Shaw AT, Engelman JA. Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med. 2014;370(26):2537-9.

17. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med. 2012;366(2):109-19.
18. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. 2014;505(7484):495-501.
19. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, et al. Comprehensive Characterization of Cancer Driver Genes and Mutations. Cell. 2018;174(4):1034-5.
20. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463(7283):899-905.
21. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373(2):123-35.
22. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373(17):1627-39.
23. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-33.
24. Hoffman GR, Rahal R, Buxton F, Xiang K, McAllister G, Frias E, et al. Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. Proc Natl Acad Sci U S A. 2014;111(8):3128-33.
25. Wilson BG, Helming KC, Wang X, Kim Y, Vazquez F, Jagani Z, et al. Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. Mol Cell Biol. 2014;34(6):1136-44.
26. Oike T, Ogiwara H, Tominaga Y, Ito K, Ando O, Tsuta K, et al. A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. Cancer Res. 2013;73(17):5508-18.
27. Helming KC, Wang, X., Wilson, BG., Vazquez, F., Haswell, JR., Manchester, HE., Kim, Y., Kryukov, G., Ghandi, M., Aguirre, AJ., Jagani, Z., Wang, Z., Garraway, LA., Hahn, WC., Roberts, WM. . ARID1B is a specific vulnerability in ARID1A-mutant cancers. Nature Medicine 2014;20:251-4.
28. Mavrakis KJ, McDonald ER, 3rd, Schlabach MR, Billy E, Hoffman GR, deWeck A, et al. Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. Science. 2016;351(6278):1208-13.
29. Marjon K, Cameron MJ, Quang P, Clasquin MF, Mandley E, Kunii K, et al. MTAP Deletions in Cancer Create Vulnerability to Targeting of the MAT2A/PRMT5/RIOK1 Axis. Cell Rep. 2016;15(3):574-87.
30. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917-21.
31. Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. Science. 2017;355(6330):1152-8.

32. Ashworth A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol. 2008;26(22):3785-90.
33. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361(2):123-34.
34. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913-7.
35. Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, Wu JN, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. Nat Med. 2015;21(12):1491-6.
36. Tagal V, Wei S, Zhang W, Brekken RA, Posner BA, Peyton M, et al. SMARCA4-inactivating mutations increase sensitivity to Aurora kinase A inhibitor VX-680 in non-small cell lung cancers. Nat Commun. 2017;8:14098.
37. Xue Y, Meehan, B., Fu, Z., Wang, XQD., Fiset, PO., Rieker, R., Levins, C., Kong, T., Zhu, X., Morin, G., Skerritt, L., Herpel, E., Venneti, S., Martinez, D., Judkins, AR., Jung, S., Camilleri-Broet, S., Gonzalez, AV., Guiot, M-C., Lockwood, WW., Spicer, JD., Agaimy, A., Pastor, WA., Dostie, J., Rak, J., Foulkes, WD., Huang, S. SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer. Nature communications. 2019;10(557).
38. Xue Y, Meehan, B., Macdonald, E., Venneti, S., Wang, XQD., Witokowski, L., Jelinic, P., Kong, T., Martinez, D., Morin, G., Firlit, M., Abedini, A., Johnson, RM., Cencic, R., Patibandla, J., Chen, H., Papadakis, AI., Auguste A., de Rink, I., Kerkhoven, RM., Bertos, N., Gotlieb, WH., Clarke, BA., Leary, A., Witcher, M., Guiot, M-C., Pelletier J., Dostie, J., Park, M., Judkins, AR., Hass, R., Levine, DA., Rak, J., Vanderhyden, B., Foulkes, WD., Huang, S. CDK4/6 inhibitors target SMARCA4-determined cyclin D1 deficiency in hypercalcemic small cell carcinoma of the ovary. Nature communications. 2019;10(558).
39. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discov. 2016;6(7):740-53.
40. Tessier-Cloutier B, Coatham M, Carey M, Nelson GS, Hamilton S, Lum A, et al. SWI/SNF-deficiency defines highly aggressive undifferentiated endometrial carcinoma. J Pathol Clin Res. 2021;7(2):144-53.
41. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, et al. Current recommendations and recent progress in endometrial cancer. CA: a cancer journal for clinicians. 2019;69(4):258-79.
42. Paul J, Toosi, B., Vizeacoumar, FS., Bhanumathy, KK., Li, Y., Gerger, C., Zawily, AE., Freyweld, T., Anderson, DH., Mousseau, D., Kanthan, R., Zhang, Z., Vizeacoumar, FJ., Freywald, A. Targeting synthetic lethality between the SRC kinase and the EPHB6 receptor may benefit cancer treatment. Oncotarget. 2016;7(31):50027-42.
43. Hart T, Brown KR, Sircoulomb F, Rottapel R, Moffat J. Measuring error rates in genomic perturbation screens: gold standards for human functional genomics. Mol Syst Biol. 2014;10:733.

44. Hirsch F, Varella-Garcia, M., Bunn, PA., Di Maria, MV., Veve, R., Bremnes, RM., Baron, AE., Zeng, C., Franklin, WA. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol. 2003;21:3798-807.
45. Hamilton E, Infante JR. Targeting CDK4/6 in patients with cancer. Cancer Treat Rev. 2016;45:129-38.
46. Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J Clin Oncol. 2004;22(16):3238-47.
47. Wang F, Wang, S., Wang, Z., Duan, J., An, T., Zhao, J., Bai, H., Wang, J., Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education). Phosphorylated EGFR expression may predict outcome of EGFR-TKIs therapy for the advanced NSCLC patients with wild-type EGFR. Journal of Experimental & Clinical Cancer Research 2012;31(65).
48. Decaudin D, Le Tourneau C. Combinations of targeted therapies in human cancers. Aging (Albany NY). 2016;8(10):2258-9.
49. Rodon J, Perez, J., Kurzrock, R. . Combining targeted therapies: practical issues to consider at the bench and bedside. . Oncologist 2010;15(1):37-50.
50. Schuster A, Erasimus H, Fritah S, Nazarov PV, van Dyck E, Niclou SP, et al. RNAi/CRISPR Screens: from a Pool to a Valid Hit. Trends Biotechnol. 2019;37(1):38-55.
51. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clin Cancer Res. 2013;19(19):5320-8.
52. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. Epidermal growth factor receptor: mechanisms of activation and signalling. Exp Cell Res. 2003;284(1):31-53.
53. Navolanic PM, Steelman LS, McCubrey JA. EGFR family signaling and its association with breast cancer development and resistance to chemotherapy (Review). Int J Oncol. 2003;22(2):237-52.
54. Shelton JG, Steelman LS, Abrams SL, White ER, Akula SM, Franklin RA, et al. Conditional EGFR promotes cell cycle progression and prevention of apoptosis in the absence of autocrine cytokines. Cell Cycle. 2005;4(6):822-30.
55. Blagosklonny MV. Analysis of FDA approved anticancer drugs reveals the future of cancer therapy. Cell Cycle. 2004;3(8):1035-42.
56. O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol. 2016;13(7):417-30.
57. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From Discovery to Therapy. Cancer Discov. 2016;6(4):353-67.
58. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov. 2015;14(2):130-46.
59. Turner NC, Ro J, Andre F, Loi S, Verma S, Iwata H, et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2015;373(3):209-19.
60. La Monica S, Fumarola C, Cretella D, Bonelli M, Minari R, Cavazzoni A, et al. Efficacy of the CDK4/6 Dual Inhibitor Abemaciclib in EGFR-Mutated NSCLC Cell Lines with Different Resistance Mechanisms to Osimertinib. Cancers (Basel). 2020;13(1).

61. Zhou J, Wu Z, Wong G, Pectasides E, Nagaraja A, Stachler M, et al. CDK4/6 or MAPK blockade enhances efficacy of EGFR inhibition in oesophageal squamous cell carcinoma. Nat Commun. 2017;8:13897.
62. Nie H, Zhou X, Shuzhang D, Nie C, Zhang X, Huang J. Palbociclib overcomes afatinib resistance in non-small cell lung cancer. Biomed Pharmacother. 2019;109:1750-7.
63. Goel S, Wang Q, Watt AC, Tolaney SM, Dillon DA, Li W, et al. Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers with CDK4/6 Inhibitors. Cancer Cell. 2016;29(3):255-69.
64. Giuliano CJ, Lin A, Girish V, Sheltzer JM. Generating Single Cell-Derived Knockout Clones in Mammalian Cells with CRISPR/Cas9. Curr Protoc Mol Biol. 2019;128(1):e100.
65. Callegaro-Filho D, Gershenson DM, Nick AM, Munsell MF, Ramirez PT, Eifel PJ, et al. Small cell carcinoma of the ovary-hypercalcemic type (SCCOHT): A review of 47 cases. Gynecol Oncol. 2016;140(1):53-7.
66. Karnezis A, Hoang, L., Coatham, M., Ravn, S., Almadani, N., Cloutier, B., Irving, J., Meng, B., Li, X., Chow, C., McAlpine, J., Kuo, K-T., Mao, T-L., Djordjevic, B., Soslow, R., Huntsman, D., Gilks, B., Koebel, M., Lee, C-H. . Loss of switch/sucrose non-fermenting complex protein expression is associated with dedifferentiation in endometrial carcinomas. Mod Pathol. 2016.
67. Arnedos M, Bayar MA, Cheaib B, Scott V, Bouakka I, Valent A, et al. Modulation of Rb phosphorylation and antiproliferative response to palbociclib: the preoperative-palbociclib (POP) randomized clinical trial. Ann Oncol. 2018;29(8):1755-62.
68. Forster JC, Harriss-Phillips WM, Douglass MJ, Bezak E. A review of the development of tumor vasculature and its effects on the tumor microenvironment. Hypoxia (Auckl). 2017;5:21-32.
69. Carrington C. Oral targeted therapy for cancer. Aust Prescr. 2015;38(5):171-6.
70. Ruiz-Garcia A, Plotka A, O'Gorman M, Wang DD. Effect of food on the bioavailability of palbociclib. Cancer Chemother Pharmacol. 2017;79(3):527-33.
71. Wegner CS, Hauge A, Andersen LMK, Huang R, Simonsen TG, Gaustad JV, et al. Increasing aggressiveness of patient-derived xenograft models of cervix carcinoma during serial transplantation. Oncotarget. 2018;9(30):21036-51.
72. Shi J, Li Y, Jia R, Fan X. The fidelity of cancer cells in PDX models: Characteristics, mechanism and clinical significance. Int J Cancer. 2020;146(8):2078-88.
73. Mandal R, Samstein RM, Lee KW, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. Science. 2019;364(6439):485-91.
74. Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. Nature. 2015;521(7550):81-4.
75. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998-1013.
76. Schneeberger VE, Allaj V, Gardner EE, Poirier JT, Rudin CM. Quantitation of Murine Stroma and Selective Purification of the Human Tumor Component of Patient-Derived Xenografts for Genomic Analysis. PloS one. 2016;11(9):e0160587.
77. Gusenbauer S, Vlaicu P, Ullrich A. HGF induces novel EGFR functions involved in resistance formation to tyrosine kinase inhibitors. Oncogene. 2013;32(33):3846-56.

78. Wang W, Li Q, Yamada T, Matsumoto K, Matsumoto I, Oda M, et al. Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. Clin Cancer Res. 2009;15(21):6630-8.
79. Guan X, LaPak KM, Hennessey RC, Yu CY, Shakya R, Zhang J, et al. Stromal Senescence By Prolonged CDK4/6 Inhibition Potentiates Tumor Growth. Mol Cancer Res. 2017;15(3):237-49.
80. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671-5.
81. Bukhari AB, Lewis CW, Pearce JJ, Luong D, Chan GK, Gamper AM. Inhibiting Wee1 and ATR kinases produces tumor-selective synthetic lethality and suppresses metastasis. J Clin Invest. 2019;129(3):1329-44.
82. Lorzadeh A, Bilenky M, Hammond C, Knapp D, Li L, Miller PH, et al. Nucleosome Density ChIP-Seq Identifies Distinct Chromatin Modification Signatures Associated with MNase Accessibility. Cell Rep. 2016;17(8):2112-24.
83. Lorzadeh A, Lopez Gutierrez R, Jackson L, Moksa M, Hirst M. Generation of Native Chromatin Immunoprecipitation Sequencing Libraries for Nucleosome Density Analysis. J Vis Exp. 2017(130).
84. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589-95.
85. Feng J, Liu, T., Quin, B., Zhang, T., Liu, XS. Identifying ChIP-seq enrichment using MACS. Nat Protoc. 2012.
86. Nguyen LV, Pellacani D, Lefort S, Kannan N, Osako T, Makarem M, et al. Barcoding reveals complex clonal dynamics of de novo transformed human mammary cells. Nature. 2015;528(7581):267-71.
87. Gascard P, Bilenky M, Sigaroudinia M, Zhao J, Li L, Carles A, et al. Epigenetic and transcriptional determinants of the human breast. Nat Commun. 2015;6:6351.

5
Conclusions and Future Directions

Improvements in the diagnostic process and advancements in therapeutic approaches are still both extremely warranted for patients with DDEC. It has become increasingly understood that DDEC patients have poor responses to conventional chemotherapy but thorough investigation of the rare gynecological malignancy at the molecular level has prolonged adoption of any sort of specialized targeted therapy. In Chapter 2, we examined patient samples by various next-generation sequencing approaches to begin uncovering the unique features of endometrial cancers with both undifferentiated and differentiated regions. In Chapter 3, having discovered chromatin remodeling protein loss as a potential driver of DDEC, I developed cell line models of DDEC by knocking out SMARCA4 that we then characterized both *in vitro* and *in vivo.* Finally, in Chapter 4, we identified and validated interacting partners with SMARCA4 that are synthetic lethal in the context of the endometrium.

5.1. Frameshift mutations leading to subsequent loss of chromatin remodelling proteins are a prominent feature of the undifferentiated regions of DDEC.

Across the series of clinical cases we examined, over two thirds were found by targeted DNA sequencing to possess frameshift mutations to one of three SWI/SNF chromatin remodeling subunits (1, 2). These findings were validated by immunohistochemistry where expression of SMARCA4, SMARCB1 and ARID1B was found to be lost only within the undifferentiated regions of DDEC neoplasms (1, 2). ARID1A has long been known to be an epigenetic regulator frequently mutated in cancers of the ovary and endometrium yet the discovery that SMARCA4 mutations may be a driver of cancer adaptation and progression in more rare and aggressive cases of cancer of the

gynecological tract has only more recently been revealed (3, 4). As SWI/SNF complexes broadly control chromatin accessibility, this chromatin remodelling complex has diverse and different functions depending on subunit composition (4, 5). The BAF SWI/SNF complex which contains ARID1A/B has been suggested to be key to the regulation of enhancer regions (5). Although, there is likely a shared mechanism by which all nine recurrently mutated subunits similarly operate as tumor suppressors in cancer (4, 6-8). Subsequent work has corroborated these novel discoveries in Chapter 2 related to SWI/SNF dysregulation that we performed on small cohorts of DDEC patients (9). Since publication of our findings in Chapter 2 in 2016, expanded series of clinical DDEC cases harboring inactivating mutations to core components of the SWI/SNF complex continue to display more aggressive clinical behavior, progressing more rapidly and frequently presenting with more extrauterine spread than SWI/SNF-intact DDEC (9). It should be noted that a higher proportion of DDEC cases, 44% were found to have co-lost ARID1A and ARID1B within the Tessier-Cloutier *et al.* study published in 2020, an increase of 20% over what was evident within the cohort published in Coatham *et al.* in 2016 (2). CRISPR gene editing approaches employed in this work that resulted in models of DDEC exhibiting SMARCA4 loss, were unsuccessful when ARID1A/B deficient cell line models of DDEC were attempted in a similar manner. Fortunately, we now have patient derived models of ARID1A/B deficient DDEC in culture that together with the information we acquired studying the cell line models of DDEC deficient in SMARCA4 in Chapter 3, can hopefully guide the successful development of similar cell line models of DDEC with ARID1A/B loss. While DDEC is still considered a rare cancer, the study of it may reveal mechanisms that are relevant and underlying many other cancers presenting with a

variety of SWI/SNF deficient forms. Despite massive improvements in our genomic understanding of the subtypes of gynecological cancers, deeper genetic profiling combined with studies confirming how observed genetic alterations may contribute to malignant disease, was necessary for more personalized treatment approaches to ever be proposed and hopefully soon, adopted.

5.2. The undifferentiated portions of DDEC tumors are characterized by markers of epithelial to mesenchymal transition and stemness

Beyond demonstrating dysregulation of chromatin remodelling in DDEC for the first time, our findings studying clinical SMARCA4 deficient DDEC confirmed previously reported features of gynecological dedifferentiation such as PAX8 and ER loss and characterized the behavior of potential novel biomarkers of DDEC (10, 11). Our gene expression profiling datasets constitute a noteworthy contribution to researchers and clinicians' understanding of DDEC. Studies performed by various groups on different patient specimens have subsequently confirmed many of the gene expression changes we observed within our cohort such as loss of claudin 4 and upregulation of EMT-related transcription factors, Zeb1/2 and Twist1 (12, 13). Other cancers afflicting adults wherein SMARCA4 loss can be linked to progression such as NSCLC and SCCOHT, have also been associated with EMT (14-16). Re-expression of SMARCA4 has even resulted in differentiation of a SCCOHT cell line in terms of expression of claudin 4 (14). These markers and their translatability into clinical biomarkers will continue to be determined as their exact prevalence across cancers, specifically gynecological carcinomas are further elucidated. Unfortunately, as DDEC can still be frequently misdiagnosed and occurs more rarely, our sample size was small, but it will be worthwhile to keep expanding our gene

expression profiling studies as we acquire more patient samples with both with same and different SWI/SNF mutation statuses.

Molecularly defining DDEC by SWI/SNF status is important as absence of SMARCA4, ARID1B, and SMARCB1 can signify a more aggressive clinical course. Other less recurrent genetic events though may still be worth translating into clinical biomarkers to further bolster diagnoses that remain reliant on histology, which is highly variable with these neoplasms. As more specific treatment options become available for DDEC, it may be useful to validate multiple marker panels that would ensure patients with DDEC are not missed for either targeted or immuno-therapies. As has been shown with proteomics-based discovery work, the performance of individual markers in IHC is typically worse than predicted by the methodologies producing large datasets (17). Our attempt to translate Sox2 into a biomarker for the differential diagnosis of DDEC proved challenging and is not an uncommon result as many potentially prognostic targets suffer due to a lack of available quality antibodies (17). Adoption of a multi-marker model holds immense promise, as targeted sequencing approaches are unlikely to enter current clinical workflows when IHC-based classification is currently both efficient and sensitive (17). While absence of MMR proteins, PAX8, ER, and claudins have all been proven to be reliable markers of the presence of undifferentiated components in an otherwise well-differentiated endometrial cancer, moving forward implementation of consistently upregulated markers within the undifferentiated cells such as ZEB1 or BMP7 could substantially improve patient outcomes by allowing for differential diagnosis based on presence rather than absence of protein expression.

5.3. *In vitro* knockout of SMARCA4 in endometrial cancer cells leads to senescence

Since 2013, CRISPR gene editing has emerged as a powerful scientific advancement allowing for the obtainment of models of disease not previously thought of as attainable (18, 19). The ability of Cas9 to introduce nicks in DNA that in turn allow NHEJ to introduce frameshift mutations in targets of interest allowed us, in Chapter 3, to produce endometrial cancer cells lacking SMARCA4 expression (18, 19). Our group and others have demonstrated on numerous occasions that DDEC preferentially occurs in a MMR deficient genetic background, which is also a common feature among commercially available endometrial cancer cell lines (20-22). In Chapter 3, we characterized three distinct SMARCA4 derivations and demonstrated that immediately following SMARCA4 knockout, endometrial cancer cells display several qualities associated with senescence. While not the first work to link SMARCA4 with senescence, we are the first to propose in the context of the endometrium that senescence induced by SMARCA4 loss is a prerequisite to complete cellular dedifferentiation. Knowledge on epigenetics influencing senescence is growing, with current work exposing a role for enhancer remodelling prompting senescence-associated gene expression (23). Interestingly, AP-1 superfamily members can preserve senescence programming through binding to enhancer chromatin and their depletion is sufficient to partially revert the phenotypic phenomenon but does not lead cells to fully re-enter the cell cycle and proliferate (23). Having demonstrated AP-1 transcription factor dysregulation in the SMARCA4 deficient HEC 116 Plasmid Derived model of DDEC upon *in vivo* serial passaging, it would be interesting to probe the senescent code of these cells utilizing ChIP-seq experiments for enhancer specific, histone modifications H3K4me1 and H3K27ac, upon *in vivo* exposure and over time.

Together with paired genomic and transcriptomic profiling it may be possible to definitively claim AP-1 imprints a reversible senescence program in DDEC.

A hallmark of senescence overlooked in the results published within this thesis is the state of the mitochondria of SMARCA4 knockout EC cells. Typically, during senescence, NAD+/NADH ratios are low, reactive oxygen species are found at higher levels and there is reduced amounts of ATP, all of which lead to an increase in glycolysis (24, 25). Classically, to fully transform into aggressive carcinomas, cancer cells must overcome conditions of mitochondrial dysfunction and be capable of regenerating NAD+ under hypoxic conditions (25, 26). Recently, STAT3 in cancer cell mitochondria has been connected to senescence with its deletion in stem cells linked to the acquisition of senescence (26, 27). As we observed with our cell line model of DDEC, activation of p53 within the undifferentiated regions of endometrial tumors, it may be critical to assess STAT3 levels within our system, as activated p53 and Rb was observed upon depletion of STAT3 in normal human fibroblasts (26). Furthermore, the metabolic enzyme complex, hydride transfer complex (HTC), consisting of malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1), and pyruvate carboxylase (PC) was also demonstrated within the same paper to play a vital role in tumor formation by promoting senescent escape, thus a deeper examination of the metabolism of our gene edited EC cells *in vitro* and upon serial passaging is warranted (26). Cycling of hydride ions from NADH to NADP through the catalytic activity of the HTC counteracts senescence and PC has been found at high levels in NSCLC (26, 28). Mutations to SWI/SNF complexes and their dysregulation on cancer cell metabolism has been demonstrated in lung cancer but little has been

published regarding mitochondrial DNA content or oxidative phosphorylation dependency of SWI/SNF deficient gynecological cancers (29).

Our work *in vitro* highlights the importance of developing models of cancer that can potentially illuminate the path well-differentiated cancer cells take upon undergoing dedifferentiation. Utilizing only existing patient samples may have resulted in missing some phenomena entirely or at the very least convoluted discoveries that would be difficult to experimentally confirm.

5.4. Knockout of SMARCA4 in endometrial cancer cells recapitulates features of clinical DDEC *in vivo*

DDEC tumors removed from patients are characterized by the following morphology within their undifferentiated regions: monotonous cells growing in solid sheets with no discernable pattern (1, 2, 9). In Chapter 3, we subcutaneously injected EC cells subjected to CRISPR gene editing into immune-compromised mice and observed tumor growth and histology over several generations. A SMARCA4 deficient model of DDEC capable of forming tumors with both undifferentiated and differentiated portions mixed has never been published but is reported here for the first time. While we ruled out some previously reported events connected to dedifferentiation in some SMARCA4 deficient cancer types such as low levels of redundant chromatin remodeling complex subunit SMARCA2 in undifferentiated cells, we have not yet ruled out clonal outgrowth as a major contributor to dedifferentiation in our model (30). It is still unclear whether dedifferentiation in the context of the endometrium is a stochastic event characterized by the accumulation of one clone and associated with a mutation. DNA barcoding experiments are currently ongoing in which cells were transduced to contain a

single barcode, which upon application of selective pressure such as serial passaging in our case, can be amplified by PCR and quantified with next generation sequencing (31, 32). There are also emerging bioinformatics pipelines which can also predict from single-cell ATAC datasets if populations of clones are dominant and in our case possibly coincide with the occurrence of dedifferentiation we observe with the SMARCA4 KO EC cells *in vivo* (33). As the field of epigenetics continues burgeoning it becomes more and more unlikely that genetic events alone result in endometrial cancer cell dedifferentiation and the likelihood of distinct populations of cells with shared epigenetic features rises.

Another mechanism we have yet to adequately probe is the degree to which undifferentiated regions arise in relation to or as a response to hypoxia. Hypoxia is a known inducer of dedifferentiation and promoter of stem cell-like phenotypes in cancer (34, 35). Prolonged low oxygen levels are a feature cells within fast-growing tumors that are exceeding vascular formation are subjected to, and this phenomenon is likely to be at play in the necrotic cell line based SMARCA4 deficient xenografts. While not shown here, immunohistochemistry for CA9, a molecular responder to hypoxia was not extremely evident in the tumor sections we profiled (36). A more thorough examination of expanded CDX sections or patient samples could be undertaken prior to testing the hypothesis that hypoxia induces dedifferentiation upon SMARCA4 loss in endometrial cancer cells. *In vitro* experimentation wherein different oxygen conditions can be applied and markers of epithelial dedifferentiation and stemness assessed by qRT-PCR or immunoblotting would ultimately be insightful. Hypoxia also has been proven to have ample effects on epigenetic modifications in cells and while the focus of the work presented in Chapter 3, centred on chromatin accessibility and gene expression changes

brought on by loss of SMARCA4, it would be worthwhile examining the cell line models of DDEC at other critical levels of epigenetic regulation such as DNA methylation and histone modifications. In preliminary data not shown within this thesis, the global DNA methylation profile of undifferentiated regions of cell line model SMARCA4 deficient neoplasms are distinct from their SMARCA4 intact counterparts. It would be particularly interesting to compare the signature genes up and downregulated within these DNA methylation analyses to the differential expressed genes found by several omics methodologies and those presented within Chapter 3. SMARCA4 tumors examined so far, such as SCCOHT and ATRT, typically exhibit a hypomethylated phenotype which changes heterochromatin structure, activating oncogenes and leading to aggressive clinical behavior (37, 38). Additionally, recent preclinical animal studies with SMARCA4 deficient cancers, such as SCCOHT and NSCLC, found these SMARCA4 mutant cancer cells were vulnerable to KDM6A/B inhibition, as they rely heavily on histone demethylases (39). Regulation of histone modifications at lysine 27 was demonstrated to be defective in addition to low expression of KDM6 enzymes (39). Immunofluorescent data not published by our group has examined H3K27me3 and H3K27ac marks in our cell line models of DDEC and while we did not observe a pattern of low levels of H3K27ac across all the paired models *in vitro*, it would be worth examining the landscape of histone modifications from both patient models and the cell line models upon serial passaging *in vivo.* Inhibition of KDM6s by GSK-J4 would be worth assessing as well to see if its strong suppression of SMARCA4 deficient tumor growth could encompass DDEC, broadening treatment options for a disease with an otherwise dismal prognosis.

5.5. CDK4 and EGFR are synthetic lethal partners with SMARCA4 in the context of the endometrium

In Chapters 2 and 3, the focus was primarily on the characterization of both clinical DDEC samples and our cell line models of DDEC, respectively. In Chapter 4, having established that our endometrial cancer cells lacking SMARCA4 expression can closely recapitulate aspects of the clinical phenotype of DDEC *in vivo*, we tested whether SMARCA4 deficient DDEC could be a candidate for synthetic lethality-based therapy. For the first time, a shRNA-based screen revealed EGFR and CDK4 may be vulnerabilities in endometrial cancers cells with absent SMARCA4 expression. *In vitro,* cell viability and clonogenic assays further confirmed that chemical inhibition of EGFR and CDK4 either alone or in combination selectively targeted SMARCA4 knockout EC cells. Encouragingly, other SWI/SNF mutant cancers have been found to be sensitive to tyrosine and cyclin dependent kinase therapies due to upregulation of EGFR phosphorylation or downregulation of cyclin D1, respectively (40-43). Surprisingly, translation of these findings into a preclinical setting demonstrated oral administration of either CDK4 or EGFR inhibitors, palbociclib and gefitinib, could not significantly slow tumor growth or prolong survival of tumor bearing mice. One of the limitations of utilizing our cell line model of DDEC for this *in vivo* work is that we observe varying degrees of necrosis particularly with the SMARCA4 deficient cells, whose tumor growth is markedly accelerated over SMARCA4 intact cells when comparing vehicle treated animals. This is a notably parallel behaviour of our cell line model of DDEC where SMARCA4 has been knocked out and SMARCA4 deficient patient neoplasms. In the future, it should be a priority to move towards orthotopic xenograft models rather than subcutaneous

implantation so that tumor formation occurs in the same location and microenvironment it does in humans (44). Unfortunately, endometrial cancer and likely our CDX model falls into those preclinical model systems whose metastatic potential is affected by subcutaneous implantation and thus our therapeutic response to SL-based inhibitors was adversely affected.

The inefficacy or resistance of cell line models to SL-based treatment *in vivo* also stresses the limitations that exist regarding targeted approaches and the utmost importance of combining SL inhibition with other promising therapeutic options. Considering the MMR deficient genetic background in which DDEC normally arises, this rare subset of EC may be an excellent candidate for select immunotherapy approaches (45, 46). Studies of breast cancer mouse models has led to the conclusion that inhibition of CDK4/6 enhanced response to PD-1 blockade and as immune checkpoint inhibitors (ICIs) have been only more recently been granted approval for treatment in combination with chemotherapies in breast cancer, results from ongoing clinical trials will be vital to follow-up (47, 48). At this point, combinations appear to be well tolerated and while established biomarkers of a complex tumor microenvironment such as increased PD-L1 and TILs are typically predictive of responses to ICIs, combination of palbociclib with immunotherapy appears to produce robust responses even when low levels of stromal components were observed (47-52). Otherwise performing phosphokinase arrays with the SMARCA4 intact and deficient HEC 116 Plasmid Derived cells both as adherent cells or spheres and subjected or not to *in vivo* forces could reveal deeper insight into the signalling pathway adaptations arising in the absence of the chromatin remodeling subunit. siRNA based screens in the cell line BIN67 revealed receptor tyrosine kinase

signalling beyond EGFR, as a heavily relied on oncogenic pathway in SMARCA4 deficient SCCOHT, therefore activation of AKT and MAPK may be worth pursuing as vulnerabilities in DDEC (53). In many cancers, SMARCA4 together with another factor independently activate transcription in a redundant manner, making SMARCA4 deficient cancers solely reliant on driving specific oncogenic signatures (54). The sensitivity of SCOOHT and SMARCA4-mutant esophageal cancers to BET inhibitors targeting BRD4 is an example that exemplifies such a cancer cell dependency (54, 55). In DDEC, transcription factors such as BRD4 and MITF together with other receptor tyrosine kinase signalling proteins are still worth investigating as potential combinatorial therapeutic strategies that may also circumvent eventual resistance mechanisms reducing targeted therapy efficacy.

5.6. Final Summary

In this thesis, a variety of novel advanced techniques such as CRISPR gene editing, single cell sequencing, and genome wide screens were employed to interrogate the molecular underpinnings of DDEC. The work presented contributes substantially towards not only improving the diagnosis and treatment of DDEC but also provides a solid foundation for understanding the dedifferentiation process in the endometrium. While our findings with our cell line-based models underscored the distinct nature of SMARCA4 deficient cells pre- and post- *in vivo* implantation, the features of the SMARCA4 knockout CDX consistently recapitulated published phenotypical features of clinical DDEC. Based on our model's sufficient similarity to patient DDEC with SMARCA4 loss, our findings in terms of the presence of a senescent intermediary, phenotype switching driven by transcription factor dysregulation, and synthetic lethal vulnerability to cell cycle-dependent and receptor tyrosine kinases provide a new lens to examine clinical

specimens under while also aligning well with various aspects emerging from continued efforts studying other SWI/SNF deficient cancers.

5.7. References

1. Karnezis A, Hoang, L., Coatham, M., Ravn, S., Almadani, N., Cloutier, B., Irving, J., Meng, B., Li, X., Chow, C., McAlpine, J., Kuo, K-T., Mao, T-L., Djordjevic, B., Soslow, R., Huntsman, D., Gilks, B., Koebel, M., Lee, C-H. . Loss of switch/sucrose non-fermenting complex protein expression is associated with dedifferentiation in endometrial carcinomas. Mod Pathol. 2016.
2. Coatham M, Li X, Karnezis AN, Hoang LN, Tessier-Cloutier B, Meng B, et al. Concurrent ARID1A and ARID1B inactivation in endometrial and ovarian dedifferentiated carcinomas. Mod Pathol. 2016;29(12):1586-93.
3. Mao TL, Shih Ie M. The roles of ARID1A in gynecologic cancer. J Gynecol Oncol. 2013;24(4):376-81.
4. Wang Y, Hoang L, Ji JX, Huntsman DG. SWI/SNF Complex Mutations in Gynecologic Cancers: Molecular Mechanisms and Models. Annu Rev Pathol. 2020;15:467-92.
5. Michel B, D'Avino, AR., Cassel, SH., Mashtalir, N., McKenzie, ZM., McBride, MJ., Valencia, AM., Zhou, Q., Bocker, M., Soares, LMM., Pan, J., Remillard, DI., Lareau, CA., Zullow, HJ., Fortoul, N., Gray, NS., Bradner, JE., Chan, HM., Kadoch, C. A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation. Nature Cell Biology. 2018;20:1410-20.
6. Bultman SJ, Herschkowitz JI, Godfrey V, Gebuhr TC, Yaniv M, Perou CM, et al. Characterization of mammary tumors from Brg1 heterozygous mice. Oncogene. 2008;27(4):460-8.
7. Mathur R, Alver BH, San Roman AK, Wilson BG, Wang X, Agoston AT, et al. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. Nat Genet. 2017;49(2):296-302.
8. Han ZY, Richer W, Freneaux P, Chauvin C, Lucchesi C, Guillemot D, et al. The occurrence of intracranial rhabdoid tumours in mice depends on temporal control of Smarcb1 inactivation. Nat Commun. 2016;7:10421.
9. Tessier-Cloutier B, Coatham M, Carey M, Nelson GS, Hamilton S, Lum A, et al. SWI/SNF-deficiency defines highly aggressive undifferentiated endometrial carcinoma. J Pathol Clin Res. 2021;7(2):144-53.
10. Ramalingam P, Masand RP, Euscher ED, Malpica A. Undifferentiated Carcinoma of the Endometrium: An Expanded Immunohistochemical Analysis Including PAX-8 and Basal-Like Carcinoma Surrogate Markers. Int J Gynecol Pathol. 2016;35(5):410-8.
11. Li Z, Zhao C. Clinicopathologic and Immunohistochemical Characterization of Dedifferentiated Endometrioid Adenocarcinoma. Applied immunohistochemistry & molecular morphology : AIMM. 2016;24(8):562-8.
12. Tessier-Cloutier B, Soslow RA, Stewart CJR, Kobel M, Lee CH. Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas. Histopathology. 2018;73(2):299-305.

13. Franceschi T, Durieux E, Morel AP, de Saint Hilaire P, Ray-Coquard I, Puisieux A, et al. Role of epithelial-mesenchymal transition factors in the histogenesis of uterine carcinomas. Virchows Arch. 2019;475(1):85-94.
14. Orlando K, Douglas, AK, Abudu, A., Wang, Y., Tessier-Cloutier, B., Su, W., Peters, A., Sherman, LS., Moore, R. Re-expression of SMARCA4/BRG1 in small cell carcinoma of ovary, hypercalcemic type (SCCOHT) promotes an epithelial-like gene signature through an AP-1-dependent mechanism. eLife. 2020.
15. Tsoukalas N, Aravantinou-Fatorou E, Tolia M, Giaginis C, Galanopoulos M, Kiakou M, et al. Epithelial-Mesenchymal Transition in Non Small-cell Lung Cancer. Anticancer research. 2017;37(4):1773-8.
16. Mahmood MQ, Ward C, Muller HK, Sohal SS, Walters EH. Epithelial mesenchymal transition (EMT) and non-small cell lung cancer (NSCLC): a mutual association with airway disease. Medical oncology (Northwood, London, England). 2017;34(3):45.
17. Dieters-Castator DZ, Rambau PF, Kelemen LE, Siegers GM, Lajoie GA, Postovit LM, et al. Proteomics-Derived Biomarker Panel Improves Diagnostic Precision to Classify Endometrioid and High-grade Serous Ovarian Carcinoma. Clin Cancer Res. 2019;25(14):4309-19.
18. Sander J, Joung, JK. CRISPR-Cas systems for genome editing, regulation and targeting. Nat Biotechnol. 2014;32(4):347-55.
19. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262-78.
20. Stewart CJ, Crook ML. SWI/SNF complex deficiency and mismatch repair protein expression in undifferentiated and dedifferentiated endometrial carcinoma. Pathology. 2015;47(5):439-45.
21. Garg K, Shih K, Barakat R, Zhou Q, Iasonos A, Soslow RA. Endometrial carcinomas in women aged 40 years and younger: tumors associated with loss of DNA mismatch repair proteins comprise a distinct clinicopathologic subset. The American journal of surgical pathology. 2009;33(12):1869-77.
22. Broaddus RR, Lynch HT, Chen LM, Daniels MS, Conrad P, Munsell MF, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer. 2006;106(1):87-94.
23. Martinez-Zamudio RI, Roux PF, de Freitas J, Robinson L, Dore G, Sun B, et al. AP-1 imprints a reversible transcriptional programme of senescent cells. Nat Cell Biol. 2020;22(7):842-55.
24. Wiley CD, Campisi J. From Ancient Pathways to Aging Cells-Connecting Metabolism and Cellular Senescence. Cell Metab. 2016;23(6):1013-21.
25. Moiseeva O, Bourdeau V, Roux A, Deschenes-Simard X, Ferbeyre G. Mitochondrial dysfunction contributes to oncogene-induced senescence. Mol Cell Biol. 2009;29(16):4495-507.
26. Igelmann S, Lessard F, Uchenunu O, Bouchard J, Fernandez-Ruiz A, Rowell MC, et al. A hydride transfer complex reprograms NAD metabolism and bypasses senescence. Mol Cell. 2021;81(18):3848-65 e19.
27. Mantel C, Messina-Graham S, Moh A, Cooper S, Hangoc G, Fu XY, et al. Mouse hematopoietic cell-targeted STAT3 deletion: stem/progenitor cell defects, mitochondrial dysfunction, ROS overproduction, and a rapid aging-like phenotype. Blood. 2012;120(13):2589-99.

28. Lao-On U, Attwood PV, Jitrapakdee S. Roles of pyruvate carboxylase in human diseases: from diabetes to cancers and infection. J Mol Med (Berl). 2018;96(3-4):237-47.
29. Lissanu Deribe Y, Sun Y, Terranova C, Khan F, Martinez-Ledesma J, Gay J, et al. Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer. Nat Med. 2018;24(7):1047-57.
30. Jancewicz I, Siedlecki JA, Sarnowski TJ, Sarnowska E. BRM: the core ATPase subunit of SWI/SNF chromatin-remodelling complex-a tumour suppressor or tumour-promoting factor? Epigenetics Chromatin. 2019;12(1):68.
31. Nguyen LV, Pellacani D, Lefort S, Kannan N, Osako T, Makarem M, et al. Barcoding reveals complex clonal dynamics of de novo transformed human mammary cells. Nature. 2015;528(7581):267-71.
32. Nguyen LV, Makarem M, Carles A, Moksa M, Kannan N, Pandoh P, et al. Clonal analysis via barcoding reveals diverse growth and differentiation of transplanted mouse and human mammary stem cells. Cell Stem Cell. 2014;14(2):253-63.
33. Nikolic A, Singhal, D., Ellestad, K., Johnston, M., Gillmor, A., Morrisy, S., Chan, JA., Neri, P., Bahlis, N., Gallo, M. Copy-scAT: Deconvoluting single-cell chromatin accessibility of genetic subclones in cancer. bioRxiv. 2021.
34. Muz B, de la Puente P, Azab F, Luderer M, Azab AK. Hypoxia promotes stem cell-like phenotype in multiple myeloma cells. Blood Cancer J. 2014;4:e262.
35. Axelson H, Fredlund E, Ovenberger M, Landberg G, Pahlman S. Hypoxia-induced dedifferentiation of tumor cells--a mechanism behind heterogeneity and aggressiveness of solid tumors. Semin Cell Dev Biol. 2005;16(4-5):554-63.
36. Olive PL, Aquino-Parsons C, MacPhail SH, Liao SY, Raleigh JA, Lerman MI, et al. Carbonic anhydrase 9 as an endogenous marker for hypoxic cells in cervical cancer. Cancer Res. 2001;61(24):8924-9.
37. Holdhof D, Johann PD, Spohn M, Bockmayr M, Safaei S, Joshi P, et al. Atypical teratoid/rhabdoid tumors (ATRTs) with SMARCA4 mutation are molecularly distinct from SMARCB1-deficient cases. Acta Neuropathol. 2021;141(2):291-301.
38. Fahiminiyah S, Witkowski, L., Nadaf, J., Carrot-Zhang, J., Goudie, C., Hasselblatt, M., Johann, P., Kool, M., Lee, RS., Gayden, T., Roberts, CWM., Biegel, JA., Jabado, N., Majewski, J., Foulkes, WD. Molecular analyses reveal close similarities between small cell carcinoma of the ovary, hypercalcemic type and atypical teratoid/rhabdoid tumor. Oncotarget. 2016;7(2):1732-40.
39. Romero O, Vilarrubi, A., Alburquerque-Bejar, JJ., Gomez, A., Andrades, A., trastulli, D., Proz, E., Setien, F., Verdura, S., Farre, L., Martin-Tejera, JF., Llabata, P., Oaknin, A., Saigi, M., Piulats, JM., Matias-Guiu, X., Medina, PP., Vidal, A., Villaneuva, A., Sanchez-Cespedes, M. SMARCA4 deficient tumours are vulnerable to KDM6A/UTX and KDM6B/JMJD3 blockade. Nature Communications 2021.
40. Huang PH. Targeting SWI/SNF mutant cancers with tyrosine kinase inhibitor therapy. Expert Rev Anticancer Ther. 2017;17(1):1-3.
41. Xue Y, Meehan, B., Fu, Z., Wang, XQD., Fiset, PO., Rieker, R., Levins, C., Kong, T., Zhu, X., Morin, G., Skerritt, L., Herpel, E., Venneti, S., Martinez, D., Judkins, AR., Jung, S., Camilleri-Broet, S., Gonzalez, AV., Guiot, M-C., Lockwood, WW., Spicer, JD., Agaimy, A., Pastor, WA., Dostie, J., Rak, J., Foulkes, WD., Huang, S. SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer. Nature communications. 2019;10(557).

42. Xue Y, Meehan, B., Macdonald, E., Venneti, S., Wang, XQD., Witokowski, L., Jelinic, P., Kong, T., Martinez, D., Morin, G., Firlit, M., Abedini, A., Johnson, RM., Cencic, R., Patibandla, J., Chen, H., Papadakis, AI., Auguste A., de Rink, I., Kerkhoven, RM., Bertos, N., Gotlieb, WH., Clarke, BA., Leary, A., Witcher, M., Guiot, M-C., Pelletier J., Dostie, J., Park, M., Judkins, AR., Hass, R., Levine, DA., Rak, J., Vanderhyden, B., Foulkes, WD., Huang, S. CDK4/6 inhibitors target SMARCA4-determined cyclin D1 deficiency in hypercalcemic small cell carcinoma of the ovary. Nature communications. 2019;10(558).
43. Darr J, Klochendler A, Isaac S, Geiger T, Eden A. Phosphoproteomic analysis reveals Smarcb1 dependent EGFR signaling in Malignant Rhabdoid tumor cells. Mol Cancer. 2015;14:167.
44. Konings GF, Saarinen N, Delvoux B, Kooreman L, Koskimies P, Krakstad C, et al. Development of an Image-Guided Orthotopic Xenograft Mouse Model of Endometrial Cancer with Controllable Estrogen Exposure. Int J Mol Sci. 2018;19(9).
45. Ono R, Nakayama, K., Nakamura, K., Yamashita, H., Ishibashi, T., Ishikawa, M., Minamoto, T., Razia, S., Ishikawa, N., Otsuki, Y., Nakayama, S., Onuma, H., Kurioka, H., Kyo, S. Dedifferentiated Endometrial Carcinoma Could be A Target for Immune Checkpoint Inhibitors
(Anti PD-1/PD-L1 Antibodies). Int J Mol Sci 2019;20(3744).
46. Goh C, Farah BL, Ho WY, Wong SL, Goh CHR, Chew SH, et al. Dedifferentiated endometrioid adenocarcinoma of the uterus: A case series and review of literature. Gynecol Oncol Rep. 2020;32:100538.
47. Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, et al. CDK4/6 inhibition triggers anti-tumour immunity. Nature. 2017;548(7668):471-5.
48. Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, et al. CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation. Cancer Discov. 2018;8(2):216-33.
49. Yuan Y, Lee JS, Yost SE, Frankel PH, Ruel C, Egelston CA, et al. Phase I/II trial of palbociclib, pembrolizumab and letrozole in patients with hormone receptor-positive metastatic breast cancer. Eur J Cancer. 2021;154:11-20.
50. Giraldo NA, Sanchez-Salas R, Peske JD, Vano Y, Becht E, Petitprez F, et al. The clinical role of the TME in solid cancer. Br J Cancer. 2019;120(1):45-53.
51. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol. 2016;27(8):1482-92.
52. Bense RD, Sotiriou C, Piccart-Gebhart MJ, Haanen J, van Vugt M, de Vries EGE, et al. Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer. J Natl Cancer Inst. 2017;109(1).
53. Lang J, Hendricks, WPD., Orlando, KA., Yin, H., Kiefer, J., Ramos, P., Sharma, R., Pirrotte, P., Raupach, EA., Sereduk, C., Tang, N., Liang, WS., Washington, M., Facista, SJ., Zismann, VL., Cousins, EM., Major, MB., Wang, Y., Karnezis, AN., Sekulic, A., Hass, R., Vanderhyden, BC>< Nair, P., Weissman, BE., Huntsman, DG., Trent, JM. . Ponatinib Shows Potent Antitumor Activity in Small Cell Carcinoma of the Ovary Hypercalcemic Type (SCCOHT) through Multikinase Inhibition. Clin Cancer Res. 2018;24:1932-43.

54. Shorstova T, Marques M, Su J, Johnston J, Kleinman CL, Hamel N, et al. SWI/SNF-Compromised Cancers Are Susceptible to Bromodomain Inhibitors. Cancer Res. 2019;79(10):2761-74.
55. Campbell J, Ryan CJ, Brough R, Bajrami I, Pemberton HN, Chong IY, et al. Large-Scale Profiling of Kinase Dependencies in Cancer Cell Lines. Cell Rep. 2016;14(10):2490-501.

Works Cited

Abcouwer, S. F., P. L. Marjon, R. K. Loper and D. L. Vander Jagt (2002). "Response of VEGF expression to amino acid deprivation and inducers of endoplasmic reticulum stress." Invest Ophthalmol Vis Sci **43**(8): 2791-2798.

Achuthan, S., T. R. Santhoshkumar, J. Prabhakar, S. A. Nair and M. R. Pillai (2011). "Drug-induced senescence generates chemoresistant stemlike cells with low reactive oxygen species." J Biol Chem **286**(43): 37813-37829.

Adam, S., J. Dabin and S. E. Polo (2015). "Chromatin plasticity in response to DNA damage: The shape of things to come." DNA Repair (Amst) **32**: 120-126.

Agaimy, A. (2014). "The Expanding Family of SMARCB1(INI1)-deficient Neoplasia: Implications of Phenotypic, Biological, and Molecular Heterogeneity." Adv Anat Pathol **21**(6): 394-410.

Agaimy, A., F. Haller, J. Frohnauer, I. M. Schaefer, P. Strobel, A. Hartmann, R. Stoehr and G. Kloppel (2015). "Pancreatic undifferentiated rhabdoid carcinoma: KRAS alterations and SMARCB1 expression status define two subtypes." Mod Pathol **28**(2): 248-260.

Ahmed, S. U., R. Carruthers, L. Gilmour, S. Yildirim, C. Watts and A. J. Chalmers (2015). "Selective Inhibition of Parallel DNA Damage Response Pathways Optimizes Radiosensitization of Glioblastoma Stem-like Cells." Cancer Res **75**(20): 4416-4428.

Al-Ejeh, F., M. Pajic, W. Shi, M. Kalimutho, M. Miranda, A. M. Nagrial, A. Chou, A. V. Biankin, S. M. Grimmond, I. Australian Pancreatic Cancer Genome, M. P. Brown and K. K. Khanna (2014). "Gemcitabine and CHK1 inhibition potentiate EGFR-directed radioimmunotherapy against pancreatic ductal adenocarcinoma." Clin Cancer Res **20**(12): 3187-3197.

Alagoz, M., Y. Katsuki, H. Ogiwara, T. Ogi, A. Shibata, A. Kakarougkas and P. Jeggo (2015). "SETDB1, HP1 and SUV39 promote repositioning of 53BP1 to extend resection during homologous recombination in G2 cells." Nucleic Acids Res **43**(16): 7931-7944.

Alessio, N., Squillaro, T., Cipollaro, M., Bagella, L., Giordano, A., Galderisi, U. (2010). "The BRG1 ATPase of chromatin remodeling complexes is involved in modulation of mesenchymal stem cell senescence through RB-P53 pathways." Oncogene **29**(40): 5452-5463.

Allis, C. D. and T. Jenuwein (2016). "The molecular hallmarks of epigenetic control." Nat Rev Genet **17**(8): 487-500.

Alsaab, H., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S., Iyer, AK. (2017). "PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome." Front Pharmacol **8**(8).

Altenberg, B., Greulich, KO. (2004). "Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes." Genomics **84**(6): 1014-1020.

Altrabulsi, B., A. Malpica, M. T. Deavers, D. C. Bodurka, R. Broaddus and E. G. Silva (2005). "Undifferentiated carcinoma of the endometrium." Am J Surg Pathol **29**(10): 1316-1321.

Anderson, A. R. A., A. M. Weaver, P. T. Cummings and V. Quaranta (2006). "Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment." Cell **127**(5): 905-915.

Arnedos, M., M. A. Bayar, B. Cheaib, V. Scott, I. Bouakka, A. Valent, J. Adam, V. Leroux-Kozal, V. Marty, A. Rapinat, C. Mazouni, B. Sarfati, I. Bieche, C. Balleyguier, D. Gentien, S. Delaloge, M. Lacroix-Triki, S. Michiels and F. Andre (2018). "Modulation of Rb phosphorylation and antiproliferative response to palbociclib: the preoperative-palbociclib (POP) randomized clinical trial." Ann Oncol **29**(8): 1755-1762.

Asakura, T., N. Yamaguchi, K. Ohkawa and K. Yoshida (2015). "Proteasome inhibitor-resistant cells cause EMT-induction via suppression of E-cadherin by miR-200 and ZEB1." Int J Oncol **46**(5): 2251-2260.

Asanoma, K., Hori, E., Yoshida, S., Yagi, H., Onoyama, I., Kodama, K., Yasunaga, M., Ohgami, T., Kaneki, E., Okugawa, K. (2019). "Mutual suppression between BHLHE40/BHLHE41 and the MIR301B-MIR130B cluster is involved in epithelial-to-mesenchymal transition of endometrial cancer cells." Oncotarget **10**(45): 4640-4654.

Asghar, U., A. K. Witkiewicz, N. C. Turner and E. S. Knudsen (2015). "The history and future of targeting cyclin-dependent kinases in cancer therapy." Nat Rev Drug Discov **14**(2): 130-146.

Ashworth, A. (2008). "A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair." J Clin Oncol **26**(22): 3785-3790.

Aubele, M., M. Schmitt, R. Napieralski, S. Paepke, J. Ettl, M. Absmaier, V. Magdolen, J. Martens, J. A. Foekens, O. G. Wilhelm and M. Kiechle (2017). "The Predictive Value of PITX2 DNA Methylation for High-Risk Breast Cancer Therapy: Current Guidelines, Medical Needs, and Challenges." Dis Markers **2017**: 4934608.

Audergon, P. N., S. Catania, A. Kagansky, P. Tong, M. Shukla, A. L. Pidoux and R. C. Allshire (2015). "Epigenetics. Restricted epigenetic inheritance of H3K9 methylation." Science **348**(6230): 132-135.

Axelson, H., E. Fredlund, M. Ovenberger, G. Landberg and S. Pahlman (2005). "Hypoxia-induced dedifferentiation of tumor cells--a mechanism behind heterogeneity and aggressiveness of solid tumors." Semin Cell Dev Biol **16**(4-5): 554-563.

Aytac, E., Y. Ozdemir and G. Ozuner (2014). "Long term outcomes of neuroendocrine carcinomas (high-grade neuroendocrine tumors) of the colon, rectum, and anal canal." J Visc Surg **151**(1): 3-7.

Bailey, M. H., C. Tokheim, E. Porta-Pardo, S. Sengupta, D. Bertrand, A. Weerasinghe, A. Colaprico, M. C. Wendl, J. Kim, B. Reardon, P. Kwok-Shing Ng, K. J. Jeong, S. Cao, Z. Wang, J. Gao, Q. Gao, F. Wang, E. M. Liu, L. Mularoni, C. Rubio-Perez, N. Nagarajan, I. Cortes-Ciriano, D. C. Zhou, W. W. Liang, J. M. Hess, V. D. Yellapantula, D. Tamborero, A. Gonzalez-Perez, C. Suphavilai, J. Y. Ko, E. Khurana, P. J. Park, E. M. Van Allen, H. Liang, M. C. W. Group, N. Cancer Genome Atlas Research, M. S. Lawrence, A. Godzik, N. Lopez-Bigas, J. Stuart, D. Wheeler, G. Getz, K. Chen, A. J. Lazar, G. B. Mills, R. Karchin and L. Ding (2018). "Comprehensive Characterization of Cancer Driver Genes and Mutations." Cell **174**(4): 1034-1035.

Bakhsh, S., Kinloch, M., Hoang, LN., Soslow, RA., Kobel, M., Lee, CH., McAlpine, JN., McConechy, MK., Blake Gilks, C. (2016). "Histopathological features of endometrial carcinomas associated with POLE mutations: implications for decisions about adjuvant therapy." Histopathology **68**: 916-924.

Bannister, A. J. and T. Kouzarides (2011). "Regulation of chromatin by histone modifications." Cell Res **21**(3): 381-395.

Bannister, A. J., P. Zegerman, J. F. Partridge, E. A. Miska, J. O. Thomas, R. C. Allshire and T. Kouzarides (2001). "Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain." Nature **410**(6824): 120-124.

Bao, S., Q. Wu, R. E. McLendon, Y. Hao, Q. Shi, A. B. Hjelmeland, M. W. Dewhirst, D. D. Bigner and J. N. Rich (2006). "Glioma stem cells promote radioresistance by preferential activation of the DNA damage response." Nature **444**(7120): 756-760.

Bao, W., H. Qiu, T. Yang, X. Luo, H. Zhang and X. Wan (2013). "Upregulation of TrkB promotes epithelial-mesenchymal transition and anoikis resistance in endometrial carcinoma." PLoS One **8**(7): e70616.

Bartucci, M., S. Svensson, P. Romania, R. Dattilo, M. Patrizii, M. Signore, S. Navarra, F. Lotti, M. Biffoni, E. Pilozzi, E. Duranti, S. Martinelli, C. Rinaldo, A. Zeuner, M. Maugeri-Sacca, A. Eramo and R. De Maria (2012). "Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy." Cell Death Differ **19**(5): 768-778.

Barutcu, A. R., B. R. Lajoie, A. J. Fritz, R. P. McCord, J. A. Nickerson, A. J. van Wijnen, J. B. Lian, J. L. Stein, J. Dekker, G. S. Stein and A. N. Imbalzano (2016). "SMARCA4 regulates gene expression and higher-order chromatin structure in proliferating mammary epithelial cells." Genome Res **26**(9): 1188-1201.

Baselga, J., J. Cortes, S. B. Kim, S. A. Im, R. Hegg, Y. H. Im, L. Roman, J. L. Pedrini, T. Pienkowski, A. Knott, E. Clark, M. C. Benyunes, G. Ross, S. M. Swain and C. S. Group (2012). "Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer." N Engl J Med **366**(2): 109-119.

Basisty, N., A. Kale, O. H. Jeon, C. Kuehnemann, T. Payne, C. Rao, A. Holtz, S. Shah, V. Sharma, L. Ferrucci, J. Campisi and B. Schilling (2020). "A proteomic atlas of senescence-associated secretomes for aging biomarker development." PLoS Biol **18**(1): e3000599.

Basu-Roy, U., E. Seo, L. Ramanathapuram, T. B. Rapp, J. A. Perry, S. H. Orkin, A. Mansukhani and C. Basilico (2012). "Sox2 maintains self renewal of tumor-initiating cells in osteosarcomas." Oncogene **31**(18): 2270-2282.

Baylin, S. B. and P. A. Jones (2011). "A decade of exploring the cancer epigenome - biological and translational implications." Nat Rev Cancer **11**(10): 726-734.

Beleut, M., R. D. Rajaram, M. Caikovski, A. Ayyanan, D. Germano, Y. Choi, P. Schneider and C. Brisken (2010). "Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland." Proc Natl Acad Sci U S A **107**(7): 2989-2994.

Bell, A. C. and G. Felsenfeld (2000). "Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene." Nature **405**(6785): 482-485.

Bense, R. D., C. Sotiriou, M. J. Piccart-Gebhart, J. Haanen, M. van Vugt, E. G. E. de Vries, C. P. Schroder and R. S. N. Fehrmann (2017). "Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer." J Natl Cancer Inst **109**(1).

Bernstein, B. E., E. L. Humphrey, R. L. Erlich, R. Schneider, P. Bouman, J. S. Liu, T. Kouzarides and S. L. Schreiber (2002). "Methylation of histone H3 Lys 4 in coding regions of active genes." Proc Natl Acad Sci U S A **99**(13): 8695-8700.

Beroukhim, R., C. H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J. S. Boehm, J. Dobson, M. Urashima, K. T. Mc Henry, R. M. Pinchback, A. H. Ligon, Y. J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M. S. Lawrence, B. A. Weir, K. E. Tanaka, D. Y. Chiang, A. J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F. J. Kaye, H. Sasaki, J. E. Tepper, J. A. Fletcher, J. Tabernero, J. Baselga, M. S. Tsao, F. Demichelis, M. A. Rubin, P. A. Janne, M. J. Daly, C. Nucera, R. L. Levine, B. L. Ebert, S. Gabriel, A. K. Rustgi, C. R. Antonescu, M. Ladanyi, A. Letai, L. A. Garraway, M. Loda, D. G. Beer, L. D. True, A. Okamoto, S. L. Pomeroy, S. Singer, T. R. Golub, E. S. Lander, G. Getz, W. R. Sellers and M. Meyerson (2010). "The landscape of somatic copy-number alteration across human cancers." Nature **463**(7283): 899-905.

Bhanu, N. V., S. Sidoli and B. A. Garcia (2016). "Histone modification profiling reveals differential signatures associated with human embryonic stem cell self-renewal and differentiation." Proteomics **16**(3): 448-458.

Bhowmick, N. A., E. G. Neilson and H. L. Moses (2004). "Stromal fibroblasts in cancer initiation and progression." Nature **432**(7015): 332-337.

Bian, Y., X. Chang, Y. Liao, J. Wang, Y. Li, K. Wang and X. Wan (2016). "Promotion of epithelial-mesenchymal transition by Frizzled2 is involved in the metastasis of endometrial cancer." Oncol Rep **36**(2): 803-810.

Bibikova, M., E. Chudin, B. Wu, L. Zhou, E. W. Garcia, Y. Liu, S. Shin, T. W. Plaia, J. M. Auerbach, D. E. Arking, R. Gonzalez, J. Crook, B. Davidson, T. C. Schulz, A. Robins, A. Khanna, P. Sartipy, J. Hyllner, P. Vanguri, S. Savant-Bhonsale, A. K. Smith, A. Chakravarti, A. Maitra, M. Rao, D. L. Barker, J. F. Loring and J. B. Fan (2006). "Human embryonic stem cells have a unique epigenetic signature." Genome Res **16**(9): 1075-1083.

Bischof, P. and A. Campana (2000). "A putative role for oncogenes in trophoblast invasion?" Hum Reprod **15 Suppl 6**: 51-58.

Black, J. C., C. Van Rechem and J. R. Whetstine (2012). "Histone lysine methylation dynamics: establishment, regulation, and biological impact." Mol Cell **48**(4): 491-507.

Blagosklonny, M. V. (2004). "Analysis of FDA approved anticancer drugs reveals the future of cancer therapy." Cell Cycle **3**(8): 1035-1042.

Borghaei, H., L. Paz-Ares, L. Horn, D. R. Spigel, M. Steins, N. E. Ready, L. Q. Chow, E. E. Vokes, E. Felip, E. Holgado, F. Barlesi, M. Kohlhaufl, O. Arrieta, M. A. Burgio, J. Fayette, H. Lena, E. Poddubskaya, D. E. Gerber, S. N. Gettinger, C. M. Rudin, N. Rizvi, L. Crino, G. R. Blumenschein, Jr., S. J. Antonia, C. Dorange, C. T. Harbison, F. Graf Finckenstein and J. R. Brahmer (2015). "Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer." N Engl J Med **373**(17): 1627-1639.

Boumahdi, S., G. Driessens, G. Lapouge, S. Rorive, D. Nassar, M. Le Mercier, B. Delatte, A. Caauwe, S. Lenglez, E. Nkusi, S. Brohee, I. Salmon, C. Dubois, V. del Marmol, F. Fuks, B. Beck and C. Blanpain (2014). "SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma." Nature **511**(7508): 246-250.

Bourgo, R. J., H. Siddiqui, S. Fox, D. Solomon, C. G. Sansam, M. Yaniv, C. Muchardt, D. Metzger, P. Chambon, C. W. Roberts and E. S. Knudsen (2009). "SWI/SNF deficiency results in aberrant chromatin organization, mitotic failure, and diminished proliferative capacity." Mol Biol Cell **20**(14): 3192-3199.

Boyer, L. A., K. Plath, J. Zeitlinger, T. Brambrink, L. A. Medeiros, T. I. Lee, S. S. Levine, M. Wernig, A. Tajonar, M. K. Ray, G. W. Bell, A. P. Otte, M. Vidal, D. K. Gifford, R. A. Young and R. Jaenisch (2006). "Polycomb complexes repress developmental regulators in murine embryonic stem cells." Nature **441**(7091): 349-353.

Bracken, C., Gregory, PA., Kolesnikoff, N., Bert, AG., Wang, J., Shannon, MF., Goodall, GJ. (2008). "A Double-Negative Feedback Loop between ZEB1-SIP1 and the microRNA-200 Family Regulates Epithelial-Mesenchymal Transition." **68**(19).

Brahmer, J., K. L. Reckamp, P. Baas, L. Crino, W. E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E. E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O.

Aren Frontera, L. Havel, M. Steins, M. C. Garassino, J. G. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C. T. Harbison, B. Lestini and D. R. Spigel (2015). "Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer." N Engl J Med **373**(2): 123-135.

Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre and A. Jemal (2018). "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." CA Cancer J Clin **68**(6): 394-424.

Brenner, D. R., H. K. Weir, A. A. Demers, L. F. Ellison, C. Louzado, A. Shaw, D. Turner, R. R. Woods, L. M. Smith and C. Canadian Cancer Statistics Advisory (2020). "Projected estimates of cancer in Canada in 2020." CMAJ **192**(9): E199-E205.

Broaddus, R. R., H. T. Lynch, L. M. Chen, M. S. Daniels, P. Conrad, M. F. Munsell, K. G. White, R. Luthra and K. H. Lu (2006). "Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma." Cancer **106**(1): 87-94.

Brooks, R. A., G. F. Fleming, R. R. Lastra, N. K. Lee, J. W. Moroney, C. H. Son, K. Tatebe and J. L. Veneris (2019). "Current recommendations and recent progress in endometrial cancer." CA Cancer J Clin **69**(4): 258-279.

Brunetti, L., M. C. Gundry and M. A. Goodell (2017). "DNMT3A in Leukemia." Cold Spring Harb Perspect Med **7**(2).

Brunner, A. H., P. Riss, G. Heinze, E. Meltzow and H. Brustmann (2011). "Immunoexpression of PAX 8 in endometrial cancer: relation to high-grade carcinoma and p53." Int J Gynecol Pathol **30**(6): 569-575.

Bryant, H. E., N. Schultz, H. D. Thomas, K. M. Parker, D. Flower, E. Lopez, S. Kyle, M. Meuth, N. J. Curtin and T. Helleday (2005). "Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase." Nature **434**(7035): 913-917.

Bukhari, A. B., C. W. Lewis, J. J. Pearce, D. Luong, G. K. Chan and A. M. Gamper (2019). "Inhibiting Wee1 and ATR kinases produces tumor-selective synthetic lethality and suppresses metastasis." J Clin Invest **129**(3): 1329-1344.

Bultman, S. J., J. I. Herschkowitz, V. Godfrey, T. C. Gebuhr, M. Yaniv, C. M. Perou and T. Magnuson (2008). "Characterization of mammary tumors from Brg1 heterozygous mice." Oncogene **27**(4): 460-468.

Burk, U., Schubert, J., Wellner, U., Schmalhofer, O., Vincan, E., Spaderna, S., Brabletz, T. (2008). "A Reciprocal Repression Between ZEB1 and Members of the miR-200 Family Promotes EMT and Invasion in Cancer Cells." Embo Reports **9**(6): 582-589.

Byron, S. A., M. Gartside, M. A. Powell, C. L. Wellens, F. Gao, D. G. Mutch, P. J. Goodfellow and P. M. Pollock (2012). "FGFR2 point mutations in 466 endometrioid

endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features." PLoS One **7**(2): e30801.

Cabrera, M. C., R. E. Hollingsworth and E. M. Hurt (2015). "Cancer stem cell plasticity and tumor hierarchy." World J Stem Cells **7**(1): 27-36.

Cacan, E. (2016). "Histone Deacetylase-1-mediated Suppression of FAS in Chemoresistant Ovarian Cancer Cells." Anticancer Res **36**(6): 2819-2826.

Cacan, E., M. W. Ali, N. H. Boyd, S. B. Hooks and S. F. Greer (2014). "Inhibition of HDAC1 and DNMT1 modulate RGS10 expression and decrease ovarian cancer chemoresistance." PLoS One **9**(1): e87455.

Cai, M. H., X. G. Xu, S. L. Yan, Z. Sun, Y. Ying, B. K. Wang and Y. X. Tu (2018). "Depletion of HDAC1, 7 and 8 by Histone Deacetylase Inhibition Confers Elimination of Pancreatic Cancer Stem Cells in Combination with Gemcitabine." Sci Rep **8**.

Cai, X. D., Y. B. Zhou, L. X. Huang, Q. L. Zeng, L. J. Zhang, Q. Q. Wang, S. L. Li, J. Q. Feng and A. J. Han (2012). "Reduced expression of Kruppel-like factor 17 is related to tumor growth and poor prognosis in lung adenocarcinoma." Biochem Biophys Res Commun **418**(1): 67-73.

Cairns, B. R., Y. J. Kim, M. H. Sayre, B. C. Laurent and R. D. Kornberg (1994). "A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast." Proc Natl Acad Sci U S A **91**(5): 1950-1954.

Calabrese, C., H. Poppleton, M. Kocak, T. L. Hogg, C. Fuller, B. Hamner, E. Y. Oh, M. W. Gaber, D. Finklestein, M. Allen, A. Frank, I. T. Bayazitov, S. S. Zakharenko, A. Gajjar, A. Davidoff and R. J. Gilbertson (2007). "A perivascular niche for brain tumor stem cells." Cancer Cell **11**(1): 69-82.

Calcagno, A. M., C. D. Salcido, J. P. Gillet, C. P. Wu, J. M. Fostel, M. D. Mumau, M. M. Gottesman, L. Varticovski and S. V. Ambudkar (2010). "Prolonged drug selection of breast cancer cells and enrichment of cancer stem cell characteristics." J Natl Cancer Inst **102**(21): 1637-1652.

Callegaro-Filho, D., D. M. Gershenson, A. M. Nick, M. F. Munsell, P. T. Ramirez, P. J. Eifel, E. D. Euscher, R. M. Marques, S. M. Nicolau and K. M. Schmeler (2016). "Small cell carcinoma of the ovary-hypercalcemic type (SCCOHT): A review of 47 cases." Gynecol Oncol **140**(1): 53-57.

Campbell, J., C. J. Ryan, R. Brough, I. Bajrami, H. N. Pemberton, I. Y. Chong, S. Costa-Cabral, J. Frankum, A. Gulati, H. Holme, R. Miller, S. Postel-Vinay, R. Rafiq, W. Wei, C. T. Williamson, D. A. Quigley, J. Tym, B. Al-Lazikani, T. Fenton, R. Natrajan, S. J. Strauss, A. Ashworth and C. J. Lord (2016). "Large-Scale Profiling of Kinase Dependencies in Cancer Cell Lines." Cell Rep **14**(10): 2490-2501.

Campisi, J. (2013). "Aging, cellular senescence, and cancer." Annu Rev Physiol **75**: 685-705.

Cancer Genome Atlas Research, N., C. Kandoth, N. Schultz, A. D. Cherniack, R. Akbani, Y. Liu, H. Shen, A. G. Robertson, I. Pashtan, R. Shen, C. C. Benz, C. Yau, P. W. Laird, L. Ding, W. Zhang, G. B. Mills, R. Kucherlapati, E. R. Mardis and D. A. Levine (2013). "Integrated genomic characterization of endometrial carcinoma." Nature **497**(7447): 67-73.

Cancer Genome Atlas Research, N., J. N. Weinstein, E. A. Collisson, G. B. Mills, K. R. Shaw, B. A. Ozenberger, K. Ellrott, I. Shmulevich, C. Sander and J. M. Stuart (2013). "The Cancer Genome Atlas Pan-Cancer analysis project." Nat Genet **45**(10): 1113-1120.

Carrington, C. (2015). "Oral targeted therapy for cancer." Aust Prescr **38**(5): 171-176.

Castilla, M., Moreno-Bueno, G., Romero-Perez, L., Van De Vijver, K., Biscuola, M., Lopez-Garcia, MA., Prat, J., Matias-Guiu, X., Cano, A., Oliva, E., Palacios, J. (2011). "Micro-RNA Signature of the Epithelial-Mesenchymal Transition in Endometrial Carcinosarcoma." J Pathol **223**(1): 72-80.

Chaffer, C. L., Marjanovic N.D., Lee, T., Bell, G., Kleer, C.G., Reinhardt, F., D'alessio, A.C., Young, R.A., Weinberg, R.A. (2013). "Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity." Cell **154**(1): 61-74.

Chan, N., Bristow, RG. (2010). ""Contextual" Synthetic Lethality and/or Loss of Heterozygosity: Tumor Hypoxia and Modification of DNA Repair." Clinical Cancer Research **16**(18).

Chandra, T., P. A. Ewels, S. Schoenfelder, M. Furlan-Magaril, S. W. Wingett, K. Kirschner, J. Y. Thuret, S. Andrews, P. Fraser and W. Reik (2015). "Global reorganization of the nuclear landscape in senescent cells." Cell Rep **10**(4): 471-483.

Chang, C. H., M. Zhang, K. Rajapakshe, C. Coarfa, D. Edwards, S. Huang and J. M. Rosen (2015). "Mammary Stem Cells and Tumor-Initiating Cells Are More Resistant to Apoptosis and Exhibit Increased DNA Repair Activity in Response to DNA Damage." Stem Cell Reports **5**(3): 378-391.

Chang, C. J., Yang, J.Y., Xia, W., Chen, C.T., Xie, X., Chao, C.H., Woodward, W.A., Hsu, J.M., Hortobagyi, G.N., Hung, M.C. (2011). "EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-β-catenin signaling." Cancer Cell **19**(1): 86-100.

Chang, H. H. Y., N. R. Pannunzio, N. Adachi and M. R. Lieber (2017). "Non-homologous DNA end joining and alternative pathways to double-strand break repair." Nat Rev Mol Cell Biol **18**(8): 495-506.

Chang, L. and S. Takada (2016). "Histone acetylation dependent energy landscapes in tri-nucleosome revealed by residue-resolved molecular simulations." Sci Rep **6**: 34441.

Charles, N., T. Ozawa, M. Squatrito, A. M. Bleau, C. W. Brennan, D. Hambardzumyan and E. C. Holland (2010). "Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells." Cell Stem Cell **6**(2): 141-152.

Chekhun, V. F., N. Y. Lukyanova, O. Kovalchuk, V. P. Tryndyak and I. P. Pogribny (2007). "Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarcinoma cells reveals novel hyper- and hypomethylated targets." Mol Cancer Ther **6**(3): 1089-1098.

Chen, A., Beetham, H., Black, MA., Priya, R., Telford, BJ., Guest, J., Wiggins, GA., Godwin, TD., Guilford, PJ. (2014). "E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition." BMC Cancer **14**.

Chen, P., Xing, T., Want, Q., Liu, A., Liu, H., Hu, Y., Ji, Y. Song, Y. Wang, D. (2019). "MicroRNA-202 Inhibits Cell Migration and Invasion Through Targeting FGF2 and Inactivating Wnt/β-catenin Signaling in Endometrial Carcinoma." Biosci Rep **39**(10).

Cheng, S. W., K. P. Davies, E. Yung, R. J. Beltran, J. Yu and G. V. Kalpana (1999). "c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function." Nat Genet **22**(1): 102-105.

Cheung, L. W., B. T. Hennessy, J. Li, S. Yu, A. P. Myers, B. Djordjevic, Y. Lu, K. Stemke-Hale, M. D. Dyer, F. Zhang, Z. Ju, L. C. Cantley, S. E. Scherer, H. Liang, K. H. Lu, R. R. Broaddus and G. B. Mills (2011). "High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability." Cancer Discov **1**(2): 170-185.

Choi, D., H. W. Lee, K. Y. Hur, J. J. Kim, G. S. Park, S. H. Jang, Y. S. Song, K. S. Jang and S. S. Paik (2009). "Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma." World J Gastroenterol **15**(18): 2258-2264.

Ciccia, A. and S. J. Elledge (2010). "The DNA damage response: making it safe to play with knives." Mol Cell **40**(2): 179-204.

Clark, S. J., J. Harrison and P. L. Molloy (1997). "Sp1 binding is inhibited by (m)Cp(m)CpG methylation." Gene **195**(1): 67-71.

Clevers, H. (2011). "The cancer stem cell: premises, promises and challenges." Nat Med **17**(3): 313-319.

Coatham, M., X. Li, A. N. Karnezis, L. N. Hoang, B. Tessier-Cloutier, B. Meng, R. A. Soslow, C. Blake Gilks, D. G. Huntsman, C. J. Stewart, L. M. Postovit, M. Kobel and C. H. Lee (2016). "Concurrent ARID1A and ARID1B inactivation in endometrial and ovarian dedifferentiated carcinomas." Mod Pathol **29**(12): 1586-1593.

Cohet, N., K. M. Stewart, R. Mudhasani, A. J. Asirvatham, C. Mallappa, K. M. Imbalzano, V. M. Weaver, A. N. Imbalzano and J. A. Nickerson (2010). "SWI/SNF chromatin remodeling enzyme ATPases promote cell proliferation in normal mammary epithelial cells." J Cell Physiol **223**(3): 667-678.

Colas, E., L. Muinelo-Romay, L. Alonso-Alconada, M. Llaurado, M. Monge, J. Barbazan, M. Gonzalez, M. Schoumacher, N. Pedrola, T. Ertekin, L. Devis, A. Ruiz, J. Castellvi, A. Doll, A. Gil-Moreno, M. Vazquez-Levin, L. Lapyckyj, R. Lopez-Lopez, S. Robine, E. Friederich, M. Castro, J. Reventos, D. Vignjevic and M. Abal (2012). "ETV5 cooperates with LPP as a sensor of extracellular signals and promotes EMT in endometrial carcinomas." Oncogene **31**(45): 4778-4788.

Collado, M., M. A. Blasco and M. Serrano (2007). "Cellular senescence in cancer and aging." Cell **130**(2): 223-233.

Cong, L., Ran, FA., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, PD., Wu, Z., Jiang, W., Marraffini, LA., Zhang, F. (2013). "Multiplex genome engineering using CRISPR/Cas systems." Science **339**(6121): 819-823.

Cote, J., J. Quinn, J. L. Workman and C. L. Peterson (1994). "Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex." Science **265**(5168): 53-60.

Cozzio, A., E. Passegue, P. M. Ayton, H. Karsunky, M. L. Cleary and I. L. Weissman (2003). "Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors." Genes Dev **17**(24): 3029-3035.

Creasman, W. T., F. Odicino, P. Maisonneuve, U. Beller, J. L. Benedet, A. P. Heintz, H. Y. Ngan, M. Sideri and S. Pecorelli (2001). "Carcinoma of the corpus uteri." J Epidemiol Biostat **6**(1): 47-86.

da Silva-Diz, V., L. Lorenzo-Sanz, A. Bernat-Peguera, M. Lopez-Cerda and P. Munoz (2018). "Cancer cell plasticity: Impact on tumor progression and therapy response." Semin Cancer Biol **53**: 48-58.

Dabin, J., A. Fortuny and S. E. Polo (2016). "Epigenome Maintenance in Response to DNA Damage." Mol Cell **62**(5): 712-727.

Dalvi, M. P., L. Wang, R. Zhong, R. K. Kollipara, H. Park, J. Bayo, P. Yenerall, Y. Zhou, B. C. Timmons, J. Rodriguez-Canales, C. Behrens, B. Mino, P. Villalobos, E. R. Parra, M. Suraokar, A. Pataer, S. G. Swisher, N. Kalhor, N. V. Bhanu, B. A. Garcia, J. V. Heymach, K. Coombes, Y. Xie, L. Girard, A. F. Gazdar, R. Kittler, Wistuba, II, J. D. Minna and E. D. Martinez (2017). "Taxane-Platin-Resistant Lung Cancers Co-develop Hypersensitivity to JumonjiC Demethylase Inhibitors." Cell Rep **19**(8): 1669-1684.

Darr, J., A. Klochendler, S. Isaac, T. Geiger and A. Eden (2015). "Phosphoproteomic analysis reveals Smarcb1 dependent EGFR signaling in Malignant Rhabdoid tumor cells." Mol Cancer **14**: 167.

Das, K., X. B. Chan, D. Epstein, B. T. Teh, K. M. Kim, S. T. Kim, S. H. Park, W. K. Kang, S. Rozen, J. Lee and P. Tan (2016). "NanoString expression profiling identifies candidate biomarkers of RAD001 response in metastatic gastric cancer." ESMO Open **1**(1): e000009.

Das, P. K., S. Pillai, M. A. Rakib, J. A. Khanam, V. Gopalan, A. K. Y. Lam and F. Islam (2020). "Plasticity of Cancer Stem Cell: Origin and Role in Disease Progression and Therapy Resistance." Stem Cell Rev Rep.

Dawson, M. A. and T. Kouzarides (2012). "Cancer epigenetics: from mechanism to therapy." Cell **150**(1): 12-27.

De Angelis, M. L., F. Francescangeli, F. La Torre and A. Zeuner (2019). "Stem Cell Plasticity and Dormancy in the Development of Cancer Therapy Resistance." Front Oncol **9**: 626.

de la Serna, I. L., Y. Ohkawa and A. N. Imbalzano (2006). "Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers." Nat Rev Genet **7**(6): 461-473.

Decaudin, D. and C. Le Tourneau (2016). "Combinations of targeted therapies in human cancers." Aging (Albany NY) **8**(10): 2258-2259.

Dechassa, M., Sabri, A., Pondugula, S., Kassabov, Sr., Chatterjee, N., Kladde, MP., Bartholomew, B. (2010). "SWI/SNF has intrinsic nucleosome dissambly activity that is dependent on adjacent nucleosomes." Mol Cell **38**(4): 590-602.

Demaria, M., M. N. O'Leary, J. Chang, L. Shao, S. Liu, F. Alimirah, K. Koenig, C. Le, N. Mitin, A. M. Deal, S. Alston, E. C. Academia, S. Kilmarx, A. Valdovinos, B. Wang, A. de Bruin, B. K. Kennedy, S. Melov, D. Zhou, N. E. Sharpless, H. Muss and J. Campisi (2017). "Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse." Cancer Discov **7**(2): 165-176.

Deng, J., E. S. Wang, R. W. Jenkins, S. Li, R. Dries, K. Yates, S. Chhabra, W. Huang, H. Liu, A. R. Aref, E. Ivanova, C. P. Paweletz, M. Bowden, C. W. Zhou, G. S. Herter-Sprie, J. A. Sorrentino, J. E. Bisi, P. H. Lizotte, A. A. Merlino, M. M. Quinn, L. E. Bufe, A. Yang, Y. Zhang, H. Zhang, P. Gao, T. Chen, M. E. Cavanaugh, A. J. Rode, E. Haines, P. J. Roberts, J. C. Strum, W. G. Richards, J. H. Lorch, S. Parangi, V. Gunda, G. M. Boland, R. Bueno, S. Palakurthi, G. J. Freeman, J. Ritz, W. N. Haining, N. E. Sharpless, H. Arthanari, G. I. Shapiro, D. A. Barbie, N. S. Gray and K. K. Wong (2018). "CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation." Cancer Discov **8**(2): 216-233.

Dennis, K., T. Fan, T. Geiman, Q. Yan and K. Muegge (2001). "Lsh, a member of the SNF2 family, is required for genome-wide methylation." Genes Dev **15**(22): 2940-2944.

Dick, J. E. (2009). "Looking ahead in cancer stem cell research." Nat Biotechnol **27**(1): 44-46.

Diehn, M., R. W. Cho, N. A. Lobo, T. Kalisky, M. J. Dorie, A. N. Kulp, D. Qian, J. S. Lam, L. E. Ailles, M. Wong, B. Joshua, M. J. Kaplan, I. Wapnir, F. M. Dirbas, G. Somlo, C. Garberoglio, B. Paz, J. Shen, S. K. Lau, S. R. Quake, J. M. Brown, I. L. Weissman and M. F. Clarke (2009). "Association of reactive oxygen species levels and radioresistance in cancer stem cells." Nature **458**(7239): 780-783.

Dieters-Castator, D. Z., P. F. Rambau, L. E. Kelemen, G. M. Siegers, G. A. Lajoie, L. M. Postovit and M. Kobel (2019). "Proteomics-Derived Biomarker Panel Improves Diagnostic Precision to Classify Endometrioid and High-grade Serous Ovarian Carcinoma." Clin Cancer Res **25**(14): 4309-4319.

Dong, P., K. Ihira, Y. Xiong, H. Watari, S. J. Hanley, T. Yamada, M. Hosaka, M. Kudo, J. Yue and N. Sakuragi (2016). "Reactivation of epigenetically silenced miR-124 reverses the epithelial-to-mesenchymal transition and inhibits invasion in endometrial cancer cells via the direct repression of IQGAP1 expression." Oncotarget **7**(15): 20260-20270.

Dong, P., M. Kaneuchi, H. Watari, J. Hamada, S. Sudo, J. Ju and N. Sakuragi (2011). "MicroRNA-194 inhibits epithelial to mesenchymal transition of endometrial cancer cells by targeting oncogene BMI-1." Mol Cancer **10**: 99.

Dong, P., Kaneuchi, M., Xiong, Y., Cao, L., Cai, M., Liu, X., Guo, S-W., Ju, J., Jia, N., Konno, Y., Watari, H., Hosaka, M., Sudo, S., Sakuragi, N. (2014). "Identification of KLF17 as a novel epithelial to mesenchymal transition inducer via direct activation of TWIST1 in endometrioid endometrial cancer." Carcinogenesis **35**(4): 760-768.

Dong, P., M. Karaayvaz, N. Jia, M. Kaneuchi, J. Hamada, H. Watari, S. Sudo, J. Ju and N. Sakuragi (2013). "Mutant p53 gain-of-function induces epithelial-mesenchymal transition through modulation of the miR-130b-ZEB1 axis." Oncogene **32**(27): 3286-3295.

Doudna, J. A. and E. Charpentier (2014). "Genome editing. The new frontier of genome engineering with CRISPR-Cas9." Science **346**(6213): 1258096.

Douma, S., T. Van Laar, J. Zevenhoven, R. Meuwissen, E. Van Garderen and D. S. Peeper (2004). "Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB." Nature **430**(7003): 1034-1039.

Dutta, I., D. Dieters-Castator, J. W. Papatzimas, A. Medina, J. Schueler, D. J. Derksen, G. Lajoie, L. M. Postovit and G. M. Siegers (2021). "ADAM protease inhibition overcomes resistance of breast cancer stem-like cells to gammadelta T cell immunotherapy." Cancer Lett **496**: 156-168.

Emran, A. A., D. M. Marzese, D. R. Menon, H. Hammerlindl, F. Ahmed, E. Richtig, P. Duijf, D. S. Hoon and H. Schaider (2019). "Commonly integrated epigenetic modifications of differentially expressed genes lead to adaptive resistance in cancer." Epigenomics **11**(7): 732-737.

Espinosa, I., A. De Leo, E. D'Angelo, J. M. Rosa-Rosa, M. Corominas, A. Gonzalez, J. Palacios and J. Prat (2018). "Dedifferentiated endometrial carcinomas with

neuroendocrine features: a clinicopathologic, immunohistochemical, and molecular genetic study." Hum Pathol **72**: 100-106.

Espinosa, I., Lee, CH., D'Angelo, E., Palacios, J., Prat, J. (2017). "Undifferentiated and Dedifferentiated EndometrialCarcinomas With POLE Exonuclease Domain MutationsHave a Favorable Prognosis." Am J Surg Pathol **41**: 1121-1128.

Esteller, M. (2008). "Epigenetics in cancer." N Engl J Med **358**(11): 1148-1159.

Esteve, P. O., H. G. Chin, A. Smallwood, G. R. Feehery, O. Gangisetty, A. R. Karpf, M. F. Carey and S. Pradhan (2006). "Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication." Genes Dev **20**(22): 3089-3103.

Eun, K., S. W. Ham and H. Kim (2017). "Cancer stem cell heterogeneity: origin and new perspectives on CSC targeting." BMB Rep **50**(3): 117-125.

Evans, T., O. Sany, P. Pearmain, R. Ganesan, A. Blann and S. Sundar (2011). "Differential trends in the rising incidence of endometrial cancer by type: data from a UK population-based registry from 1994 to 2006." Br J Cancer **104**(9): 1505-1510.

Fader, A., Diaz, LA., Armstrong, DK., Tanner. EJ., Uram, J., Eyring, A., Wang, H., Fisher, G., Grenten, T., Le, D. (2016). "Preliminary results of a phase II study: PD-1 blockade in mismatch repair–deficient, recurrent or
persistent endometrial cancer." Gynecol Oncol **141**: 206-207.

Fahiminiyah, S., Witkowski, L., Nadaf, J., Carrot-Zhang, J., Goudie, C., Hasselblatt, M., Johann, P., Kool, M., Lee, RS., Gayden, T., Roberts, CWM., Biegel, JA., Jabado, N., Majewski, J., Foulkes, WD. (2016). "Molecular analyses reveal close similarities between small cell carcinoma of the ovary, hypercalcemic type and atypical teratoid/rhabdoid tumor." Oncotarget **7**(2): 1732-1740.

Fane, M. E., Y. Chhabra, A. G. Smith and R. A. Sturm (2019). "BRN2, a POUerful driver of melanoma phenotype switching and metastasis." Pigment Cell Melanoma Res **32**(1): 9-24.

Fang, Y. Y., M. R. Tan, J. Zhou, L. Liang, X. Y. Liu, K. Zhao and E. C. Bao (2019). "miR-214-3p inhibits epithelial-to-mesenchymal transition and metastasis of endometrial cancer cells by targeting TWIST1." Onco Targets Ther **12**: 9449-9458.

Farmer, H., N. McCabe, C. J. Lord, A. N. Tutt, D. A. Johnson, T. B. Richardson, M. Santarosa, K. J. Dillon, I. Hickson, C. Knights, N. M. Martin, S. P. Jackson, G. C. Smith and A. Ashworth (2005). "Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy." Nature **434**(7035): 917-921.

Feng, J., Liu, T., Quin, B., Zhang, T., Liu, XS. (2012). "Identifying ChIP-seq enrichment using MACS." Nat Protoc.

Feng, Z., H. Gan, Z. Cai, N. Li, Z. Yang, G. Lu and J. Chen (2013). "Aberrant expression of hypoxia-inducible factor 1alpha, TWIST and E-cadherin is associated with aggressive tumor phenotypes in endometrioid endometrial carcinoma." Jpn J Clin Oncol **43**(4): 396-403.

Fierz, B. and M. G. Poirier (2019). "Biophysics of Chromatin Dynamics." Annu Rev Biophys **48**: 321-345.

Filipowicz, W., S. N. Bhattacharyya and N. Sonenberg (2008). "Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?" Nat Rev Genet **9**(2): 102-114.

Findlay, S. D., K. M. Vincent, J. R. Berman and L. M. Postovit (2016). "A Digital PCR-Based Method for Efficient and Highly Specific Screening of Genome Edited Cells." PLoS One **11**(4): e0153901.

Flavahan, W. A., E. Gaskell and B. E. Bernstein (2017). "Epigenetic plasticity and the hallmarks of cancer." Science **357**(6348).

Flowers, S., N. G. Nagl, Jr., G. R. Beck, Jr. and E. Moran (2009). "Antagonistic roles for BRM and BRG1 SWI/SNF complexes in differentiation." J Biol Chem **284**(15): 10067-10075.

Fong, P. C., D. S. Boss, T. A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau, M. J. O'Connor, A. Ashworth, J. Carmichael, S. B. Kaye, J. H. Schellens and J. S. de Bono (2009). "Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers." N Engl J Med **361**(2): 123-134.

Forster, J. C., W. M. Harriss-Phillips, M. J. Douglass and E. Bezak (2017). "A review of the development of tumor vasculature and its effects on the tumor microenvironment." Hypoxia (Auckl) **5**: 21-32.

Foulkes, W. D., Clarke, BA., Hasselblatt, M., Majewski, J., Albrecht, S., McCluggage, WG. (2014). "No Small Surprise - Small Cell Carcinoma of the Ovary, Hypercalcaemic Type, Is a Malignant Rhabdoid Tumour." J Pathol **233**(3): 209-214.

Franceschi, T., E. Durieux, A. P. Morel, P. de Saint Hilaire, I. Ray-Coquard, A. Puisieux and M. Devouassoux-Shisheboran (2019). "Role of epithelial-mesenchymal transition factors in the histogenesis of uterine carcinomas." Virchows Arch **475**(1): 85-94.

Frank, N. Y., T. Schatton and M. H. Frank (2010). "The therapeutic promise of the cancer stem cell concept." Journal of Clinical Investigation **120**(1): 41-50.

Fu, D., Wang, ZM., Chen-Li, Wang, BL., Shen, ZZ., Huang, W., Shao, ZM. (2009). "Sox17, the canonical Wnt antagonist, is epigenetically inactivated by promoter methylation in human breast cancer." Breeast Cancer Research and Treatment **119**: 601-612.

Fu, X., P. Zhang and B. Yu (2017). "Advances toward LSD1 inhibitors for cancer therapy." Future Med Chem **9**(11): 1227-1242.

Funato, K., T. Major, P. W. Lewis, C. D. Allis and V. Tabar (2014). "Use of human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation." Science **346**(6216): 1529-1533.

Gaj, T., S. J. Sirk, S. L. Shui and J. Liu (2016). "Genome-Editing Technologies: Principles and Applications." Cold Spring Harb Perspect Biol **8**(12).

Gallmeier, E., P. C. Hermann, M. T. Mueller, J. G. Machado, A. Ziesch, E. N. De Toni, A. Palagyi, C. Eisen, J. W. Ellwart, J. Rivera, B. Rubio-Viqueira, M. Hidalgo, F. Bunz, B. Goke and C. Heeschen (2011). "Inhibition of ataxia telangiectasia- and Rad3-related function abrogates the in vitro and in vivo tumorigenicity of human colon cancer cells through depletion of the CD133(+) tumor-initiating cell fraction." Stem Cells **29**(3): 418-429.

Gao, X., Y. Cai and R. An (2018). "miR215 promotes epithelial to mesenchymal transition and proliferation by regulating LEFTY2 in endometrial cancer." Int J Mol Med **42**(3): 1229-1236.

Gao, X., P. Tate, P. Hu, R. Tjian, W. C. Skarnes and Z. Wang (2008). "ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a." Proc Natl Acad Sci U S A **105**(18): 6656-6661.

Garg, K., K. Shih, R. Barakat, Q. Zhou, A. Iasonos and R. A. Soslow (2009). "Endometrial carcinomas in women aged 40 years and younger: tumors associated with loss of DNA mismatch repair proteins comprise a distinct clinicopathologic subset." Am J Surg Pathol **33**(12): 1869-1877.

Gascard, P., M. Bilenky, M. Sigaroudinia, J. Zhao, L. Li, A. Carles, A. Delaney, A. Tam, B. Kamoh, S. Cho, M. Griffith, A. Chu, G. Robertson, D. Cheung, I. Li, A. Heravi-Moussavi, M. Moksa, M. Mingay, A. Hussainkhel, B. Davis, R. P. Nagarajan, C. Hong, L. Echipare, H. O'Geen, M. J. Hangauer, J. B. Cheng, D. Neel, D. Hu, M. T. McManus, R. Moore, A. Mungall, Y. Ma, P. Plettner, E. Ziv, T. Wang, P. J. Farnham, S. J. Jones, M. A. Marra, T. D. Tlsty, J. F. Costello and M. Hirst (2015). "Epigenetic and transcriptional determinants of the human breast." Nat Commun **6**: 6351.

Geiss, G. K., R. E. Bumgarner, B. Birditt, T. Dahl, N. Dowidar, D. L. Dunaway, H. P. Fell, S. Ferree, R. D. George, T. Grogan, J. J. James, M. Maysuria, J. D. Mitton, P. Oliveri, J. L. Osborn, T. Peng, A. L. Ratcliffe, P. J. Webster, E. H. Davidson, L. Hood and K. Dimitrov (2008). "Direct multiplexed measurement of gene expression with color-coded probe pairs." Nat Biotechnol **26**(3): 317-325.

Gerdes, M. J., A. Sood, C. Sevinsky, A. D. Pris, M. I. Zavodszky and F. Ginty (2014). "Emerging understanding of multiscale tumor heterogeneity." Front Oncol **4**: 366.

Gerlinger, M. and C. Swanton (2010). "How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine." British Journal of Cancer **103**(8): 1139-1143.

Ghuwalewala, S., Ghatak, D., Das, P., Dey, S., Sarkar, S., Alam, N., Panda, CK., Roychoudhury, S. (2016). "CD44highCD24low molecular signature determines the Cancer Stem Cell and EMT phenotype in Oral Squamous Cell Carcinoma." Stem Cell Research **16**(2): 405-417.

Giordano, G., D'Adda, T., Bottarelli, L., Lombardi, M., Brigati, M., Berretta, R., Merisio, C. (2012). "Two cases of low-grade endometrioid carcinoma associated with undifferentiated carcinoma of the uterus (dedifferentiated carcinoma): a molecular study." Pathol Oncol Res **2**: 523-528.

Giraldo, N. A., R. Sanchez-Salas, J. D. Peske, Y. Vano, E. Becht, F. Petitprez, P. Validire, A. Ingels, X. Cathelineau, W. H. Fridman and C. Sautes-Fridman (2019). "The clinical role of the TME in solid cancer." Br J Cancer **120**(1): 45-53.

Giuliano, C. J., A. Lin, V. Girish and J. M. Sheltzer (2019). "Generating Single Cell-Derived Knockout Clones in Mammalian Cells with CRISPR/Cas9." Curr Protoc Mol Biol **128**(1): e100.

Goel, S., M. J. DeCristo, A. C. Watt, H. BrinJones, J. Sceneay, B. B. Li, N. Khan, J. M. Ubellacker, S. Xie, O. Metzger-Filho, J. Hoog, M. J. Ellis, C. X. Ma, S. Ramm, I. E. Krop, E. P. Winer, T. M. Roberts, H. J. Kim, S. S. McAllister and J. J. Zhao (2017). "CDK4/6 inhibition triggers anti-tumour immunity." Nature **548**(7668): 471-475.

Goel, S., Q. Wang, A. C. Watt, S. M. Tolaney, D. A. Dillon, W. Li, S. Ramm, A. C. Palmer, H. Yuzugullu, V. Varadan, D. Tuck, L. N. Harris, K. K. Wong, X. S. Liu, P. Sicinski, E. P. Winer, I. E. Krop and J. J. Zhao (2016). "Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers with CDK4/6 Inhibitors." Cancer Cell **29**(3): 255-269.

Goh, C., B. L. Farah, W. Y. Ho, S. L. Wong, C. H. R. Goh, S. H. Chew, R. Nadarajah, Y. K. Lim and T. H. Ho (2020). "Dedifferentiated endometrioid adenocarcinoma of the uterus: A case series and review of literature." Gynecol Oncol Rep **32**: 100538.

Gong, F. D. and K. M. Miller (2013). "Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation." Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis **750**(1-2): 23-30.

Gonzalez-Meljem, J. M., J. R. Apps, H. C. Fraser and J. P. Martinez-Barbera (2018). "Paracrine roles of cellular senescence in promoting tumourigenesis." Br J Cancer **118**(10): 1283-1288.

Gottesman, M. M., T. Fojo and S. E. Bates (2002). "Multidrug resistance in cancer: role of ATP-dependent transporters." Nat Rev Cancer **2**(1): 48-58.

Graff, J. (1997). "Embryonic patterning: To BMP or not to BMP, that is the question." Cell **89**(2): 171-174.

Granja, J., Corces, MR., Pierce, SE., Bagdatli, T., Choudhry, H., Change HY, Greenleaf, WJ. (2021). "ArchR is a scalable software package for integrative single-cell chromatin accessibility analysis." Nature Genetics **53**: 403-411.

Greaves, M. (2015). "Evolutionary determinants of cancer." Cancer Discov **5**(8): 806-820.

Greer, E. L. and Y. Shi (2012). "Histone methylation: a dynamic mark in health, disease and inheritance." Nat Rev Genet **13**(5): 343-357.

Grunstein, M. (1997). "Histone acetylation in chromatin structure and transcription." Nature **389**(6649): 349-352.

Gu, C. J., F. Xie, B. Zhang, H. L. Yang, J. Cheng, Y. Y. He, X. Y. Zhu, D. J. Li and M. Q. Li (2018). "High Glucose Promotes Epithelial-Mesenchymal Transition of Uterus Endometrial Cancer Cells by Increasing ER/GLUT4-Mediated VEGF Secretion." Cell Physiol Biochem **50**(2): 706-720.

Guan, H., J. Li, R. Sun, W. Liu, M. Feng, H. Ma and C. Li (2019). "Expression Of BMP7 In Ovarian Cancer And Biological Effect Of BMP7 Knockdown On Ovarian Cancer Cells." Onco Targets Ther **12**: 7897-7909.

Guan, X., K. M. LaPak, R. C. Hennessey, C. Y. Yu, R. Shakya, J. Zhang and C. E. Burd (2017). "Stromal Senescence By Prolonged CDK4/6 Inhibition Potentiates Tumor Growth." Mol Cancer Res **15**(3): 237-249.

Guillon, J., C. Petit, M. Moreau, B. Toutain, C. Henry, H. Roche, N. Bonichon-Lamichhane, J. P. Salmon, J. Lemonnier, M. Campone, V. Verriele, E. Lelievre, C. Guette and O. Coqueret (2019). "Regulation of senescence escape by TSP1 and CD47 following chemotherapy treatment." Cell Death Dis **10**(3): 199.

Guler, G. D., C. A. Tindell, R. Pitti, C. Wilson, K. Nichols, T. KaiWai Cheung, H. J. Kim, M. Wongchenko, Y. Yan, B. Haley, T. Cuellar, J. Webster, N. Alag, G. Hegde, E. Jackson, T. L. Nance, P. G. Giresi, K. B. Chen, J. Liu, S. Jhunjhunwala, J. Settleman, J. P. Stephan, D. Arnott and M. Classon (2017). "Repression of Stress-Induced LINE-1 Expression Protects Cancer Cell Subpopulations from Lethal Drug Exposure." Cancer Cell **32**(2): 221-237 e213.

Gumireddy, K., Li, A., Gimotty, P.A., Klein-Szanto, A.J., Showe, L.C., Katsaros, D., Coukos, G., Zhang, L., Huang, Q. (2009). "KLF17 is a negative regulator of epithelial–mesenchymal transition and metastasis in breast cancer." Nature Cell Biology: 1297–1304.

Gunes, C., Paszkowski-Rogacz, M., Rahmig, S., Camgoz, A., Wermke, M., Dahl, A., Bornhauser, M., Waskow, C., Bucholz, F. (2019). "Comparative RNAi Screens in Isogenic

Human Stem Cells Reveal SMARCA4 as a Differential Regulator." Stem Cell Reports **12**(5): 1084-1098.

Guo, B., R. E. Sallis, A. Greenall, M. M. Petit, E. Jansen, L. Young, W. J. Van de Ven and A. D. Sharrocks (2006). "The LIM domain protein LPP is a coactivator for the ETS domain transcription factor PEA3." Mol Cell Biol **26**(12): 4529-4538.

Guo, J., B. Wang, Z. Fu, J. Wei and W. Lu (2016). "Hypoxic Microenvironment Induces EMT and Upgrades Stem-Like Properties of Gastric Cancer Cells." Technol Cancer Res Treat **15**(1): 60-68.

Guo, J., F. Ye, X. Jiang, H. Guo, W. Xie, Y. Zhang and X. Sheng (2020). "Drp1 mediates high glucose-induced mitochondrial dysfunction and epithelial-mesenchymal transition in endometrial cancer cells." Exp Cell Res **389**(1): 111880.

Gusenbauer, S., P. Vlaicu and A. Ullrich (2013). "HGF induces novel EGFR functions involved in resistance formation to tyrosine kinase inhibitors." Oncogene **32**(33): 3846-3856.

Hacking, S., C. Jin, M. Komforti, S. Liang and M. Nasim (2019). "MMR deficient undifferentiated/dedifferentiated endometrial carcinomas showing significant programmed death ligand-1 expression (sp 142) with potential therapeutic implications." Pathol Res Pract **215**(10): 152552.

Hamada, S., A. Masamune, T. Takikawa, N. Suzuki, K. Kikuta, M. Hirota, H. Hamada, M. Kobune, K. Satoh and T. Shimosegawa (2012). "Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells." Biochem Biophys Res Commun **421**(2): 349-354.

Hamburger, A., Salmon, SE. (1977). "Primary bioassay of human tumor stem cells." Science **197**: 461-463.

Hamilton, E. and J. R. Infante (2016). "Targeting CDK4/6 in patients with cancer." Cancer Treat Rev **45**: 129-138.

Han, X., X. Fang, X. Lou, D. Hua, W. Ding, G. Foltz, L. Hood, Y. Yuan and B. Lin (2012). "Silencing SOX2 induced mesenchymal-epithelial transition and its expression predicts liver and lymph node metastasis of CRC patients." PLoS One **7**(8): e41335.

Han, Z. Y., W. Richer, P. Freneaux, C. Chauvin, C. Lucchesi, D. Guillemot, C. Grison, D. Lequin, G. Pierron, J. Masliah-Planchon, A. Nicolas, D. Ranchere-Vince, P. Varlet, S. Puget, I. Janoueix-Lerosey, O. Ayrault, D. Surdez, O. Delattre and F. Bourdeaut (2016). "The occurrence of intracranial rhabdoid tumours in mice depends on temporal control of Smarcb1 inactivation." Nat Commun **7**: 10421.

Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." Cell **144**(5): 646-674.

Hansen, K. D., W. Timp, H. C. Bravo, S. Sabunciyan, B. Langmead, O. G. McDonald, B. Wen, H. Wu, Y. Liu, D. Diep, E. Briem, K. Zhang, R. A. Irizarry and A. P. Feinberg (2011). "Increased methylation variation in epigenetic domains across cancer types." Nat Genet **43**(8): 768-775.

Hark, A. T., C. J. Schoenherr, D. J. Katz, R. S. Ingram, J. M. Levorse and S. M. Tilghman (2000). "CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus." Nature **405**(6785): 486-489.

Hart, T., K. R. Brown, F. Sircoulomb, R. Rottapel and J. Moffat (2014). "Measuring error rates in genomic perturbation screens: gold standards for human functional genomics." Mol Syst Biol **10**: 733.

Hayford, C. E., D. R. Tyson, C. J. Robbins, 3rd, P. L. Frick, V. Quaranta and L. A. Harris (2021). "An in vitro model of tumor heterogeneity resolves genetic, epigenetic, and stochastic sources of cell state variability." PLoS Biol **19**(6): e3000797.

He, S., Z. Wu, Y. Tian, Z. Yu, J. Yu, X. Wang, J. Li, B. Liu and Y. Xu (2020). "Structure of nucleosome-bound human BAF complex." Science **367**(6480): 875-881.

Heintzman, N. D., R. K. Stuart, G. Hon, Y. Fu, C. W. Ching, R. D. Hawkins, L. O. Barrera, S. Van Calcar, C. Qu, K. A. Ching, W. Wang, Z. Weng, R. D. Green, G. E. Crawford and B. Ren (2007). "Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome." Nat Genet **39**(3): 311-318.

Helming, K., Wang, X., Wilson, BG., Vazquez, F., Haswell, JR., Manchester, HE., Kim, Y., Kryukov, GV., Ghandi, M., Aguirre, AJ., Jagani, Z., Garraway, LA., Hahn, WC., Roberts, CWM. (2014). "ARID1B is a specific vulnerability in ARID1A-mutant cancers." Nat Med **20**: 251-254.

Helming, K. C., Wang, X., Wilson, BG., Vazquez, F., Haswell, JR., Manchester, HE., Kim, Y., Kryukov, G., Ghandi, M., Aguirre, AJ., Jagani, Z., Wang, Z., Garraway, LA., Hahn, WC., Roberts, WM. (2014). "ARID1B is a specific vulnerability in ARID1A-mutant cancers." Nature Medicine **20**: 251-254.

Hernandez-Camarero, P., G. Jimenez, E. Lopez-Ruiz, S. Barungi, J. A. Marchal and M. Peran (2018). "Revisiting the dynamic cancer stem cell model: Importance of tumour edges." Crit Rev Oncol Hematol **131**: 35-45.

Herpel, E., R. J. Rieker, H. Dienemann, T. Muley, M. Meister, A. Hartmann, A. Warth and A. Agaimy (2017). "SMARCA4 and SMARCA2 deficiency in non-small cell lung cancer: immunohistochemical survey of 316 consecutive specimens." Ann Diagn Pathol **26**: 47-51.

Herreros-Villanueva, M., J. S. Zhang, A. Koenig, E. V. Abel, T. C. Smyrk, W. R. Bamlet, A. A. de Narvajas, T. S. Gomez, D. M. Simeone, L. Bujanda and D. D. Billadeau (2013). "SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells." Oncogenesis **2**: e61.

Hezroni, H., I. Tzchori, A. Davidi, A. Mattout, A. Biran, M. Nissim-Rafinia, H. Westphal and E. Meshorer (2011). "H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells." Nucleus **2**(4): 300-309.

Hidalgo, M., F. Amant, A. V. Biankin, E. Budinska, A. T. Byrne, C. Caldas, R. B. Clarke, S. de Jong, J. Jonkers, G. M. Maelandsmo, S. Roman-Roman, J. Seoane, L. Trusolino and A. Villanueva (2014). "Patient-derived xenograft models: an emerging platform for translational cancer research." Cancer Discov **4**(9): 998-1013.

Hinohara, K., H. J. Wu, S. Vigneau, T. O. McDonald, K. J. Igarashi, K. N. Yamamoto, T. Madsen, A. Fassl, S. B. Egri, M. Papanastasiou, L. Ding, G. Peluffo, O. Cohen, S. C. Kales, M. Lal-Nag, G. Rai, D. J. Maloney, A. Jadhav, A. Simeonov, N. Wagle, M. Brown, A. Meissner, P. Sicinski, J. D. Jaffe, R. Jeselsohn, A. A. Gimelbrant, F. Michor and K. Polyak (2018). "KDM5 Histone Demethylase Activity Links Cellular Transcriptomic Heterogeneity to Therapeutic Resistance." Cancer Cell **34**(6): 939-953 e939.

Hirsch, F., Varella-Garcia, M., Bunn, PA., Di Maria, MV., Veve, R., Bremnes, RM., Baron, AE., Zeng, C., Franklin, WA. (2003). "Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis." J Clin Oncol **21**: 3798-3807.

Hoadley, K. A., C. Yau, T. Hinoue, D. M. Wolf, A. J. Lazar, E. Drill, R. Shen, A. M. Taylor, A. D. Cherniack, V. Thorsson, R. Akbani, R. Bowlby, C. K. Wong, M. Wiznerowicz, F. Sanchez-Vega, A. G. Robertson, B. G. Schneider, M. S. Lawrence, H. Noushmehr, T. M. Malta, N. Cancer Genome Atlas, J. M. Stuart, C. C. Benz and P. W. Laird (2018). "Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer." Cell **173**(2): 291-304 e296.

Hoang, L., Han, G., McConechy, M., Lau, S., Chow, C., Blake Gilks, C., Huntsman, DG., Kobel, M., Lee, CH. (2014). "Immunohistochemical characterization of prototypical endometrial clear cell carcinoma-diagnostic utility of HNF-1beta and oestrogen receptor." Histopathology **64**(6): 585-596.

Hoang, L. N., Y. S. Lee, A. N. Karnezis, B. Tessier-Cloutier, N. Almandani, M. Coatham, C. B. Gilks, R. A. Soslow, C. J. Stewart, M. Kobel and C. H. Lee (2016). "Immunophenotypic features of dedifferentiated endometrial carcinoma - insights from BRG1/INI1-deficient tumours." Histopathology **69**(4): 560-569.

Hoang, L. N., M. K. McConechy, B. Meng, J. B. McIntyre, C. Ewanowich, C. B. Gilks, D. G. Huntsman, M. Kobel and C. H. Lee (2015). "Targeted mutation analysis of endometrial clear cell carcinoma." Histopathology **66**(5): 664-674.

Hoare, M., Narita, M. (2018). "The Power Behind the Throne: Senescence and the Hallmarks of Cancer." Annual Review of Cancer Biology **2**: 175-194.

Hodges, C., J. G. Kirkland and G. R. Crabtree (2016). "The Many Roles of BAF (mSWI/SNF) and PBAF Complexes in Cancer." Cold Spring Harb Perspect Med **6**(8).

Hoffman, G., Rahal, R., Buxton, F., Xiang, K., McAllister, G., Frias, E., Bagdasarian, L., Huber, J., Lindeman, A., Chen, D., Romero, R., Ramadan, N., Phadke, T., Haas, K., Jaskelioff, M., Wilson, BG., Meyer, MJ., Saenz-Vash, V., Zhai, H., Myer, VE., Porter, JA., Keen, N., McLaughlin, ME., Mickanin, C., Roberts, CWM., Stegmeier, F., Jagani, Z. (2014). "Functional Epigenetics Approach Identifies BRM/SMARCA2 as a Critical Synthetic Lethal Target in BRG1-deficient Cancers." Proc Natl Acad Sci USA **111**(8): 3128-3133.

Hoffman, G. R., R. Rahal, F. Buxton, K. Xiang, G. McAllister, E. Frias, L. Bagdasarian, J. Huber, A. Lindeman, D. Chen, R. Romero, N. Ramadan, T. Phadke, K. Haas, M. Jaskelioff, B. G. Wilson, M. J. Meyer, V. Saenz-Vash, H. Zhai, V. E. Myer, J. A. Porter, N. Keen, M. E. McLaughlin, C. Mickanin, C. W. Roberts, F. Stegmeier and Z. Jagani (2014). "Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers." Proc Natl Acad Sci U S A **111**(8): 3128-3133.

Holdhof, D., P. D. Johann, M. Spohn, M. Bockmayr, S. Safaei, P. Joshi, J. Masliah-Planchon, B. Ho, M. Andrianteranagna, F. Bourdeaut, A. Huang, M. Kool, S. A. Upadhyaya, A. E. Bendel, D. Indenbirken, W. D. Foulkes, J. W. Bush, D. Creytens, U. Kordes, M. C. Fruhwald, M. Hasselblatt and U. Schuller (2021). "Atypical teratoid/rhabdoid tumors (ATRTs) with SMARCA4 mutation are molecularly distinct from SMARCB1-deficient cases." Acta Neuropathol **141**(2): 291-301.

Holliday, R. and J. E. Pugh (1975). "DNA modification mechanisms and gene activity during development." Science **187**(4173): 226-232.

Hong, H., K. Takahashi, T. Ichisaka, T. Aoi, O. Kanagawa, M. Nakagawa, K. Okita and S. Yamanaka (2009). "Suppression of induced pluripotent stem cell generation by the p53-p21 pathway." Nature **460**(7259): 1132-1135.

Horibata, S., T. V. Vo, V. Subramanian, P. R. Thompson and S. A. Coonrod (2015). "Utilization of the Soft Agar Colony Formation Assay to Identify Inhibitors of Tumorigenicity in Breast Cancer Cells." J Vis Exp(99): e52727.

Hornick, J., Dal Cin, P., Fletcher, CDM. (2009). "Loss of INI1 Expression Is Characteristic of Both Conventional and Proximal-Type Epithelioid Sarcoma." Am J Surg Pathol **33**(4): 542-550.

Hsu, P. D., E. S. Lander and F. Zhang (2014). "Development and applications of CRISPR-Cas9 for genome engineering." Cell **157**(6): 1262-1278.

Huang, A., Garraway, L.A., Ashworth, A., Weber, B. (2019). "Synthetic lethality as an engine for cancer drug target discovery." Nature Reviews Drug Discovery **19**: 23-28.

Huang, P. H. (2017). "Targeting SWI/SNF mutant cancers with tyrosine kinase inhibitor therapy." Expert Rev Anticancer Ther **17**(1): 1-3.

Huang, Y. and N. Yang (2018). "MicroRNA-20a-5p inhibits epithelial to mesenchymal transition and invasion of endometrial cancer cells by targeting STAT3." Int J Clin Exp Pathol **11**(12): 5715-5724.

Huyen, Y., O. Zgheib, R. A. Ditullio, Jr., V. G. Gorgoulis, P. Zacharatos, T. J. Petty, E. A. Sheston, H. S. Mellert, E. S. Stavridi and T. D. Halazonetis (2004). "Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks." Nature **432**(7015): 406-411.

Igelmann, S., F. Lessard, O. Uchenunu, J. Bouchard, A. Fernandez-Ruiz, M. C. Rowell, S. Lopes-Paciencia, D. Papadopoli, A. Fouillen, K. J. Ponce, G. Huot, L. Mignacca, M. Benfdil, P. Kalegari, H. M. Wahba, J. Pencik, N. Vuong, J. Quenneville, J. Guillon, V. Bourdeau, L. Hulea, E. Gagnon, L. Kenner, R. Moriggl, A. Nanci, M. N. Pollak, J. G. Omichinski, I. Topisirovic and G. Ferbeyre (2021). "A hydride transfer complex reprograms NAD metabolism and bypasses senescence." Mol Cell **81**(18): 3848-3865 e3819.

Iniguez, A. B., G. Alexe, E. J. Wang, G. Roti, S. Patel, L. Chen, S. Kitara, A. Conway, A. L. Robichaud, B. Stolte, P. Bandopadhayay, A. Goodale, S. Pantel, Y. Lee, D. M. Cheff, M. D. Hall, R. Guha, M. I. Davis, M. Menard, N. Nasholm, W. A. Weiss, J. Qi, R. Beroukhim, F. Piccioni, C. Johannessen and K. Stegmaier (2018). "Resistance to Epigenetic-Targeted Therapy Engenders Tumor Cell Vulnerabilities Associated with Enhancer Remodeling." Cancer Cell **34**(6): 922-938 e927.

Inoue, H., H. Takahashi, M. Hashimura, K. Eshima, M. Akiya, T. Matsumoto and M. Saegusa (2016). "Cooperation of Sox4 with beta-catenin/p300 complex in transcriptional regulation of the Slug gene during divergent sarcomatous differentiation in uterine carcinosarcoma." BMC Cancer **16**: 53.

International Cancer Genome, C., T. J. Hudson, W. Anderson, A. Artez, A. D. Barker, C. Bell, R. R. Bernabe, M. K. Bhan, F. Calvo, I. Eerola, D. S. Gerhard, A. Guttmacher, M. Guyer, F. M. Hemsley, J. L. Jennings, D. Kerr, P. Klatt, P. Kolar, J. Kusada, D. P. Lane, F. Laplace, L. Youyong, G. Nettekoven, B. Ozenberger, J. Peterson, T. S. Rao, J. Remacle, A. J. Schafer, T. Shibata, M. R. Stratton, J. G. Vockley, K. Watanabe, H. Yang, M. M. Yuen, B. M. Knoppers, M. Bobrow, A. Cambon-Thomsen, L. G. Dressler, S. O. Dyke, Y. Joly, K. Kato, K. L. Kennedy, P. Nicolas, M. J. Parker, E. Rial-Sebbag, C. M. Romeo-Casabona, K. M. Shaw, S. Wallace, G. L. Wiesner, N. Zeps, P. Lichter, A. V. Biankin, C. Chabannon, L. Chin, B. Clement, E. de Alava, F. Degos, M. L. Ferguson, P. Geary, D. N. Hayes, T. J. Hudson, A. L. Johns, A. Kasprzyk, H. Nakagawa, R. Penny, M. A. Piris, R. Sarin, A. Scarpa, T. Shibata, M. van de Vijver, P. A. Futreal, H. Aburatani, M. Bayes, D. D. Botwell, P. J. Campbell, X. Estivill, D. S. Gerhard, S. M. Grimmond, I. Gut, M. Hirst, C. Lopez-Otin, P. Majumder, M. Marra, J. D. McPherson, H. Nakagawa, Z. Ning, X. S. Puente, Y. Ruan, T. Shibata, M. R. Stratton, H. G. Stunnenberg, H. Swerdlow, V. E. Velculescu, R. K. Wilson, H. H. Xue, L. Yang, P. T. Spellman, G. D. Bader, P. C. Boutros, P. J. Campbell, P. Flicek, G. Getz, R. Guigo, G. Guo, D. Haussler, S. Heath, T. J. Hubbard, T. Jiang, S. M. Jones, Q. Li, N. Lopez-Bigas, R. Luo, L. Muthuswamy, B. F. Ouellette, J. V. Pearson, X. S. Puente, V. Quesada, B. J. Raphael, C. Sander, T. Shibata, T. P. Speed, L. D. Stein, J. M. Stuart, J. W. Teague, Y. Totoki, T. Tsunoda, A. Valencia,

D. A. Wheeler, H. Wu, S. Zhao, G. Zhou, L. D. Stein, R. Guigo, T. J. Hubbard, Y. Joly, S. M. Jones, A. Kasprzyk, M. Lathrop, N. Lopez-Bigas, B. F. Ouellette, P. T. Spellman, J. W. Teague, G. Thomas, A. Valencia, T. Yoshida, K. L. Kennedy, M. Axton, S. O. Dyke, P. A. Futreal, D. S. Gerhard, C. Gunter, M. Guyer, T. J. Hudson, J. D. McPherson, L. J. Miller, B. Ozenberger, K. M. Shaw, A. Kasprzyk, L. D. Stein, J. Zhang, S. A. Haider, J. Wang, C. K. Yung, A. Cros, Y. Liang, S. Gnaneshan, J. Guberman, J. Hsu, M. Bobrow, D. R. Chalmers, K. W. Hasel, Y. Joly, T. S. Kaan, K. L. Kennedy, B. M. Knoppers, W. W. Lowrance, T. Masui, P. Nicolas, E. Rial-Sebbag, L. L. Rodriguez, C. Vergely, T. Yoshida, S. M. Grimmond, A. V. Biankin, D. D. Bowtell, N. Cloonan, A. deFazio, J. R. Eshleman, D. Etemadmoghadam, B. B. Gardiner, J. G. Kench, A. Scarpa, R. L. Sutherland, M. A. Tempero, N. J. Waddell, P. J. Wilson, J. D. McPherson, S. Gallinger, M. S. Tsao, P. A. Shaw, G. M. Petersen, D. Mukhopadhyay, L. Chin, R. A. DePinho, S. Thayer, L. Muthuswamy, K. Shazand, T. Beck, M. Sam, L. Timms, V. Ballin, Y. Lu, J. Ji, X. Zhang, F. Chen, X. Hu, G. Zhou, Q. Yang, G. Tian, L. Zhang, X. Xing, X. Li, Z. Zhu, Y. Yu, J. Yu, H. Yang, M. Lathrop, J. Tost, P. Brennan, I. Holcatova, D. Zaridze, A. Brazma, L. Egevard, E. Prokhortchouk, R. E. Banks, M. Uhlen, A. Cambon-Thomsen, J. Viksna, F. Ponten, K. Skryabin, M. R. Stratton, P. A. Futreal, E. Birney, A. Borg, A. L. Borresen-Dale, C. Caldas, J. A. Foekens, S. Martin, J. S. Reis-Filho, A. L. Richardson, C. Sotiriou, H. G. Stunnenberg, G. Thoms, M. van de Vijver, L. van't Veer, F. Calvo, D. Birnbaum, H. Blanche, P. Boucher, S. Boyault, C. Chabannon, I. Gut, J. D. Masson-Jacquemier, M. Lathrop, I. Pauporte, X. Pivot, A. Vincent-Salomon, E. Tabone, C. Theillet, G. Thomas, J. Tost, I. Treilleux, F. Calvo, P. Bioulac-Sage, B. Clement, T. Decaens, F. Degos, D. Franco, I. Gut, M. Gut, S. Heath, M. Lathrop, D. Samuel, G. Thomas, J. Zucman-Rossi, P. Lichter, R. Eils, B. Brors, J. O. Korbel, A. Korshunov, P. Landgraf, H. Lehrach, S. Pfister, B. Radlwimmer, G. Reifenberger, M. D. Taylor, C. von Kalle, P. P. Majumder, R. Sarin, T. S. Rao, M. K. Bhan, A. Scarpa, P. Pederzoli, R. A. Lawlor, M. Delledonne, A. Bardelli, A. V. Biankin, S. M. Grimmond, T. Gress, D. Klimstra, G. Zamboni, T. Shibata, Y. Nakamura, H. Nakagawa, J. Kusada, T. Tsunoda, S. Miyano, H. Aburatani, K. Kato, A. Fujimoto, T. Yoshida, E. Campo, C. Lopez-Otin, X. Estivill, R. Guigo, S. de Sanjose, M. A. Piris, E. Montserrat, M. Gonzalez-Diaz, X. S. Puente, P. Jares, A. Valencia, H. Himmelbauer, V. Quesada, S. Bea, M. R. Stratton, P. A. Futreal, P. J. Campbell, A. Vincent-Salomon, A. L. Richardson, J. S. Reis-Filho, M. van de Vijver, G. Thomas, J. D. Masson-Jacquemier, S. Aparicio, A. Borg, A. L. Borresen-Dale, C. Caldas, J. A. Foekens, H. G. Stunnenberg, L. van't Veer, D. F. Easton, P. T. Spellman, S. Martin, A. D. Barker, L. Chin, F. S. Collins, C. C. Compton, M. L. Ferguson, D. S. Gerhard, G. Getz, C. Gunter, A. Guttmacher, M. Guyer, D. N. Hayes, E. S. Lander, B. Ozenberger, R. Penny, J. Peterson, C. Sander, K. M. Shaw, T. P. Speed, P. T. Spellman, J. G. Vockley, D. A. Wheeler, R. K. Wilson, T. J. Hudson, L. Chin, B. M. Knoppers, E. S. Lander, P. Lichter, L. D. Stein, M. R. Stratton, W. Anderson, A. D. Barker, C. Bell, M. Bobrow, W. Burke, F. S. Collins, C. C. Compton, R. A. DePinho, D. F. Easton, P. A. Futreal, D. S. Gerhard, A. R. Green, M. Guyer, S. R. Hamilton, T. J. Hubbard, O. P. Kallioniemi, K. L. Kennedy, T. J. Ley, E. T. Liu, Y. Lu, P. Majumder, M. Marra, B. Ozenberger, J. Peterson, A. J. Schafer, P. T. Spellman, H. G. Stunnenberg, B. J. Wainwright, R. K. Wilson and H. Yang (2010). "International network of cancer genome projects." Nature **464**(7291): 993-998.

Ishak, C. A., M. Classon and D. D. De Carvalho (2018). "Deregulation of Retroelements as an Emerging Therapeutic Opportunity in Cancer." Trends in Cancer **4**(8): 583-597.

Iwatsuki, M., K. Mimori, T. Yokobori, H. Ishi, T. Beppu, S. Nakamori, H. Baba and M. Mori (2010). "Epithelial-mesenchymal transition in cancer development and its clinical significance." Cancer Sci **101**(2): 293-299.

Iyoda, A., K. Hiroshima, T. Toyozaki, Y. Haga, T. Fujisawa and H. Ohwada (2001). "Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology." Cancer **91**(11): 1992-2000.

Jackson, S. P. and J. Bartek (2009). "The DNA-damage response in human biology and disease." Nature **461**(7267): 1071-1078.

Jagani, Z., E. L. Mora-Blanco, C. G. Sansam, E. S. McKenna, B. Wilson, D. Chen, J. Klekota, P. Tamayo, P. T. Nguyen, M. Tolstorukov, P. J. Park, Y. J. Cho, K. Hsiao, S. Buonamici, S. L. Pomeroy, J. P. Mesirov, H. Ruffner, T. Bouwmeester, S. J. Luchansky, J. Murtie, J. F. Kelleher, M. Warmuth, W. R. Sellers, C. W. Roberts and M. Dorsch (2010). "Loss of the tumor suppressor Snf5 leads to aberrant activation of the Hedgehog-Gli pathway." Nat Med **16**(12): 1429-1433.

Jancewicz, I., J. A. Siedlecki, T. J. Sarnowski and E. Sarnowska (2019). "BRM: the core ATPase subunit of SWI/SNF chromatin-remodelling complex-a tumour suppressor or tumour-promoting factor?" Epigenetics Chromatin **12**(1): 68.

Janssen, S. M., R. Moscona, M. Elchebly, A. I. Papadakis, M. Redpath, H. Wang, E. Rubin, L. C. van Kempen and A. Spatz (2020). "BORIS/CTCFL promotes a switch from a proliferative towards an invasive phenotype in melanoma cells." Cell Death Discov **6**: 1.

Jelinic, P., J. J. Mueller, N. Olvera, F. Dao, S. N. Scott, R. Shah, J. Gao, N. Schultz, M. Gonen, R. A. Soslow, M. F. Berger and D. A. Levine (2014). "Recurrent SMARCA4 mutations in small cell carcinoma of the ovary." Nat Genet **46**(5): 424-426.

Jemal, A., F. Bray, M. M. Center, J. Ferlay, E. Ward and D. Forman (2011). "Global cancer statistics." CA Cancer J Clin **61**(2): 69-90.

Jia, H., M. Osak, G. K. Bogu, L. W. Stanton, R. Johnson and L. Lipovich (2010). "Genome-wide computational identification and manual annotation of human long noncoding RNA genes." RNA **16**(8): 1478-1487.

Jiang, F. Z., Y. Y. He, H. H. Wang, H. L. Zhang, J. Zhang, X. F. Yan, X. J. Wang, Q. Che, J. Q. Ke, Z. Chen, H. Tong, Y. L. Zhang, F. Y. Wang, Y. R. Li and X. P. Wan (2015). "Mutant p53 induces EZH2 expression and promotes epithelial-mesenchymal transition by disrupting p68-Drosha complex assembly and attenuating miR-26a processing." Oncotarget **6**(42): 44660-44674.

Jiang, W., Bikard, D., Cox, D., Zhang, F., Marraffini, LA. (2013). "RNA-guided editing of bacterial genomes using CRISPR-Cas systems." Nature Biotechnology **31**: 233-239.

Jin, J., Y. Xu, L. Huo, L. Ma, A. W. Scott, M. P. Pizzi, Y. Li, Y. Wang, X. Yao, S. Song and J. A. Ajani (2020). "An improved strategy for CRISPR/Cas9 gene knockout and subsequent wildtype and mutant gene rescue." PLoS One **15**(2): e0228910.

Jones, S., Li, M., Parsons, D.W., Zhang, Z., Wesseling, J., Kristel, P., Schmidt, M.K., Markowitz, S., Yan, H., Bigner, D., Hruban, R.H., Eshleman, J.R., Iacobuzio-Donahue, C.A., Goggins, M., Maitra, A., Malek, S.N., Powell, S. Vogelstein, B., Kinzler, K.W., Velculescu, V.E., Papadopoulos, N. (2012). "Somatic Mutations in the Chromatin Remodeling Gene ARID1A Occur in Several Tumor Types." Hum Mutat **33**(1): 100-103.

Jorissen, R. N., F. Walker, N. Pouliot, T. P. Garrett, C. W. Ward and A. W. Burgess (2003). "Epidermal growth factor receptor: mechanisms of activation and signalling." Exp Cell Res **284**(1): 31-53.

Kadoch, C. and G. R. Crabtree (2015). "Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics." Sci Adv **1**(5): e1500447.

Kadoch, C., D. C. Hargreaves, C. Hodges, L. Elias, L. Ho, J. Ranish and G. R. Crabtree (2013). "Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy." Nat Genet **45**(6): 592-601.

Kaffer, C. R., M. Srivastava, K. Y. Park, E. Ives, S. Hsieh, J. Batlle, A. Grinberg, S. P. Huang and K. Pfeifer (2000). "A transcriptional insulator at the imprinted H19/Igf2 locus." Genes Dev **14**(15): 1908-1919.

Kang, M. K., S. Mehrazarin, N. H. Park and C. Y. Wang (2017). "Epigenetic gene regulation by histone demethylases: emerging role in oncogenesis and inflammation." Oral Dis **23**(6): 709-720.

Kardos, J., S. Chai, L. E. Mose, S. R. Selitsky, B. Krishnan, R. Saito, M. D. Iglesia, M. I. Milowsky, J. S. Parker, W. Y. Kim and B. G. Vincent (2016). "Claudin-low bladder tumors are immune infiltrated and actively immune suppressed." JCI Insight **1**(3): e85902.

Karnezis, A., Hoang, L., Coatham, M., Ravn, S., Almadani, N., Cloutier, B., Irving, J., Meng, B., Li, X., Chow, C., McAlpine, J., Kuo, K-T., Mao, T-L., Djordjevic, B., Soslow, R., Huntsman, D., Gilks, B., Koebel, M., Lee, C-H. (2016). "Loss of switch/sucrose non-fermenting complex protein expression is associated with dedifferentiation in endometrial carcinomas." Mod Pathol.

Karnezis, A. N., Wang, Y., Ramos, P., Hendricks, W.Pd., Oliva, E., D'Angelo, E., Prat, J., Nucci, M.R., Nielsen, T.O., Chow, C., Leung, S., Kommoss, F., Kommoss, S., Silva, A., Ronnett, B.M., Rabban, J.T., Bowtell, D.D., Weissman, B.E., Trent, J.M., Gilks, C.B., Huntsman, D.G. (2016). "Dual Loss of the SWI/SNF Complex ATPases SMARCA4/BRG1 and SMARCA2/BRM Is Highly Sensitive and Specific for Small Cell Carcinoma of the Ovary, Hypercalcaemic Type." J Pathol **238**(3): 389-400.

Kemme, C. A., R. Marquez, R. H. Luu and J. Iwahara (2017). "Potential role of DNA methylation as a facilitator of target search processes for transcription factors through interplay with methyl-CpG-binding proteins." Nucleic Acids Res **45**(13): 7751-7759.

Kim, H., Kim, J-S. (2014). "A guide to genome engineering with programmable nucleases." Nature Reviews Genetics **15**: 321-334.

Kim, J., M. Guermah, R. K. McGinty, J. S. Lee, Z. Tang, T. A. Milne, A. Shilatifard, T. W. Muir and R. G. Roeder (2009). "RAD6-Mediated Transcription-Coupled H2B Ubiquitylation Directly Stimulates H3K4 Methylation in Human Cells." Cell **137**(3): 459-471.

Kim, K. H., W. Kim, T. P. Howard, F. Vazquez, A. Tsherniak, J. N. Wu, W. Wang, J. R. Haswell, L. D. Walensky, W. C. Hahn, S. H. Orkin and C. W. Roberts (2015). "SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2." Nat Med **21**(12): 1491-1496.

Kim, K. H. and C. W. Roberts (2016). "Targeting EZH2 in cancer." Nat Med **22**(2): 128-134.

Kim, Y. S., B. R. Yi, N. H. Kim and K. C. Choi (2014). "Role of the epithelial-mesenchymal transition and its effects on embryonic stem cells." Exp Mol Med **46**: e108.

Kitami, K., M. Yoshihara, Y. Koya, M. Sugiyama, S. Iyoshi, K. Uno, K. Mogi, S. Tano, H. Fujimoto, A. Nawa, F. Kikkawa and H. Kajiyama (2020). "Microphthalmia-Associated Transcription Factor-Dependent Melanoma Cell Adhesion Molecule Activation Promotes Peritoneal Metastasis of Ovarian Cancer." Int J Mol Sci **21**(24).

Kitazawa, S., R. Kitazawa and S. Maeda (1999). "Transcriptional regulation of rat cyclin D1 gene by CpG methylation status in promoter region." J Biol Chem **274**(40): 28787-28793.

Kobel, M., Meng, B., Hoang, L.N., Almadani, N., Li, X., Soslow, R.A., Gilks, C.B., Lee, C.H. (2016). "Molecular Analysis of Mixed Endometrial Carcinomas Shows Clonality in Most Cases." Am J Surg Pathol **40**(2): 166-180.

Kohlberger, P., Gitsch, G., Loesch, A., Tempfer, C., Kaider, A., Reinthaller, A., Kainz, C., Breitenecker, G. (1996). "p53 protein overexpression in early stage endometrial cancer " Gynecol Oncol **62**: 213-217.

Konings, G. F., N. Saarinen, B. Delvoux, L. Kooreman, P. Koskimies, C. Krakstad, K. E. Fasmer, I. S. Haldorsen, A. Zaffagnini, M. R. Hakkinen, S. Auriola, L. Dubois, N. Lieuwes, F. Verhaegen, L. E. Schyns, R. F. Kruitwagen, E. Consortium, S. Xanthoulea and A. Romano (2018). "Development of an Image-Guided Orthotopic Xenograft Mouse Model of Endometrial Cancer with Controllable Estrogen Exposure." Int J Mol Sci **19**(9).

Konno, Y., Dong, P., Xiong, Y., Suzuki, F., Lu, J., Cai, M., Watari, H., Mitamura, T., Hosaka, M., Hanley, SJB., Kudo, M., Sakuragi, N. (2014). "MicroRNA-101 Targets EZH2,

MCL-1 and FOS to Suppress Proliferation, Invasion and Stem Cell-Like Phenotype of Aggressive Endometrial Cancer Cells." Oncotarget **5**(15): 6049-6062.

Kosicki, M., K. Tomberg and A. Bradley (2018). "Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements." Nat Biotechnol **36**(8): 765-771.

Kreso, A. and J. E. Dick (2014). "Evolution of the cancer stem cell model." Cell Stem Cell **14**(3): 275-291.

Krivtsov, A. V., D. Twomey, Z. Feng, M. C. Stubbs, Y. Wang, J. Faber, J. E. Levine, J. Wang, W. C. Hahn, D. G. Gilliland, T. R. Golub and S. A. Armstrong (2006). "Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9." Nature **442**(7104): 818-822.

Kudo-Saito, C., Shirako, H., Takeuchi, T., Kawakami, Y. (2009). "Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells." Cancer Cell **15**(3): 195-206.

Kuhn, E., A. Ayhan, A. Bahadirli-Talbott, C. Zhao and M. Shih Ie (2014). "Molecular characterization of undifferentiated carcinoma associated with endometrioid carcinoma." Am J Surg Pathol **38**(5): 660-665.

Kuhn, E., R. C. Wu, B. Guan, G. Wu, J. Zhang, Y. Wang, L. Song, X. Yuan, L. Wei, R. B. Roden, K. T. Kuo, K. Nakayama, B. Clarke, P. Shaw, N. Olvera, R. J. Kurman, D. A. Levine, T. L. Wang and M. Shih Ie (2012). "Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses." J Natl Cancer Inst **104**(19): 1503-1513.

Kulkarni, M. M. (2011). "Digital Multiplexed Gene Expression Analysis Using the NanoString nCounter System." Current Protocols in Molecular Biology **94**(1): 25B.10.21-25B.10.17.

Kumari, R. and P. Jat (2021). "Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype." Front Cell Dev Biol **9**: 645593.

Kuramoto, H., M. Nishida, T. Morisawa, M. Hamano, H. Hata, Y. Kato, E. Ohno and T. Iida (1991). "Establishment and characterization of human endometrial cancer cell lines." Ann N Y Acad Sci **622**: 402-421.

La Monica, S., C. Fumarola, D. Cretella, M. Bonelli, R. Minari, A. Cavazzoni, G. Digiacomo, M. Galetti, F. Volta, M. Mancini, P. G. Petronini, M. Tiseo and R. Alfieri (2020). "Efficacy of the CDK4/6 Dual Inhibitor Abemaciclib in EGFR-Mutated NSCLC Cell Lines with Different Resistance Mechanisms to Osimertinib." Cancers (Basel) **13**(1).

Laisne, M., N. Gupta, O. Kirsh, S. Pradhan and P. A. Defossez (2018). "Mechanisms of DNA Methyltransferase Recruitment in Mammals." Genes (Basel) **9**(12).

Lamouille, S., Xu, J., Derynck, R. (2014). "Molecular mechamisms of epithelial-mesenchymal transition." Nature Reviews Molecular Cell Biology **15**: 178-196.

Lang, J., Hendricks, WPD., Orlando, KA., Yin, H., Kiefer, J., Ramos, P., Sharma, R., Pirrotte, P., Raupach, EA., Sereduk, C., Tang, N., Liang, WS., Washington, M., Facista, SJ., Zismann, VL., Cousins, EM., Major, MB., Wang, Y., Karnezis, AN., Sekulic, A., Hass, R., Vanderhyden, BC>< Nair, P., Weissman, BE., Huntsman, DG., Trent, JM. (2018). "Ponatinib Shows Potent Antitumor Activity in Small Cell Carcinoma of the Ovary Hypercalcemic Type (SCCOHT) through Multikinase Inhibition." Clin Cancer Res **24**: 1932-1943.

Lans, H., J. A. Marteijn and W. Vermeulen (2012). "ATP-dependent chromatin remodeling in the DNA-damage response." Epigenetics Chromatin **5**: 4.

Lao-On, U., P. V. Attwood and S. Jitrapakdee (2018). "Roles of pyruvate carboxylase in human diseases: from diabetes to cancers and infection." J Mol Med (Berl) **96**(3-4): 237-247.

Lau, M. T., C. Klausen and P. C. Leung (2011). "E-cadherin inhibits tumor cell growth by suppressing PI3K/Akt signaling via beta-catenin-Egr1-mediated PTEN expression." Oncogene **30**(24): 2753-2766.

Laugesen, A. and K. Helin (2014). "Chromatin repressive complexes in stem cells, development, and cancer." Cell Stem Cell **14**(6): 735-751.

Laury, A. R., R. Perets, H. Piao, J. F. Krane, J. A. Barletta, C. French, L. R. Chirieac, R. Lis, M. Loda, J. L. Hornick, R. Drapkin and M. S. Hirsch (2011). "A comprehensive analysis of PAX8 expression in human epithelial tumors." Am J Surg Pathol **35**(6): 816-826.

Lawrence, M. S., P. Stojanov, C. H. Mermel, J. T. Robinson, L. A. Garraway, T. R. Golub, M. Meyerson, S. B. Gabriel, E. S. Lander and G. Getz (2014). "Discovery and saturation analysis of cancer genes across 21 tumour types." Nature **505**(7484): 495-501.

Lax, S. F. and R. J. Kurman (1997). "A dualistic model for endometrial carcinogenesis based on immunohistochemical and molecular genetic analyses." Verh Dtsch Ges Pathol **81**: 228-232.

Le, D. T., J. N. Uram, H. Wang, B. R. Bartlett, H. Kemberling, A. D. Eyring, A. D. Skora, B. S. Luber, N. S. Azad, D. Laheru, B. Biedrzycki, R. C. Donehower, A. Zaheer, G. A. Fisher, T. S. Crocenzi, J. J. Lee, S. M. Duffy, R. M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R. H. Hruban, L. D. Wood, N. Cuka, D. M. Pardoll, N. Papadopoulos, K. W. Kinzler, S. Zhou, T. C. Cornish, J. M. Taube, R. A. Anders, J. R. Eshleman, B. Vogelstein and L. A. Diaz, Jr. (2015). "PD-1 Blockade in Tumors with Mismatch-Repair Deficiency." N Engl J Med **372**(26): 2509-2520.

Le Gallo, M., A. J. O'Hara, M. L. Rudd, M. E. Urick, N. F. Hansen, N. J. O'Neil, J. C. Price, S. Zhang, B. M. England, A. K. Godwin, D. C. Sgroi, N. I. H. I. S. C. C. S. Program, P.

Hieter, J. C. Mullikin, M. J. Merino and D. W. Bell (2012). "Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes." Nat Genet **44**(12): 1310-1315.

Leadem, B. R., I. Kagiampakis, C. Wilson, T. K. Cheung, D. Arnott, P. Trojer, M. Classon, H. Easwaran and S. B. Baylin (2018). "A KDM5 Inhibitor Increases Global H3K4 Trimethylation Occupancy and Enhances the Biological Efficacy of 5-Aza-2'-Deoxycytidine." Cancer Res **78**(5): 1127-1139.

Lee, J., I. Sohn, I. G. Do, K. M. Kim, S. H. Park, J. O. Park, Y. S. Park, H. Y. Lim, T. S. Sohn, J. M. Bae, M. G. Choi, D. H. Lim, B. H. Min, J. H. Lee, P. L. Rhee, J. J. Kim, D. I. Choi, I. B. Tan, K. Das, P. Tan, S. H. Jung, W. K. Kang and S. Kim (2014). "Nanostring-based multigene assay to predict recurrence for gastric cancer patients after surgery." PLoS One **9**(3): e90133.

Lee, S. and C. A. Schmitt (2019). "The dynamic nature of senescence in cancer." Nat Cell Biol **21**(1): 94-101.

Lee, S. H., S. Y. Oh, S. I. Do, H. J. Lee, H. J. Kang, Y. S. Rho, W. J. Bae and Y. C. Lim (2014). "SOX2 regulates self-renewal and tumorigenicity of stem-like cells of head and neck squamous cell carcinoma." Br J Cancer **111**(11): 2122-2130.

Lee, T. I., R. G. Jenner, L. A. Boyer, M. G. Guenther, S. S. Levine, R. M. Kumar, B. Chevalier, S. E. Johnstone, M. F. Cole, K. Isono, H. Koseki, T. Fuchikami, K. Abe, H. L. Murray, J. P. Zucker, B. Yuan, G. W. Bell, E. Herbolsheimer, N. M. Hannett, K. Sun, D. T. Odom, A. P. Otte, T. L. Volkert, D. P. Bartel, D. A. Melton, D. K. Gifford, R. Jaenisch and R. A. Young (2006). "Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells." Cell **125**(2): 301-313.

Lei, X., L. Wang, J. Yang and L. Z. Sun (2009). "TGFbeta signaling supports survival and metastasis of endometrial cancer cells." Cancer Manag Res **2009**(1): 15-24.

Leibovici, J. (1984). "Serial passage of tumors in mice in the study of tumor progression and testing of antineoplastic drugs." Cancer Res **44**(5): 1981-1984.

Levine, R. L., C. B. Cargile, M. S. Blazes, B. van Rees, R. J. Kurman and L. H. Ellenson (1998). "PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma." Cancer Res **58**(15): 3254-3258.

Lewis, P. W., M. M. Muller, M. S. Koletsky, F. Cordero, S. Lin, L. A. Banaszynski, B. A. Garcia, T. W. Muir, O. J. Becher and C. D. Allis (2013). "Inhibition of PRC2 Activity by a Gain-of-Function H3 Mutation Found in Pediatric Glioblastoma." Science **340**(6134): 857-861.

Li, B., M. Gogol, M. Carey, D. Lee, C. Seidel and J. L. Workman (2007). "Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin." Science **316**(5827): 1050-1054.

Li, E., T. H. Bestor and R. Jaenisch (1992). "Targeted mutation of the DNA methyltransferase gene results in embryonic lethality." Cell **69**(6): 915-926.

Li, H. and R. Durbin (2010). "Fast and accurate long-read alignment with Burrows-Wheeler transform." Bioinformatics **26**(5): 589-595.

Li, H., Y. Yang, W. Hong, M. Huang, M. Wu and X. Zhao (2020). "Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects." Signal Transduct Target Ther **5**(1): 1.

Li, X., L. Pang, Z. Yang, J. Liu, W. Li and D. Wang (2019). "LncRNA HOTAIRM1/HOXA1 Axis Promotes Cell Proliferation, Migration And Invasion In Endometrial Cancer." Onco Targets Ther **12**: 10997-11015.

Li, X., Y. Xu, Y. Chen, S. Chen, X. Jia, T. Sun, Y. Liu, X. Li, R. Xiang and N. Li (2013). "SOX2 promotes tumor metastasis by stimulating epithelial-to-mesenchymal transition via regulation of WNT/beta-catenin signal network." Cancer Lett **336**(2): 379-389.

Li, Y., D. An, Y. X. Guan and S. Kang (2017). "Aberrant Methylation of the E-Cadherin Gene Promoter Region in Endometrium and Ovarian Endometriotic Cysts of Patients with Ovarian Endometriosis." Gynecol Obstet Invest **82**(1): 78-85.

Li, Y., Q. Che, Y. Bian, Q. Zhou, F. Jiang, H. Tong, J. Ke, K. Wang and X. P. Wan (2015). "Autocrine motility factor promotes epithelial-mesenchymal transition in endometrial cancer via MAPK signaling pathway." Int J Oncol **47**(3): 1017-1024.

Li, Y., J. Huo, X. Pan, C. Wang and X. Ma (2018). "MicroRNA 302b-3p/302c-3p/302d-3p inhibits epithelial-mesenchymal transition and promotes apoptosis in human endometrial carcinoma cells." Onco Targets Ther **11**: 1275-1284.

Li, Y., D. Sun, J. Gao, Z. Shi, P. Chi, Y. Meng, C. Zou and Y. Wang (2018). "MicroRNA-373 promotes the development of endometrial cancer by targeting LATS2 and activating the Wnt/beta-Catenin pathway." J Cell Biochem.

Li, Z. and C. Zhao (2016). "Clinicopathologic and Immunohistochemical Characterization of Dedifferentiated Endometrioid Adenocarcinoma." Appl Immunohistochem Mol Morphol **24**(8): 562-568.

Liau, B. B., C. Sievers, L. K. Donohue, S. M. Gillespie, W. A. Flavahan, T. E. Miller, A. S. Venteicher, C. H. Hebert, C. D. Carey, S. J. Rodig, S. J. Shareef, F. J. Najm, P. van Galen, H. Wakimoto, D. P. Cahill, J. N. Rich, J. C. Aster, M. L. Suva, A. P. Patel and B. E. Bernstein (2017). "Adaptive Chromatin Remodeling Drives Glioblastoma Stem Cell Plasticity and Drug Tolerance." Cell Stem Cell **20**(2): 233-246 e237.

Lim, D., P. P. Ip, A. N. Cheung, T. Kiyokawa and E. Oliva (2015). "Immunohistochemical Comparison of Ovarian and Uterine Endometrioid Carcinoma, Endometrioid Carcinoma With Clear Cell Change, and Clear Cell Carcinoma." Am J Surg Pathol **39**(8): 1061-1069.

Lin, X., Qui, J., Hua, K. (2018). "Long non-coding RNAs as emerging regulators of epithelial to mesenchymal transition in gynecologic cancers." BioScience Trends **12**(4): 342-353.

Lissanu Deribe, Y., Y. Sun, C. Terranova, F. Khan, J. Martinez-Ledesma, J. Gay, G. Gao, R. A. Mullinax, T. Khor, N. Feng, Y. H. Lin, C. C. Wu, C. Reyes, Q. Peng, F. Robinson, A. Inoue, V. Kochat, C. G. Liu, J. M. Asara, C. Moran, F. Muller, J. Wang, B. Fang, V. Papadimitrakopoulou, Wistuba, II, K. Rai, J. Marszalek and P. A. Futreal (2018). "Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer." Nat Med **24**(7): 1047-1057.

Liu, F. Y., Y. L. Deng, Y. Li, D. Zeng, Z. Z. Zhou, D. A. Tian and M. Liu (2013). "Down-regulated KLF17 expression is associated with tumor invasion and poor prognosis in hepatocellular carcinoma." Med Oncol **30**(1): 425.

Liu, L., Zhang, J., Yang, X., Fang, C., Xu, H., Xi, X. (2015). "SALL4 as an Epithelial-Mesenchymal Transition and Drug Resistance Inducer Through the Regulation of c-Myc in Endometrial Cancer." PLoS One **10**(9).

Liu, P., C. Wang, C. Ma, Q. Wu, W. Zhang and G. Lao (2016). "MicroRNA-23a regulates epithelial-to-mesenchymal transition in endometrial endometrioid adenocarcinoma by targeting SMAD3." Cancer Cell Int **16**(1): 67.

Liu, Q., K. Chen, Z. Liu, Y. Huang, R. Zhao, L. Wei, X. Yu, J. He, J. Liu, J. Qi, Y. Qin and B. Li (2017). "BORIS up-regulates OCT4 via histone methylation to promote cancer stem cell-like properties in human liver cancer cells." Cancer Lett **403**: 165-174.

Liu, X., Y. Yu, J. Zhang, C. Lu, L. Wang, P. Liu and H. Song (2018). "HDAC1 Silencing in Ovarian Cancer Enhances the Chemotherapy Response." Cell Physiol Biochem **48**(4): 1505-1518.

Liu, Y., J. Wang, T. Ni, L. Wang, Y. Wang and X. Sun (2016). "CCL20 mediates RANK/RANKL-induced epithelial-mesenchymal transition in endometrial cancer cells." Oncotarget **7**(18): 25328-25339.

Liu, Y., Zhao, R., Chi, S., Zhang, W., Xiao, C., Zhou, X., Zhao, Y., Wang, H. (2020). "UBE2C Is Upregulated by Estrogen and Promotes Epithelial–Mesenchymal Transition via p53 in Endometrial Cancer." Mol Cancer Res **18**(2).

Loh, C. Y., J. Y. Chai, T. F. Tang, W. F. Wong, G. Sethi, M. K. Shanmugam, P. P. Chong and C. Y. Looi (2019). "The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges." Cells **8**(10).

Lord, C. J. and A. Ashworth (2017). "PARP inhibitors: Synthetic lethality in the clinic." Science **355**(6330): 1152-1158.

Lorzadeh, A., M. Bilenky, C. Hammond, D. Knapp, L. Li, P. H. Miller, A. Carles, A. Heravi-Moussavi, S. Gakkhar, M. Moksa, C. J. Eaves and M. Hirst (2016). "Nucleosome Density ChIP-Seq Identifies Distinct Chromatin Modification Signatures Associated with MNase Accessibility." Cell Rep **17**(8): 2112-2124.

Lorzadeh, A., R. Lopez Gutierrez, L. Jackson, M. Moksa and M. Hirst (2017). "Generation of Native Chromatin Immunoprecipitation Sequencing Libraries for Nucleosome Density Analysis." J Vis Exp(130).

Louka, M., E. Boutou, V. Bakou, V. Pappa, A. Georgoulis, H. W. Sturzbecher, C. E. Vorgias and D. Vlachodimitropoulos (2015). "DNA Damage Response/Repair in Cancer Stem Cells - Potential vs. Controversies." Advances in DNA Repair: 415-444.

Lu, R., P. Wang, T. Parton, Y. Zhou, K. Chrysovergis, S. Rockowitz, W. Y. Chen, O. Abdel-Wahab, P. A. Wade, D. Zheng and G. G. Wang (2016). "Epigenetic Perturbations by Arg882-Mutated DNMT3A Potentiate Aberrant Stem Cell Gene-Expression Program and Acute Leukemia Development." Cancer Cell **30**(1): 92-107.

Lu, Y. H., Y. F. Liu, S. Oeck and P. M. Glazer (2018). "Hypoxia Promotes Resistance to EGFR Inhibition in NSCLC Cells via the Histone Demethylases, LSD1 and PLU-1." Molecular Cancer Research **16**(10): 1458-1469.

Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). "Crystal structure of the nucleosome core particle at 2.8 A resolution." Nature **389**(6648): 251-260.

Lyon, M. F. (1961). "Gene action in the X-chromosome of the mouse (Mus musculus L.)." Nature **190**: 372-373.

Maemondo, M., A. Inoue, K. Kobayashi, S. Sugawara, S. Oizumi, H. Isobe, A. Gemma, M. Harada, H. Yoshizawa, I. Kinoshita, Y. Fujita, S. Okinaga, H. Hirano, K. Yoshimori, T. Harada, T. Ogura, M. Ando, H. Miyazawa, T. Tanaka, Y. Saijo, K. Hagiwara, S. Morita, T. Nukiwa and G. North-East Japan Study (2010). "Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR." N Engl J Med **362**(25): 2380-2388.

Magee, J. A., E. Piskounova and S. J. Morrison (2012). "Cancer stem cells: impact, heterogeneity, and uncertainty." Cancer Cell **21**(3): 283-296.

Mahmood, M. Q., C. Ward, H. K. Muller, S. S. Sohal and E. H. Walters (2017). "Epithelial mesenchymal transition (EMT) and non-small cell lung cancer (NSCLC): a mutual association with airway disease." Med Oncol **34**(3): 45.

Mali, P., L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville and G. M. Church (2013). "RNA-guided human genome engineering via Cas9." Science **339**(6121): 823-826.

Malpica, A. (2016). "How to approach the many faces of endometrioid carcinoma." Modern Pathology **29**: doi.org/10.1038/modpathol.2015.1142.

Mancini, D. N., S. M. Singh, T. K. Archer and D. I. Rodenhiser (1999). "Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors." Oncogene **18**(28): 4108-4119.

Mandal, R., R. M. Samstein, K. W. Lee, J. J. Havel, H. Wang, C. Krishna, E. Y. Sabio, V. Makarov, F. Kuo, P. Blecua, A. T. Ramaswamy, J. N. Durham, B. Bartlett, X. Ma, R. Srivastava, S. Middha, A. Zehir, J. F. Hechtman, L. G. Morris, N. Weinhold, N. Riaz, D. T. Le, L. A. Diaz, Jr. and T. A. Chan (2019). "Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response." Science **364**(6439): 485-491.

Mani, S. A., W. Guo, M. J. Liao, E. N. Eaton, A. Ayyanan, A. Y. Zhou, M. Brooks, F. Reinhard, C. C. Zhang, M. Shipitsin, L. L. Campbell, K. Polyak, C. Brisken, J. Yang and R. A. Weinberg (2008). "The epithelial-mesenchymal transition generates cells with properties of stem cells." Cell **133**(4): 704-715.

Mantel, C., S. Messina-Graham, A. Moh, S. Cooper, G. Hangoc, X. Y. Fu and H. E. Broxmeyer (2012). "Mouse hematopoietic cell-targeted STAT3 deletion: stem/progenitor cell defects, mitochondrial dysfunction, ROS overproduction, and a rapid aging-like phenotype." Blood **120**(13): 2589-2599.

Manzotti, G., A. Ciarrocchi and V. Sancisi (2019). "Inhibition of BET Proteins and Histone Deacetylase (HDACs): Crossing Roads in Cancer Therapy." Cancers (Basel) **11**(3).

Mao, T. L. and M. Shih Ie (2013). "The roles of ARID1A in gynecologic cancer." J Gynecol Oncol **24**(4): 376-381.

Marjanovic, N. D., R. A. Weinberg and C. L. Chaffer (2013). "Cell plasticity and heterogeneity in cancer." Clin Chem **59**(1): 168-179.

Marjon, K., M. J. Cameron, P. Quang, M. F. Clasquin, E. Mandley, K. Kunii, M. McVay, S. Choe, A. Kernytsky, S. Gross, Z. Konteatis, J. Murtie, M. L. Blake, J. Travins, M. Dorsch, S. A. Biller and K. M. Marks (2016). "MTAP Deletions in Cancer Create Vulnerability to Targeting of the MAT2A/PRMT5/RIOK1 Axis." Cell Rep **15**(3): 574-587.

Markowska, A., M. Pawalowska, J. Lubin and J. Markowska (2014). "Signalling pathways in endometrial cancer." Contemp Oncol (Pozn) **18**(3): 143-148.

Martin, C. and Y. Zhang (2005). "The diverse functions of histone lysine methylation." Nat Rev Mol Cell Biol **6**(11): 838-849.

Martinez-Zamudio, R. I., P. F. Roux, J. de Freitas, L. Robinson, G. Dore, B. Sun, D. Belenki, M. Milanovic, U. Herbig, C. A. Schmitt, J. Gil and O. Bischof (2020). "AP-1 imprints a reversible transcriptional programme of senescent cells." Nat Cell Biol **22**(7): 842-855.

Marusyk, A., V. Almendro and K. Polyak (2012). "Intra-tumour heterogeneity: a looking glass for cancer?" Nat Rev Cancer **12**(5): 323-334.

Mashtalir, N., D'Avino, AR., Michel, BC., Luo, J., Pan, J., Otto, JE., Zullow, HJ., McKenzie, ZM., Kubiak, RL., St Pierre, R., Valencia, AM., Poynter, SJ., Cassel, SH., Ranish, JA., Kadoch, C. (2018). "Modular organization and assembly of SWI/SNF family chromatin remodeling complexes." Cell **175**(5): 1272-1288.

Masliah-Planchon, J., Bieche, J., Guinebretiere, J-M., Bourdeaut, F., Delattre, O. (2015). "SWI/SNF Chromatin Remodeling and Human Malignancies." Annu Rev Pathol **10**(145-71).

Mathews, L. A., S. M. Cabarcas, E. M. Hurt, X. Zhang, E. M. Jaffee and W. L. Farrar (2011). "Increased expression of DNA repair genes in invasive human pancreatic cancer cells." Pancreas **40**(5): 730-739.

Mathur, R., B. H. Alver, A. K. San Roman, B. G. Wilson, X. Wang, A. T. Agoston, P. J. Park, R. A. Shivdasani and C. W. Roberts (2017). "ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice." Nat Genet **49**(2): 296-302.

Matsubara, D., Kishaba, Y., Ishikawa, S., Sakatani, T., Oguni, S., Tamura, T., Hoshino, H., Sugiyama, Y., Endo, S., Murakami, Y., Aburatani, H., Fukayama, M., Niki, T. (2013). "Lung Cancer With Loss of BRG1/BRM, Shows Epithelial Mesenchymal Transition Phenotype and Distinct Histologic and Genetic Features." Cancer Sci **104**(2): 266-273.

Mavrakis, K. J., E. R. McDonald, 3rd, M. R. Schlabach, E. Billy, G. R. Hoffman, A. deWeck, D. A. Ruddy, K. Venkatesan, J. Yu, G. McAllister, M. Stump, R. deBeaumont, S. Ho, Y. Yue, Y. Liu, Y. Yan-Neale, G. Yang, F. Lin, H. Yin, H. Gao, D. R. Kipp, S. Zhao, J. T. McNamara, E. R. Sprague, B. Zheng, Y. Lin, Y. S. Cho, J. Gu, K. Crawford, D. Ciccone, A. C. Vitari, A. Lai, V. Capka, K. Hurov, J. A. Porter, J. Tallarico, C. Mickanin, E. Lees, R. Pagliarini, N. Keen, T. Schmelzle, F. Hofmann, F. Stegmeier and W. R. Sellers (2016). "Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5." Science **351**(6278): 1208-1213.

McConechy, M., Ding, JR., Cheang, MC., Wiegand, K., Senz, J., Tone, A., Yang, W., Prentice, L., Tse, K., Zeng, T., McDonald, H., Schmidt, AP., Mutch, DG., McAlpine, JN., Hirst, M., Shah, SP., Lee, CH., Goodfellow, PJ., Gilks, CB. Huntsman, D.G. (2012). "Use of mutation profiles to refine the classification of endometrial carcinomas." J Pathol **228**(1): 20-30.

Meacham, C. E. and S. J. Morrison (2013). "Tumour heterogeneity and cancer cell plasticity." Nature **501**(7467): 328-337.

Meng, E., A. Mitra, K. Tripathi, M. A. Finan, J. Scalici, S. McClellan, L. Madeira da Silva, E. Reed, L. A. Shevde, K. Palle and R. P. Rocconi (2014). "ALDH1A1 maintains ovarian cancer stem cell-like properties by altered regulation of cell cycle checkpoint and DNA repair network signaling." PLoS One **9**(9): e107142.

Merritt, M. A. and D. W. Cramer (2010). "Molecular pathogenesis of endometrial and ovarian cancer." Cancer Biomark **9**(1-6): 287-305.

Mhawech-Fauceglia, P., Yan, L., Liu, S., Pejovic, T. (2013). "ER+/PR+/TFF3+/IMP3-immunoprofile distinguishes endometrioid from serous and clear cell carcinomas of the endometrium: a study of 401 cases." Histopathology **62**(7): 976-985.

Michel, B., D'Avino, AR., Cassel, SH., Mashtalir, N., McKenzie, ZM., McBride, MJ., Valencia, AM., Zhou, Q., Bocker, M., Soares, LMM., Pan, J., Remillard, DI., Lareau, CA., Zullow, HJ., Fortoul, N., Gray, NS., Bradner, JE., Chan, HM., Kadoch, C. (2018). "A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation." Nature Cell Biology **20**: 1410-1420.

Michor, F. and K. Polyak (2010). "The origins and implications of intratumor heterogeneity." Cancer Prev Res (Phila) **3**(11): 1361-1364.

Mikkelsen, T. S., M. Ku, D. B. Jaffe, B. Issac, E. Lieberman, G. Giannoukos, P. Alvarez, W. Brockman, T. K. Kim, R. P. Koche, W. Lee, E. Mendenhall, A. O'Donovan, A. Presser, C. Russ, X. Xie, A. Meissner, M. Wernig, R. Jaenisch, C. Nusbaum, E. S. Lander and B. E. Bernstein (2007). "Genome-wide maps of chromatin state in pluripotent and lineage-committed cells." Nature **448**(7153): 553-560.

Milanovic, M., D. N. Y. Fan, D. Belenki, J. H. M. Dabritz, Z. Zhao, Y. Yu, J. R. Dorr, L. Dimitrova, D. Lenze, I. A. Monteiro Barbosa, M. A. Mendoza-Parra, T. Kanashova, M. Metzner, K. Pardon, M. Reimann, A. Trumpp, B. Dorken, J. Zuber, H. Gronemeyer, M. Hummel, G. Dittmar, S. Lee and C. A. Schmitt (2018). "Senescence-associated reprogramming promotes cancer stemness." Nature **553**(7686): 96-100.

Miller, W. R. (1980). "Effects of serial passage on the endocrine response and steroid metabolism of a rat mammary carcinoma." Br J Cancer **42**(2): 326-330.

Mirantes, C., I. Espinosa, I. Ferrer, X. Dolcet, J. Prat and X. Matias-Guiu (2013). "Epithelial-to-mesenchymal transition and stem cells in endometrial cancer." Hum Pathol **44**(10): 1973-1981.

Mitsudomi, T., S. Morita, Y. Yatabe, S. Negoro, I. Okamoto, J. Tsurutani, T. Seto, M. Satouchi, H. Tada, T. Hirashima, K. Asami, N. Katakami, M. Takada, H. Yoshioka, K. Shibata, S. Kudoh, E. Shimizu, H. Saito, S. Toyooka, K. Nakagawa, M. Fukuoka and G. West Japan Oncology (2010). "Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial." Lancet Oncol **11**(2): 121-128.

Modena, P., Lualdi, E., Facchinetti, F., Galli, L., Teixeira, MR., Pilotti, S., Sozzi, G. "SMARCB1/INI1 Tumor Suppressor Gene Is Frequently Inactivated in Epithelioid Sarcomas." Cancer Res **65**(10): 4012-4019.

Mohn, F., M. Weber, M. Rebhan, T. C. Roloff, J. Richter, M. B. Stadler, M. Bibel and D. Schubeler (2008). "Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors." Mol Cell **30**(6): 755-766.

Moiseeva, O., V. Bourdeau, A. Roux, X. Deschenes-Simard and G. Ferbeyre (2009). "Mitochondrial dysfunction contributes to oncogene-induced senescence." Mol Cell Biol **29**(16): 4495-4507.

Mok, T. S., Y. L. Wu, S. Thongprasert, C. H. Yang, D. T. Chu, N. Saijo, P. Sunpaweravong, B. Han, B. Margono, Y. Ichinose, Y. Nishiwaki, Y. Ohe, J. J. Yang, B. Chewaskulyong, H. Jiang, E. L. Duffield, C. L. Watkins, A. A. Armour and M. Fukuoka (2009). "Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma." N Engl J Med **361**(10): 947-957.

Montserrat, N., A. Mozos, D. Llobet, X. Dolcet, C. Pons, A. G. de Herreros, X. Matias-Guiu and J. Prat (2012). "Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma." Hum Pathol **43**(5): 632-643.

Morioka, S., Y. Tanase, R. Kawaguchi, T. Uchiyama and H. Kobayash (2018). "Two Cases of Dedifferentiated Endometrioid Carcinoma: Case Presentation and Brief Review of the Literature." Case Rep Obstet Gynecol **2018**: 7624785.

Morton, J. P., P. Timpson, S. A. Karim, R. A. Ridgway, D. Athineos, B. Doyle, N. B. Jamieson, K. A. Oien, A. M. Lowy, V. G. Brunton, M. C. Frame, T. R. Evans and O. J. Sansom (2010). "Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer." Proc Natl Acad Sci U S A **107**(1): 246-251.

Muinelo-Romay, L., E. Colas, J. Barbazan, L. Alonso-Alconada, M. Alonso-Nocelo, M. Bouso, T. Curiel, J. Cueva, U. Anido, J. Forteza, A. Gil-Moreno, J. Reventos, R. Lopez-Lopez and M. Abal (2011). "High-risk endometrial carcinoma profiling identifies TGF-beta1 as a key factor in the initiation of tumor invasion." Mol Cancer Ther **10**(8): 1357-1366.

Murali, R., B. Davidson, O. Fadare, J. A. Carlson, C. P. Crum, C. B. Gilks, J. A. Irving, A. Malpica, X. Matias-Guiu, W. G. McCluggage, K. Mittal, E. Oliva, V. Parkash, J. K. L. Rutgers, P. N. Staats, C. J. R. Stewart, C. Tornos and R. A. Soslow (2019). "High-grade Endometrial Carcinomas: Morphologic and Immunohistochemical Features, Diagnostic Challenges and Recommendations." Int J Gynecol Pathol **38 Suppl 1**: S40-S63.

Muz, B., P. de la Puente, F. Azab, M. Luderer and A. K. Azab (2014). "Hypoxia promotes stem cell-like phenotype in multiple myeloma cells." Blood Cancer J **4**: e262.

Myant, K., A. Termanis, A. Y. Sundaram, T. Boe, C. Li, C. Merusi, J. Burrage, J. I. de Las Heras and I. Stancheva (2011). "LSH and G9a/GLP complex are required for developmentally programmed DNA methylation." Genome Res **21**(1): 83-94.

Naidu, S., Love, IM., Imbalzano, AN., Grossman, SR., Androphy, EJ. (2009). "The SWI/SNF chromatin remodeling subunit BRG1 is a critical regulator of p53 necessary for proliferation of malignant cells." Oncogene **28**: 2492-2501.

Nakayama, R. T. P., J.L.; Valencia A.M et al., (2017). "SMARCB1 is required for widespread BAF complex–mediated activation of enhancers and bivalent promoters." Nature Genetics **49**(11): 1613-1623.

Narita, M., M. Narita, V. Krizhanovsky, S. Nunez, A. Chicas, S. A. Hearn, M. P. Myers and S. W. Lowe (2006). "A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation." Cell **126**(3): 503-514.

Narita, M., S. Nunez, E. Heard, M. Narita, A. W. Lin, S. A. Hearn, D. L. Spector, G. J. Hannon and S. W. Lowe (2003). "Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence." Cell **113**(6): 703-716.

Nass, S. J., J. G. Herman, E. Gabrielson, P. W. Iversen, F. F. Parl, N. E. Davidson and J. R. Graff (2000). "Aberrant methylation of the estrogen receptor and E-cadherin 5' CpG islands increases with malignant progression in human breast cancer." Cancer Res **60**(16): 4346-4348.

Navolanic, P. M., L. S. Steelman and J. A. McCubrey (2003). "EGFR family signaling and its association with breast cancer development and resistance to chemotherapy (Review)." Int J Oncol **22**(2): 237-252.

Nelson, B., McAlpine, JN. (2015). "The more tumors change, the more theystay tame: do T cells keep POLE ultramutated endometrialcarcinomas in check?" Gynecol Oncol **138**: 1-2.

Nguyen, L. V., M. Makarem, A. Carles, M. Moksa, N. Kannan, P. Pandoh, P. Eirew, T. Osako, M. Kardel, A. M. Cheung, W. Kennedy, K. Tse, T. Zeng, Y. Zhao, R. K. Humphries, S. Aparicio, C. J. Eaves and M. Hirst (2014). "Clonal analysis via barcoding reveals diverse growth and differentiation of transplanted mouse and human mammary stem cells." Cell Stem Cell **14**(2): 253-263.

Nguyen, L. V., D. Pellacani, S. Lefort, N. Kannan, T. Osako, M. Makarem, C. L. Cox, W. Kennedy, P. Beer, A. Carles, M. Moksa, M. Bilenky, S. Balani, S. Babovic, I. Sun, M. Rosin, S. Aparicio, M. Hirst and C. J. Eaves (2015). "Barcoding reveals complex clonal dynamics of de novo transformed human mammary cells." Nature **528**(7581): 267-271.

Nick McElhinny, S., Gordenin, DA., Stith, CM., Burgers, PMJ., Kunkel, TA. (2008). "Division of labor at the eukaryotic replication fork." Mol Cell **30**(2): 137-144.

Nie, H., X. Zhou, D. Shuzhang, C. Nie, X. Zhang and J. Huang (2019). "Palbociclib overcomes afatinib resistance in non-small cell lung cancer." Biomed Pharmacother **109**: 1750-1757.

Nikolic, A., Singhal, D., Ellestad, K., Johnston, M., Gillmor, A., Morrisy, S., Chan, JA., Neri, P., Bahlis, N., Gallo, M. (2021). "Copy-scAT: Deconvoluting single-cell chromatin accessibility of genetic subclones in cancer." bioRxiv.

Noguchi, H., C. Miyagi-Shiohira and Y. Nakashima (2018). "Induced Tissue-Specific Stem Cells and Epigenetic Memory in Induced Pluripotent Stem Cells." Int J Mol Sci **19**(4).

Novak, D., L. Huser, J. J. Elton, V. Umansky, P. Altevogt and J. Utikal (2019). "SOX2 in development and cancer biology." Semin Cancer Biol.

Nowell, P. C. (1976). "The clonal evolution of tumor cell populations." Science **194**(4260): 23-28.

O'Hagan, H. M. (2014). "Chromatin modifications during repair of environmental exposure-induced DNA damage: a potential mechanism for stable epigenetic alterations." Environ Mol Mutagen **55**(3): 278-291.

O'Leary, B., R. S. Finn and N. C. Turner (2016). "Treating cancer with selective CDK4/6 inhibitors." Nat Rev Clin Oncol **13**(7): 417-430.

O'Driscoll, M., Jeggo, PA. (2006). "The role of double-strand break repair—insights from human genetics." Nat Rev Genet **7**(1): 45-54.

Obel, J. C., G. Friberg and G. F. Fleming (2006). "Chemotherapy in endometrial cancer." Clin Adv Hematol Oncol **4**(6): 459-468.

Ohno, S., W. D. Kaplan and R. Kinosita (1959). "Formation of the sex chromatin by a single X-chromosome in liver cells of Rattus norvegicus." Exp Cell Res **18**: 415-418.

Oike, T., H. Ogiwara, Y. Tominaga, K. Ito, O. Ando, K. Tsuta, T. Mizukami, Y. Shimada, H. Isomura, M. Komachi, K. Furuta, S. Watanabe, T. Nakano, J. Yokota and T. Kohno (2013). "A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1." Cancer Res **73**(17): 5508-5518.

Okano, M., D. W. Bell, D. A. Haber and E. Li (1999). "DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development." Cell **99**(3): 247-257.

Olive, P. L., C. Aquino-Parsons, S. H. MacPhail, S. Y. Liao, J. A. Raleigh, M. I. Lerman and E. J. Stanbridge (2001). "Carbonic anhydrase 9 as an endogenous marker for hypoxic cells in cervical cancer." Cancer Res **61**(24): 8924-8929.

Onder, S., O. C. Taskin, F. Sen, S. Topuz, S. Kucucuk, H. Sozen, R. Ilhan, S. Tuzlali and E. Yavuz (2017). "High expression of SALL4 and fascin, and loss of E-cadherin expression in undifferentiated/dedifferentiated carcinomas of the endometrium: An immunohistochemical and clinicopathologic study." Medicine (Baltimore) **96**(10): e6248.

Onder, T. T., P. B. Gupta, S. A. Mani, J. Yang, E. S. Lander and R. A. Weinberg (2008). "Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways." Cancer Res **68**(10): 3645-3654.

Ono, R., Nakayama, K., Nakamura, K., Yamashita, H., Ishibashi, T., Ishikawa, M., Minamoto, T., Razia, S., Ishikawa, N., Otsuki, Y., Nakayama, S., Onuma, H., Kurioka, H., Kyo, S. (2019). "Dedifferentiated Endometrial Carcinoma Could be A Target for Immune Checkpoint Inhibitors
(Anti PD-1/PD-L1 Antibodies)." Int J Mol Sci **20**(3744).

Orlando, K., Douglas, AK, Abudu, A., Wang, Y., Tessier-Cloutier, B., Su, W., Peters, A., Sherman, LS., Moore, R. (2020). "Re-expression of SMARCA4/BRG1 in small cell carcinoma of ovary, hypercalcemic type (SCCOHT) promotes an epithelial-like gene signature through an AP-1-dependent mechanism." Elife.

Orvis, T., A. Hepperla, V. Walter, S. Song, J. Simon, J. Parker, M. D. Wilkerson, N. Desai, M. B. Major, D. N. Hayes, I. J. Davis and B. Weissman (2014). "BRG1/SMARCA4 inactivation promotes non-small cell lung cancer aggressiveness by altering chromatin organization." Cancer Res **74**(22): 6486-6498.

Ouchi, R., S. Okabe, T. Migita, I. Nakano and H. Seimiya (2016). "Senescence from glioma stem cell differentiation promotes tumor growth." Biochem Biophys Res Commun **470**(2): 275-281.

Padilla-Benavides, T., B. T. Nasipak and A. N. Imbalzano (2015). "Brg1 Controls the Expression of Pax7 to Promote Viability and Proliferation of Mouse Primary Myoblasts." J Cell Physiol **230**(12): 2990-2997.

Paluvai, H., E. Di Giorgio and C. Brancolini (2020). "The Histone Code of Senescence." Cells **9**(2).

Pan, X. Y., B. Wang, Y. C. Che, Z. P. Weng, H. Y. Dai and W. Peng (2007). "Expression of claudin-3 and claudin-4 in normal, hyperplastic, and malignant endometrial tissue." Int J Gynecol Cancer **17**(1): 233-241.

Papamichos-Chronakis, M. and C. L. Peterson (2013). "Chromatin and the genome integrity network." Nat Rev Genet **14**(1): 62-75.

Park, S.-A., Kim, LK., Kim, YT., Heo, T-H., Kim, HJ. (2020). "Long non-coding RNA steroid receptor activator promotes the progression of endometrial cancer via Wnt/ β-catenin signaling pathway." Int J Biol Sci **16**(1): 99-115.

Parsons, D. W., S. Jones, X. Zhang, J. C. Lin, R. J. Leary, P. Angenendt, P. Mankoo, H. Carter, I. M. Siu, G. L. Gallia, A. Olivi, R. McLendon, B. A. Rasheed, S. Keir, T. Nikolskaya, Y. Nikolsky, D. A. Busam, H. Tekleab, L. A. Diaz, Jr., J. Hartigan, D. R. Smith, R. L. Strausberg, S. K. Marie, S. M. Shinjo, H. Yan, G. J. Riggins, D. D. Bigner, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V. E. Velculescu and K. W. Kinzler (2008). "An integrated genomic analysis of human glioblastoma multiforme." Science **321**(5897): 1807-1812.

Patnaik, A., L. S. Rosen, S. M. Tolaney, A. W. Tolcher, J. W. Goldman, L. Gandhi, K. P. Papadopoulos, M. Beeram, D. W. Rasco, J. F. Hilton, A. Nasir, R. P. Beckmann, A. E.

Schade, A. D. Fulford, T. S. Nguyen, R. Martinez, P. Kulanthaivel, L. Q. Li, M. Frenzel, D. M. Cronier, E. M. Chan, K. T. Flaherty, P. Y. Wen and G. I. Shapiro (2016). "Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors." Cancer Discov **6**(7): 740-753.

Paul, J., Toosi, B., Vizeacoumar, FS., Bhanumathy, KK., Li, Y., Gerger, C., Zawily, AE., Freyweld, T., Anderson, DH., Mousseau, D., Kanthan, R., Zhang, Z., Vizeacoumar, FJ., Freywald, A. (2016). "Targeting synthetic lethality between the SRC kinase and the EPHB6 receptor may benefit cancer treatment." Oncotarget **7**(31): 50027-50042.

Pedrola, N., L. Devis, M. Llaurado, I. Campoy, E. Martinez-Garcia, M. Garcia, L. Muinelo-Romay, L. Alonso-Alconada, M. Abal, F. Alameda, G. Mancebo, R. Carreras, J. Castellvi, S. Cabrera, A. Gil-Moreno, X. Matias-Guiu, J. L. Iovanna, E. Colas, J. Reventos and A. Ruiz (2015). "Nidogen 1 and Nuclear Protein 1: novel targets of ETV5 transcription factor involved in endometrial cancer invasion." Clin Exp Metastasis **32**(5): 467-478.

Perez-Soler, R., A. Chachoua, L. A. Hammond, E. K. Rowinsky, M. Huberman, D. Karp, J. Rigas, G. M. Clark, P. Santabarbara and P. Bonomi (2004). "Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer." J Clin Oncol **22**(16): 3238-3247.

Peterson, C. L. and G. Almouzni (2013). "Nucleosome Dynamics as Modular Systems that Integrate DNA Damage and Repair." Cold Spring Harbor Perspectives in Biology **5**(9).

Phelan, M. L., S. Sif, G. J. Narlikar and R. E. Kingston (1999). "Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits." Mol Cell **3**(2): 247-253.

Pinner, S., P. Jordan, K. Sharrock, L. Bazley, L. Collinson, R. Marais, E. Bonvin, C. Goding and E. Sahai (2009). "Intravital imaging reveals transient changes in pigment production and Brn2 expression during metastatic melanoma dissemination." Cancer Res **69**(20): 7969-7977.

Pitt, J. M., A. Marabelle, A. Eggermont, J. C. Soria, G. Kroemer and L. Zitvogel (2016). "Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy." Ann Oncol **27**(8): 1482-1492.

Piunti, A. and A. Shilatifard (2016). "Epigenetic balance of gene expression by Polycomb and COMPASS families." Science **352**(6290): aad9780.

Planaguma, J., M. Abal, A. Gil-Moreno, M. Diaz-Fuertes, M. Monge, A. Garcia, T. Baro, J. Xercavins, J. Reventos and F. Alameda (2005). "Up-regulation of ERM/ETV5 correlates with the degree of myometrial infiltration in endometrioid endometrial carcinoma." J Pathol **207**(4): 422-429.

Poli, V. F., L.; Zippo A. (2018). "Tumorigenic Cell Reprogramming and Cancer Plasticity: Interplay between Signaling, Microenvironment, and Epigenetics." Stem Cells International.

Poltavets, V., M. Kochetkova, S. M. Pitson and M. S. Samuel (2018). "The Role of the Extracellular Matrix and Its Molecular and Cellular Regulators in Cancer Cell Plasticity." Front Oncol **8**: 431.

Ponnusamy, L. M., P.K.S.; Chang, Y-W.; Singh K.P. (2019). "Role of cellular reprogramming and epigenetic dysregulation in acquired chemoresistance in breast cancer." Cancer Drug Resistance **2**: 297-312.

Ponti, D., N. Zaffaroni, C. Capelli and M. G. Daidone (2006). "Breast cancer stem cells: an overview." Eur J Cancer **42**(9): 1219-1224.

Prat, A., J. S. Parker, O. Karginova, C. Fan, C. Livasy, J. I. Herschkowitz, X. He and C. M. Perou (2010). "Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer." Breast Cancer Res **12**(5): R68.

Price, B. D. and A. D. D'Andrea (2013). "Chromatin Remodeling at DNA Double-Strand Breaks." Cell **152**(6): 1344-1354.

Ragunathan, K., G. Jih and D. Moazed (2015). "Epigenetics. Epigenetic inheritance uncoupled from sequence-specific recruitment." Science **348**(6230): 1258699.

Ramalingam, P., R. P. Masand, E. D. Euscher and A. Malpica (2016). "Undifferentiated Carcinoma of the Endometrium: An Expanded Immunohistochemical Analysis Including PAX-8 and Basal-Like Carcinoma Surrogate Markers." Int J Gynecol Pathol **35**(5): 410-418.

Ramos, P., A. N. Karnezis, D. W. Craig, A. Sekulic, M. L. Russell, W. P. Hendricks, J. J. Corneveaux, M. T. Barrett, K. Shumansky, Y. Yang, S. P. Shah, L. M. Prentice, M. A. Marra, J. Kiefer, V. L. Zismann, T. A. McEachron, B. Salhia, J. Prat, E. D'Angelo, B. A. Clarke, J. G. Pressey, J. H. Farley, S. P. Anthony, R. B. Roden, H. E. Cunliffe, D. G. Huntsman and J. M. Trent (2014). "Small cell carcinoma of the ovary, hypercalcemic type, displays frequent inactivating germline and somatic mutations in SMARCA4." Nat Genet **46**(5): 427-429.

Rao, A., Barkley, D., Franca, GS., Yanai, I. (2021). "Exploring tissue architecture using spatial transcriptomics." Nature **596**: 211-220.

Rao, Q., Q. Y. Xia, Q. Shen, S. S. Shi, P. Tu, Q. L. Shi and X. J. Zhou (2014). "Coexistent loss of INI1 and BRG1 expression in a rhabdoid renal cell carcinoma (RCC): implications for a possible role of SWI/SNF complex in the pathogenesis of RCC." Int J Clin Exp Pathol **7**(4): 1782-1787.

Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun, M. Schmid, S. Opravil, K. Mechtler, C. P. Ponting, C. D. Allis and T. Jenuwein (2000). "Regulation of chromatin structure by site-specific histone H3 methyltransferases." Nature **406**(6796): 593-599.

Reck, M., D. Rodriguez-Abreu, A. G. Robinson, R. Hui, T. Csoszi, A. Fulop, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M. A. Leiby, G. M. Lubiniecki, Y. Shentu, R. Rangwala, J. R. Brahmer and K.-. Investigators (2016). "Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer." N Engl J Med **375**(19): 1823-1833.

Reisman, D. N., J. Sciarrotta, W. Wang, W. K. Funkhouser and B. E. Weissman (2003). "Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis." Cancer Res **63**(3): 560-566.

Rhinn, M., B. Ritschka and W. M. Keyes (2019). "Cellular senescence in development, regeneration and disease." Development **146**(20).

Rich, J. N. (2016). "Cancer stem cells: understanding tumor hierarchy and heterogeneity." Medicine (Baltimore) **95**(1 Suppl 1): S2-7.

Richer, J. K., B. M. Jacobsen, N. G. Manning, M. G. Abel, D. M. Wolf and K. B. Horwitz (2002). "Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells." J Biol Chem **277**(7): 5209-5218.

Riggs, A. D. (1975). "X inactivation, differentiation, and DNA methylation." Cytogenet Cell Genet **14**(1): 9-25.

Risom, T., E. M. Langer, M. P. Chapman, J. Rantala, A. J. Fields, C. Boniface, M. J. Alvarez, N. D. Kendsersky, C. R. Pelz, K. Johnson-Camacho, L. E. Dobrolecki, K. Chin, A. J. Aswani, N. J. Wang, A. Califano, M. T. Lewis, C. J. Tomlin, P. T. Spellman, A. Adey, J. W. Gray and R. C. Sears (2018). "Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer." Nature Communications **9**.

Ritschka, B., M. Storer, A. Mas, F. Heinzmann, M. C. Ortells, J. P. Morton, O. J. Sansom, L. Zender and W. M. Keyes (2017). "The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration." Genes Dev **31**(2): 172-183.

Rodon, J., Perez, J., Kurzrock, R. (2010). "Combining targeted therapies: practical issues to consider at the bench and bedside. ." Oncologist **15**(1): 37-50.

Romero, O., Vilarrubi, A., Alburquerque-Bejar, JJ., Gomez, A., Andrades, A., trastulli, D., Proz, E., Setien, F., Verdura, S., Farre, L., Martin-Tejera, JF., Llabata, P., Oaknin, A., Saigi, M., Piulats, JM., Matias-Guiu, X., Medina, PP., Vidal, A., Villaneuva, A., Sanchez-Cespedes, M. (2021). "SMARCA4 deficient tumours are vulnerable to KDM6A/UTX and KDM6B/JMJD3 blockade." Nature Communications

Romero, O. A. and M. Sanchez-Cespedes (2014). "The SWI/SNF genetic blockade: effects in cell differentiation, cancer and developmental diseases." Oncogene **33**(21): 2681-2689.

Romero-Perez, L., Lopez-Garcia, MA., Diaz-Martin, J., Biscuola, M., Castilla, MA., Tafe, LJ., Garg, K., Oliva, E., Matias-Guiu, X., Soslow, RA., Palacios, J. (2013). "ZEB1 overexpression associated with E-cadherin and microRNA-200 downregulation is characteristic of undifferentiated endometrial carcinoma." Mod Pathol **26**(11).

Rosell, R., E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, R. Palmero, R. Garcia-Gomez, C. Pallares, J. M. Sanchez, R. Porta, M. Cobo, P. Garrido, F. Longo, T. Moran, A. Insa, F. De Marinis, R. Corre, I. Bover, A. Illiano, E. Dansin, J. de Castro, M. Milella, N. Reguart, G. Altavilla, U. Jimenez, M. Provencio, M. A. Moreno, J. Terrasa, J. Munoz-Langa, J. Valdivia, D. Isla, M. Domine, O. Molinier, J. Mazieres, N. Baize, R. Garcia-Campelo, G. Robinet, D. Rodriguez-Abreu, G. Lopez-Vivanco, V. Gebbia, L. Ferrera-Delgado, P. Bombaron, R. Bernabe, A. Bearz, A. Artal, E. Cortesi, C. Rolfo, M. Sanchez-Ronco, A. Drozdowskyj, C. Queralt, I. de Aguirre, J. L. Ramirez, J. J. Sanchez, M. A. Molina, M. Taron, L. Paz-Ares, P.-C. Spanish Lung Cancer Group in collaboration with Groupe Francais de and T. Associazione Italiana Oncologia (2012). "Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial." Lancet Oncol **13**(3): 239-246.

Rosso, M., B. Majem, L. Devis, L. Lapyckyj, M. J. Besso, M. Llaurado, M. F. Abascal, M. L. Matos, L. Lanau, J. Castellvi, J. L. Sanchez, A. Perez Benavente, A. Gil-Moreno, J. Reventos, A. Santamaria Margalef, M. Rigau and M. H. Vazquez-Levin (2017). "E-cadherin: A determinant molecule associated with ovarian cancer progression, dissemination and aggressiveness." PLoS One **12**(9): e0184439.

Rotili, D. and A. Mai (2011). "Targeting Histone Demethylases: A New Avenue for the Fight against Cancer." Genes Cancer **2**(6): 663-679.

Rouet, P., F. Smih and M. Jasin (1994). "Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells." Proc Natl Acad Sci U S A **91**(13): 6064-6068.

Ruiz-Garcia, A., A. Plotka, M. O'Gorman and D. D. Wang (2017). "Effect of food on the bioavailability of palbociclib." Cancer Chemother Pharmacol **79**(3): 527-533.

Ryan, R. J., Y. Drier, H. Whitton, M. J. Cotton, J. Kaur, R. Issner, S. Gillespie, C. B. Epstein, V. Nardi, A. R. Sohani, E. P. Hochberg and B. E. Bernstein (2015). "Detection of Enhancer-Associated Rearrangements Reveals Mechanisms of Oncogene Dysregulation in B-cell Lymphoma." Cancer Discov **5**(10): 1058-1071.

Rybak, A. P. and D. Tang (2013). "SOX2 plays a critical role in EGFR-mediated self-renewal of human prostate cancer stem-like cells." Cell Signal **25**(12): 2734-2742.

Saegusa, M., M. Hashimura, T. Kuwata and I. Okayasu (2009). "Requirement of the Akt/beta-catenin pathway for uterine carcinosarcoma genesis, modulating E-cadherin expression through the transactivation of slug." Am J Pathol **174**(6): 2107-2115.

Saha, A., J. Wittmeyer and B. R. Cairns (2006). "Chromatin remodelling: the industrial revolution of DNA around histones." Nat Rev Mol Cell Biol **7**(6): 437-447.

Sanchez-Tillo, E., A. Lazaro, R. Torrent, M. Cuatrecasas, E. C. Vaquero, A. Castells, P. Engel and A. Postigo (2010). "ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1." Oncogene **29**(24): 3490-3500.

Sander, J., Joung, JK. (2014). "CRISPR-Cas systems for genome editing, regulation and targeting." Nat Biotechnol **32**(4): 347-355.

Sanjuan-Pla, A., C. Bueno, C. Prieto, P. Acha, R. W. Stam, R. Marschalek and P. Menendez (2015). "Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia." Blood **126**(25): 2676-2685.

Santini, R., S. Pietrobono, S. Pandolfi, V. Montagnani, M. D'Amico, J. Y. Penachioni, M. C. Vinci, L. Borgognoni and B. Stecca (2014). "SOX2 regulates self-renewal and tumorigenicity of human melanoma-initiating cells." Oncogene **33**(38): 4697-4708.

Santos-Rosa, H., R. Schneider, A. J. Bannister, J. Sherriff, B. E. Bernstein, N. C. Emre, S. L. Schreiber, J. Mellor and T. Kouzarides (2002). "Active genes are tri-methylated at K4 of histone H3." Nature **419**(6905): 407-411.

Schaefer, I. M., A. Agaimy, C. D. Fletcher and J. L. Hornick (2017). "Claudin-4 expression distinguishes SWI/SNF complex-deficient undifferentiated carcinomas from sarcomas." Mod Pathol **30**(4): 539-548.

Schep, A. N., B. Wu, J. D. Buenrostro and W. J. Greenleaf (2017). "chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data." Nat Methods **14**(10): 975-978.

Schick, S., D. Fournier, S. Thakurela, S. K. Sahu, A. Garding and V. K. Tiwari (2015). "Dynamics of chromatin accessibility and epigenetic state in response to UV damage." J Cell Sci **128**(23): 4380-4394.

Schneeberger, V. E., V. Allaj, E. E. Gardner, J. T. Poirier and C. M. Rudin (2016). "Quantitation of Murine Stroma and Selective Purification of the Human Tumor Component of Patient-Derived Xenografts for Genomic Analysis." PLoS One **11**(9): e0160587.

Schneider, C. A., W. S. Rasband and K. W. Eliceiri (2012). "NIH Image to ImageJ: 25 years of image analysis." Nat Methods **9**(7): 671-675.

Schotta, G., M. Lachner, K. Sarma, A. Ebert, R. Sengupta, G. Reuter, D. Reinberg and T. Jenuwein (2004). "A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin." Genes Dev **18**(11): 1251-1262.

Schulz, A., F. Meyer, A. Dubrovska and K. Borgmann (2019). "Cancer Stem Cells and Radioresistance: DNA Repair and Beyond." Cancers (Basel) **11**(6).

Schuster, A., H. Erasimus, S. Fritah, P. V. Nazarov, E. van Dyck, S. P. Niclou and A. Golebiewska (2019). "RNAi/CRISPR Screens: from a Pool to a Valid Hit." Trends Biotechnol **37**(1): 38-55.

Schwitalla, S. (2014). "Tumor cell plasticity: the challenge to catch a moving target." J Gastroenterol **49**(4): 618-627.

Scully, R. and A. Xie (2013). "Double strand break repair functions of histone H2AX." Mutat Res **750**(1-2): 5-14.

Seeber, A., M. Hauer and S. M. Gasser (2013). "Nucleosome remodelers in double-strand break repair." Curr Opin Genet Dev **23**(2): 174-184.

Seligson, D. B., S. Horvath, M. A. McBrian, V. Mah, H. Yu, S. Tze, Q. Wang, D. Chia, L. Goodglick and S. K. Kurdistani (2009). "Global levels of histone modifications predict prognosis in different cancers." Am J Pathol **174**(5): 1619-1628.

Sen, P., Y. Lan, C. Y. Li, S. Sidoli, G. Donahue, Z. Dou, B. Frederick, Q. Chen, L. J. Luense, B. A. Garcia, W. Dang, F. B. Johnson, P. D. Adams, D. C. Schultz and S. L. Berger (2019). "Histone Acetyltransferase p300 Induces De Novo Super-Enhancers to Drive Cellular Senescence." Mol Cell **73**(4): 684-698 e688.

Sequist, L. V., J. C. Yang, N. Yamamoto, K. O'Byrne, V. Hirsh, T. Mok, S. L. Geater, S. Orlov, C. M. Tsai, M. Boyer, W. C. Su, J. Bennouna, T. Kato, V. Gorbunova, K. H. Lee, R. Shah, D. Massey, V. Zazulina, M. Shahidi and M. Schuler (2013). "Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations." J Clin Oncol **31**(27): 3327-3334.

Shang, X., X. Lin, E. Alvarez, G. Manorek and S. B. Howell (2012). "Tight junction proteins claudin-3 and claudin-4 control tumor growth and metastases." Neoplasia **14**(10): 974-985.

Sharma, S. V., D. Y. Lee, B. Li, M. P. Quinlan, F. Takahashi, S. Maheswaran, U. McDermott, N. Azizian, L. Zou, M. A. Fischbach, K. K. Wong, K. Brandstetter, B. Wittner, S. Ramaswamy, M. Classon and J. Settleman (2010). "A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations." Cell **141**(1): 69-80.

Shaw, A. T. and J. A. Engelman (2014). "Ceritinib in ALK-rearranged non-small-cell lung cancer." N Engl J Med **370**(26): 2537-2539.

Shaw, A. T., D. W. Kim, K. Nakagawa, T. Seto, L. Crino, M. J. Ahn, T. De Pas, B. Besse, B. J. Solomon, F. Blackhall, Y. L. Wu, M. Thomas, K. J. O'Byrne, D. Moro-Sibilot, D. R. Camidge, T. Mok, V. Hirsh, G. J. Riely, S. Iyer, V. Tassell, A. Polli, K. D. Wilner and P. A. Janne (2013). "Crizotinib versus chemotherapy in advanced ALK-positive lung cancer." N Engl J Med **368**(25): 2385-2394.

Shelton, J. G., L. S. Steelman, S. L. Abrams, E. R. White, S. M. Akula, R. A. Franklin, F. E. Bertrand, M. McMahon and J. A. McCubrey (2005). "Conditional EGFR promotes cell cycle progression and prevention of apoptosis in the absence of autocrine cytokines." Cell Cycle **4**(6): 822-830.

Sheppard, K. E. and G. A. McArthur (2013). "The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma." Clin Cancer Res **19**(19): 5320-5328.

Sherr, C. J., D. Beach and G. I. Shapiro (2016). "Targeting CDK4 and CDK6: From Discovery to Therapy." Cancer Discov **6**(4): 353-367.

Shi, J., Y. Li, R. Jia and X. Fan (2020). "The fidelity of cancer cells in PDX models: Characteristics, mechanism and clinical significance." Int J Cancer **146**(8): 2078-2088.

Shi, J., W. A. Whyte, C. J. Zepeda-Mendoza, J. P. Milazzo, C. Shen, J. S. Roe, J. L. Minder, F. Mercan, E. Wang, M. A. Eckersley-Maslin, A. E. Campbell, S. Kawaoka, S. Shareef, Z. Zhu, J. Kendall, M. Muhar, C. Haslinger, M. Yu, R. G. Roeder, M. H. Wigler, G. A. Blobel, J. Zuber, D. L. Spector, R. A. Young and C. R. Vakoc (2013). "Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation." Genes Dev **27**(24): 2648-2662.

Shibue, T. and R. A. Weinberg (2017). "EMT, CSCs, and drug resistance: the mechanistic link and clinical implications." Nat Rev Clin Oncol **14**(10): 611-629.

Shilatifard, A. (2012). "The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis." Annu Rev Biochem **81**: 65-95.

Shlush, L. I., S. Zandi, A. Mitchell, W. C. Chen, J. M. Brandwein, V. Gupta, J. A. Kennedy, A. D. Schimmer, A. C. Schuh, K. W. Yee, J. L. McLeod, M. Doedens, J. J. Medeiros, R. Marke, H. J. Kim, K. Lee, J. D. McPherson, T. J. Hudson, H. P.-L. G. P. Consortium, A. M. Brown, F. Yousif, Q. M. Trinh, L. D. Stein, M. D. Minden, J. C. Wang and J. E. Dick (2014). "Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia." Nature **506**(7488): 328-333.

Shorstova, T., M. Marques, J. Su, J. Johnston, C. L. Kleinman, N. Hamel, S. Huang, M. A. Alaoui-Jamali, W. D. Foulkes and M. Witcher (2019). "SWI/SNF-Compromised Cancers Are Susceptible to Bromodomain Inhibitors." Cancer Res **79**(10): 2761-2774.

Silva, E. G., M. T. Deavers, D. C. Bodurka and A. Malpica (2006). "Association of low-grade endometrioid carcinoma of the uterus and ovary with undifferentiated carcinoma: a new type of dedifferentiated carcinoma?" Int J Gynecol Pathol **25**(1): 52-58.

Silva, E. G., M. T. Deavers and A. Malpica (2007). "Undifferentiated carcinoma of the endometrium: a review." Pathology **39**(1): 134-138.

Silva, E. G., Deavers, M.T., Bodurka, D.C., Malpica, A. (2006). "Association of Low-Grade Endometrioid Carcinoma of the Uterus and Ovary With Undifferentiated Carcinoma: A New Type of Dedifferentiated Carcinoma?" Int J Gynecol Pathol **25**(1): 52-58.

Simon, J. A. and R. E. Kingston (2013). "Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put." Mol Cell **49**(5): 808-824.

Singer, E., J. Judkins, N. Salomonis, L. Matlaf, P. Soteropoulos, S. McAllister and L. Soroceanu (2015). "Reactive oxygen species-mediated therapeutic response and resistance in glioblastoma." Cell Death & Disease **6**.

Singh, A. and J. Settleman (2010). "EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer." Oncogene **29**(34): 4741-4751.

Singh, S., J. Trevino, N. Bora-Singhal, D. Coppola, E. Haura, S. Altiok and S. P. Chellappan (2012). "EGFR/Src/Akt signaling modulates Sox2 expression and self-renewal of stem-like side-population cells in non-small cell lung cancer." Mol Cancer **11**: 73.

Singhal, N., J. Graumann, G. Wu, M. J. Arauzo-Bravo, D. W. Han, B. Greber, L. Gentile, M. Mann and H. R. Scholer (2010). "Chromatin-Remodeling Components of the BAF Complex Facilitate Reprogramming." Cell **141**(6): 943-955.

Skvortsov, S., P. Debbage, P. Lukas and I. Skvortsova (2015). "Crosstalk between DNA repair and cancer stem cell (CSC) associated intracellular pathways." Seminars in Cancer Biology **31**: 36-42.

Smeenk, G. and H. van Attikum (2013). "The chromatin response to DNA breaks: leaving a mark on genome integrity." Annu Rev Biochem **82**: 55-80.

Sneeringer, C. J., M. P. Scott, K. W. Kuntz, S. K. Knutson, R. M. Pollock, V. M. Richon and R. A. Copeland (2010). "Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas." Proc Natl Acad Sci U S A **107**(49): 20980-20985.

So, K., Min, KJ., Hong, JH., Lee, J-K. (2015). "Interleukin-6 Expression by Interactions Between Gynecologic Cancer Cells and Human Mesenchymal Stem Cells Promotes Epithelial-Mesenchymal Transition." Int J Oncol **47**(4): 1451-1459.

Soldi, M., T. Mari, L. Nicosia, D. Musiani, G. Sigismondo, A. Cuomo, G. Pavesi and T. Bonaldi (2017). "Chromatin proteomics reveals novel combinatorial histone modification signatures that mark distinct subpopulations of macrophage enhancers." Nucleic Acids Res **45**(21): 12195-12213.

Solomon, B. J., T. Mok, D. W. Kim, Y. L. Wu, K. Nakagawa, T. Mekhail, E. Felip, F. Cappuzzo, J. Paolini, T. Usari, S. Iyer, A. Reisman, K. D. Wilner, J. Tursi, F. Blackhall and P. Investigators (2014). "First-line crizotinib versus chemotherapy in ALK-positive lung cancer." N Engl J Med **371**(23): 2167-2177.

Somervaille, T. C. P., C. J. Matheny, G. J. Spencer, M. Iwasaki, J. L. Rinn, D. M. Witten, H. Y. Chang, S. A. Shurtleff, J. R. Downing and M. L. Cleary (2009). "Hierarchical Maintenance of MLL Myeloid Leukemia Stem Cells Employs a Transcriptional Program Shared with Embryonic Rather Than Adult Stem Cells." Cell Stem Cell **4**(2): 129-140.

Soria, G., S. E. Polo and G. Almouzni (2012). "Prime, Repair, Restore: The Active Role of Chromatin in the DNA Damage Response." Molecular Cell **46**(6): 722-734.

Soria, J. C., Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewaskulyong, K. H. Lee, A. Dechaphunkul, F. Imamura, N. Nogami, T. Kurata, I. Okamoto, C. Zhou, B. C. Cho, Y. Cheng, E. K. Cho, P. J. Voon, D. Planchard, W. C. Su, J. E. Gray, S. M. Lee, R. Hodge, M. Marotti, Y. Rukazenkov, S. S. Ramalingam and F. Investigators (2018). "Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer." N Engl J Med **378**(2): 113-125.

Sozen, H., D. Vatansever, A. C. Iyibozkurt, S. Topuz, M. Ozsurmeli, Y. Salihoglu, B. Guzelbey and S. Berkman (2015). "Clinicopathologic and survival analyses of synchronous primary endometrial and epithelial ovarian cancers." J Obstet Gynaecol Res **41**(11): 1813-1819.

Spoelstra, N., Manning, NG., Higashi, Y., Darling, D., Singh, M., Shroyer, KR., Broaddus, RR., Horwitz, KB., Richer JK. (2006). "The Transcription Factor ZEB1 Is Aberrantly Expressed in Aggressive Uterine Cancers." **66**(7): 3893-3902.

St Pierre, R. and C. Kadoch (2017). "Mammalian SWI/SNF complexes in cancer: emerging therapeutic opportunities." Curr Opin Genet Dev **42**: 56-67.

Staberg, M., R. D. Rasmussen, S. R. Michaelsen, H. Pedersen, K. E. Jensen, M. Villingshoj, J. Skjoth-Rasmussen, J. Brennum, K. Vitting-Seerup, H. S. Poulsen and P. Hamerlik (2018). "Targeting glioma stem-like cell survival and chemoresistance through inhibition of lysine-specific histone demethylase KDM2B." Molecular Oncology **12**(3): 406-420.

Steger, D. J., M. I. Lefterova, L. Ying, A. J. Stonestrom, M. Schupp, D. Zhuo, A. L. Vakoc, J. E. Kim, J. Chen, M. A. Lazar, G. A. Blobel and C. R. Vakoc (2008). "DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells." Mol Cell Biol **28**(8): 2825-2839.

Stewart, C. J. and M. L. Crook (2015). "SWI/SNF complex deficiency and mismatch repair protein expression in undifferentiated and dedifferentiated endometrial carcinoma." Pathology **47**(5): 439-445.

Strehl, J. D., D. L. Wachter, J. Fiedler, E. Heimerl, M. W. Beckmann, A. Hartmann and A. Agaimy (2015). "Pattern of SMARCB1 (INI1) and SMARCA4 (BRG1) in poorly differentiated endometrioid adenocarcinoma of the uterus: analysis of a series with emphasis on a novel SMARCA4-deficient dedifferentiated rhabdoid variant." Ann Diagn Pathol **19**(4): 198-202.

Sullivan, N. J., A. K. Sasser, A. E. Axel, F. Vesuna, V. Raman, N. Ramirez, T. M. Oberyszyn and B. M. Hall (2009). "Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells." Oncogene **28**(33): 2940-2947.

Supek, F. and B. Lehner (2015). "Differential DNA mismatch repair underlies mutation rate variation across the human genome." Nature **521**(7550): 81-84.

Suva, M. L., N. Riggi and B. E. Bernstein (2013). "Epigenetic reprogramming in cancer." Science **339**(6127): 1567-1570.

Suzuki, T., M. Terashima, S. Tange and A. Ishimura (2013). "Roles of histone methyl-modifying enzymes in development and progression of cancer." Cancer Sci **104**(7): 795-800.

Swanton, C. (2012). "Intratumor Heterogeneity: Evolution through Space and Time." Cancer Research **72**(19): 4875-4882.

Tafe, L. J., K. Garg, I. Chew, C. Tornos and R. A. Soslow (2010). "Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms." Mod Pathol **23**(6): 781-789.

Tagal, V., S. Wei, W. Zhang, R. A. Brekken, B. A. Posner, M. Peyton, L. Girard, T. Hwang, D. A. Wheeler, J. D. Minna, M. A. White, A. F. Gazdar and M. G. Roth (2017). "SMARCA4-inactivating mutations increase sensitivity to Aurora kinase A inhibitor VX-680 in non-small cell lung cancers." Nat Commun **8**: 14098.

Tate, J. G., S. Bamford, H. C. Jubb, Z. Sondka, D. M. Beare, N. Bindal, H. Boutselakis, C. G. Cole, C. Creatore, E. Dawson, P. Fish, B. Harsha, C. Hathaway, S. C. Jupe, C. Y. Kok, K. Noble, L. Ponting, C. C. Ramshaw, C. E. Rye, H. E. Speedy, R. Stefancsik, S. L. Thompson, S. Wang, S. Ward, P. J. Campbell and S. A. Forbes (2019). "COSMIC: the Catalogue Of Somatic Mutations In Cancer." Nucleic Acids Res **47**(D1): D941-D947.

Teng, M. W., S. F. Ngiow, A. Ribas and M. J. Smyth (2015). "Classifying Cancers Based on T-cell Infiltration and PD-L1." Cancer Res **75**(11): 2139-2145.

Tessier-Cloutier, B., M. Coatham, M. Carey, G. S. Nelson, S. Hamilton, A. Lum, R. A. Soslow, C. J. Stewart, L. M. Postovit, M. Kobel and C. H. Lee (2021). "SWI/SNF-deficiency defines highly aggressive undifferentiated endometrial carcinoma." J Pathol Clin Res **7**(2): 144-153.

Tessier-Cloutier, B., Coatham, M., Carey, M., Nelson, GC., Hamilton, S., Lum, A., Soslow, RA., Stewart, CJR., Postovit, LM., Koebel, M., Lee, C-H. (2020). "SWI/SNF-deficiency defines highly aggressive undifferentiated endometrial carcinoma." J Pathol Clin Res.

Tessier-Cloutier, B., R. A. Soslow, C. J. R. Stewart, M. Kobel and C. H. Lee (2018). "Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas." Histopathology **73**(2): 299-305.

Thiery, J. P. (2002). "Epithelial-mesenchymal transitions in tumor progression." Nat Rev Cancer **2**(6): 442-454.

Tian, X., S. Zhang, H. M. Liu, Y. B. Zhang, C. A. Blair, D. Mercola, P. Sassone-Corsi and X. Zi (2013). "Histone lysine-specific methyltransferases and demethylases in carcinogenesis: new targets for cancer therapy and prevention." Curr Cancer Drug Targets **13**(5): 558-579.

Tierney, B., McCann, GA., Naidu, S., Rath, KS., Saini, U., Wanner, R., Kuppusamy, P., Suarez, A., Goodfellow, PJ., Cohn, DE., Selvendiran, K. (2014). "Aberrantly activated pSTAT3-Ser727 in human endometrial cancer is suppressed by HO-3867, a novel STAT3 inhibitor." Gynecologic Oncology **135**(1): 133-141.

Tomita, H., K. Tanaka, T. Tanaka and A. Hara (2016). "Aldehyde dehydrogenase 1A1 in stem cells and cancer." Oncotarget **7**(10): 11018-11032.

Torres, C. M., A. Biran, M. J. Burney, H. Patel, T. Henser-Brownhill, A. S. Cohen, Y. Li, R. Ben-Hamo, E. Nye, B. Spencer-Dene, P. Chakravarty, S. Efroni, N. Matthews, T. Misteli, E. Meshorer and P. Scaffidi (2016). "The linker histone H1.0 generates epigenetic and functional intratumor heterogeneity." Science **353**(6307).

Travaglino, A., A. Raffone, M. Mascolo, M. Guida, L. Insabato, G. F. Zannoni and F. Zullo (2020). "TCGA Molecular Subgroups in Endometrial Undifferentiated/Dedifferentiated Carcinoma." Pathol Oncol Res **26**(3): 1411-1416.

Trotter, K. W. and T. K. Archer (2008). "The BRG1 transcriptional coregulator." Nucl Recept Signal **6**: e004.

Trouche, D., C. Le Chalony, C. Muchardt, M. Yaniv and T. Kouzarides (1997). "RB and hbrm cooperate to repress the activation functions of E2F1." Proc Natl Acad Sci U S A **94**(21): 11268-11273.

Tsai, H. C. and S. B. Baylin (2011). "Cancer epigenetics: linking basic biology to clinical medicine." Cell Res **21**(3): 502-517.

Tsoukalas, N., E. Aravantinou-Fatorou, M. Tolia, C. Giaginis, M. Galanopoulos, M. Kiakou, I. D. Kostakis, E. Dana, I. Vamvakaris, A. Korogiannos, E. Tsiambas, N. Salemis, G. Kyrgias, A. Karameris and S. Theocharis (2017). "Epithelial-Mesenchymal Transition in Non Small-cell Lung Cancer." Anticancer Res **37**(4): 1773-1778.

Turner, N. C., J. Ro, F. Andre, S. Loi, S. Verma, H. Iwata, N. Harbeck, S. Loibl, C. Huang Bartlett, K. Zhang, C. Giorgetti, S. Randolph, M. Koehler, M. Cristofanilli and P. S. Group (2015). "Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer." N Engl J Med **373**(3): 209-219.

Urick, M., Rudd, ML., Godwin, AK., Sgroi, D., Merino, M., Bell, DW. (2011). "PIK3R1 (p85α) Is Somatically Mutated at High Frequency in Primary Endometrial Cancer." Cancer Res **71**(12): 4061-4067.

Van Campenhout, C., Cabochette, P., Veillard, A-C., Laczik, M., Zelisko-Schmidt, A., Sabatel, C., Dhainaut, M., Vanhollebeke, B., Gueydan, C., Kruys, V. (2019). "Guidelines for optimized gene knockout using CRISPR/Cas9." BioTechniques **66**(6).

van der Horst, P., Wang, W., Vandenput, I., Kuhne, LC., Ewing, PC., van IJcken, WFJ., van der Zee, M., Amant, F., Burger, CW., Blok, LJ. (2012). "Progesterone Inhibits Epithelial-to-Mesenchymal Transition in Endometrial Cancer." PLos One **7**(1).

van Gool, I. C., F. A. Eggink, L. Freeman-Mills, E. Stelloo, E. Marchi, M. de Bruyn, C. Palles, R. A. Nout, C. D. de Kroon, E. M. Osse, P. Klenerman, C. L. Creutzberg, I. P. Tomlinson, V. T. Smit, H. W. Nijman, T. Bosse and D. N. Church (2015). "POLE Proofreading Mutations Elicit an Antitumor Immune Response in Endometrial Cancer." Clin Cancer Res **21**(14): 3347-3355.

Van Itallie, C., Rahner, C., Anderson, JM. (2001). "Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability." J Clin Invest **107**: 1319-1327.

Velegzhaninov, I. O., V. A. Ievlev, Y. I. Pylina, D. M. Shadrin and O. M. Vakhrusheva (2018). "Programming of Cell Resistance to Genotoxic and Oxidative Stress." Biomedicines **6**(1).

Venere, M., P. Hamerlik, Q. Wu, R. D. Rasmussen, L. A. Song, A. Vasanji, N. Tenley, W. A. Flavahan, A. B. Hjelmeland, J. Bartek and J. N. Rich (2014). "Therapeutic targeting of constitutive PARP activation compromises stem cell phenotype and survival of glioblastoma-initiating cells." Cell Death Differ **21**(2): 258-269.

Venhuizen, J. H., F. J. C. Jacobs, P. N. Span and M. M. Zegers (2020). "P120 and E-cadherin: Double-edged swords in tumor metastasis." Semin Cancer Biol **60**: 107-120.

Vermeulen, L., F. de Sousa e Melo, D. J. Richel and J. P. Medema (2012). "The developing cancer stem-cell model: clinical challenges and opportunities." Lancet Oncol **13**(2): e83-89.

Vierbuchen, T., E. Ling, C. J. Cowley, C. H. Couch, X. Wang, D. A. Harmin, C. W. M. Roberts and M. E. Greenberg (2017). "AP-1 Transcription Factors and the BAF Complex Mediate Signal-Dependent Enhancer Selection." Mol Cell **68**(6): 1067-1082 e1012.

Vinogradova, M., V. S. Gehling, A. Gustafson, S. Arora, C. A. Tindell, C. Wilson, K. E. Williamson, G. D. Guler, P. Gangurde, W. Manieri, J. Busby, E. M. Flynn, F. Lan, H. J. Kim, S. Odate, A. G. Cochran, Y. Liu, M. Wongchenko, Y. Yang, T. K. Cheung, T. M. Maile, T. Lau, M. Costa, G. V. Hegde, E. Jackson, R. Pitti, D. Arnott, C. Bailey, S. Bellon, R. T. Cummings, B. K. Albrecht, J. C. Harmange, J. R. Kiefer, P. Trojer and M. Classon (2016). "An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells." Nat Chem Biol **12**(7): 531-538.

Vita, G., L. Borgia, L. Di Giovannantonio and M. Bisceglia (2011). "Dedifferentiated endometrioid adenocarcinoma of the uterus: a clinicopathologic study of a case." Int J Surg Pathol **19**(5): 649-652.

Vitale, I., G. Manic, R. De Maria, G. Kroemer and L. Galluzzi (2017). "DNA Damage in Stem Cells." Mol Cell **66**(3): 306-319.

Waclaw, B., I. Bozic, M. E. Pittman, R. H. Hruban, B. Vogelstein and M. A. Nowak (2015). "A spatial model predicts that dispersal and cell turnover limit intratumour heterogeneity." Nature **525**(7568): 261-+.

Wainwright, E. N. and P. Scaffidi (2017). "Epigenetics and Cancer Stem Cells: Unleashing, Hijacking, and Restricting Cellular Plasticity." Trends Cancer **3**(5): 372-386.

Wang, C., Q. Li and Y. He (2020). "MicroRNA215p promotes epithelial to mesenchymal transition by targeting SRYbox 17 in endometrial cancer." Oncol Rep **43**(6): 1897-1905.

Wang, C., K. Su, Y. Zhang, W. Zhang, Q. Zhao, D. Chu and R. Guo (2019). "IR-A/IGF-1R-mediated signals promote epithelial-mesenchymal transition of endometrial carcinoma cells by activating PI3K/AKT and ERK pathways." Cancer Biol Ther **20**(3): 295-306.

Wang, F., Wang, S., Wang, Z., Duan, J., An, T., Zhao, J., Bai, H., Wang, J., Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education) (2012). "Phosphorylated EGFR expression may predict outcome of EGFR-TKIs therapy for the advanced NSCLC patients with wild-type EGFR." Journal of Experimental & Clinical Cancer Research **31**(65).

Wang, H., W. Bao, F. Jiang, Q. Che, Z. Chen, F. Wang, H. Tong, C. Dai, X. He, Y. Liao, B. Liu, J. Sun and X. Wan (2015). "Mutant p53 (p53-R248Q) functions as an oncogene in promoting endometrial cancer by up-regulating REGgamma." Cancer Lett **360**(2): 269-279.

Wang, J., L. Zhang, W. Jiang, R. Zhang, B. Zhang, A. Silayiding and X. Duan (2020). "MicroRNA-135a promotes proliferation, migration, invasion and induces chemoresistance of endometrial cancer cells." Eur J Obstet Gynecol Reprod Biol X **5**: 100103.

Wang, K., J. Kan, S. T. Yuen, S. T. Shi, K. M. Chu, S. Law, T. L. Chan, Z. Kan, A. S. Chan, W. Y. Tsui, S. P. Lee, S. L. Ho, A. K. Chan, G. H. Cheng, P. C. Roberts, P. A.

Rejto, N. W. Gibson, D. J. Pocalyko, M. Mao, J. Xu and S. Y. Leung (2011). "Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer." Nat Genet **43**(12): 1219-1223.

Wang, K. C., Y. W. Yang, B. Liu, A. Sanyal, R. Corces-Zimmerman, Y. Chen, B. R. Lajoie, A. Protacio, R. A. Flynn, R. A. Gupta, J. Wysocka, M. Lei, J. Dekker, J. A. Helms and H. Y. Chang (2011). "A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression." Nature **472**(7341): 120-124.

Wang, W., J. Cote, Y. Xue, S. Zhou, P. A. Khavari, S. R. Biggar, C. Muchardt, G. V. Kalpana, S. P. Goff, M. Yaniv, J. L. Workman and G. R. Crabtree (1996). "Purification and biochemical heterogeneity of the mammalian SWI-SNF complex." EMBO J **15**(19): 5370-5382.

Wang, W., Q. Li, T. Yamada, K. Matsumoto, I. Matsumoto, M. Oda, G. Watanabe, Y. Kayano, Y. Nishioka, S. Sone and S. Yano (2009). "Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors." Clin Cancer Res **15**(21): 6630-6638.

Wang, X., Zhang, W., Sun, X., Lin, Y., Chen, W. (2018). "Cancer-associated Fibroblasts Induce Epithelial-Mesenchymal Transition Through Secreted Cytokines in Endometrial Cancer Cells." Oncol Lett **15**(4): 5694-5702.

Wang, X. L., R.S.; Alver, B.H.; et al., (2017). "SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation." Nature Genetics **49**(2): 289-295.

Wang, Y., L. Hoang, J. X. Ji and D. G. Huntsman (2020). "SWI/SNF Complex Mutations in Gynecologic Cancers: Molecular Mechanisms and Models." Annu Rev Pathol **15**: 467-492.

Wang, Z., W. Wang, K. Huang, Y. Wang, J. Li and X. Yang (2017). "MicroRNA-34a inhibits cells proliferation and invasion by downregulating Notch1 in endometrial cancer." Oncotarget **8**(67): 111258-111270.

Wegner, C. S., A. Hauge, L. M. K. Andersen, R. Huang, T. G. Simonsen, J. V. Gaustad and E. K. Rofstad (2018). "Increasing aggressiveness of patient-derived xenograft models of cervix carcinoma during serial transplantation." Oncotarget **9**(30): 21036-21051.

Weina, K. and J. Utikal (2014). "SOX2 and cancer: current research and its implications in the clinic." Clin Transl Med **3**: 19.

Wen, Y., J. Cai, Y. Hou, Z. Huang and Z. Wang (2017). "Role of EZH2 in cancer stem cells: from biological insight to a therapeutic target." Oncotarget **8**(23): 37974-37990.

White, J. C. P., P.; Crea, F. (2019). "The role of histone lysine demethylases in cancer cells' resistance to tyrosine kinase inhibitors." Cancer Drug Resistance **2**: 326-334.

Wik, E., M. B. Raeder, C. Krakstad, J. Trovik, E. Birkeland, E. A. Hoivik, S. Mjos, H. M. Werner, M. Mannelqvist, I. M. Stefansson, A. M. Oyan, K. H. Kalland, L. A. Akslen and H. B. Salvesen (2013). "Lack of estrogen receptor-alpha is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma." Clin Cancer Res **19**(5): 1094-1105.

Wiley, C. D. and J. Campisi (2016). "From Ancient Pathways to Aging Cells-Connecting Metabolism and Cellular Senescence." Cell Metab **23**(6): 1013-1021.

Wilson, B. G., K. C. Helming, X. Wang, Y. Kim, F. Vazquez, Z. Jagani, W. C. Hahn and C. W. Roberts (2014). "Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation." Mol Cell Biol **34**(6): 1136-1144.

Wilson, B. G. and C. W. Roberts (2011). "SWI/SNF nucleosome remodellers and cancer." Nat Rev Cancer **11**(7): 481-492.

Wilting, R. H. and J. H. Dannenberg (2012). "Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance." Drug Resist Updat **15**(1-2): 21-38.

Witkowski, L., J. Carrot-Zhang, S. Albrecht, S. Fahiminiya, N. Hamel, E. Tomiak, D. Grynspan, E. Saloustros, J. Nadaf, B. Rivera, C. Gilpin, E. Castellsague, R. Silva-Smith, F. Plourde, M. Wu, A. Saskin, M. Arseneault, R. G. Karabakhtsian, E. A. Reilly, F. R. Ueland, A. Margiolaki, K. Pavlakis, S. M. Castellino, J. Lamovec, H. J. Mackay, L. M. Roth, T. M. Ulbright, T. A. Bender, V. Georgoulias, M. Longy, A. Berchuck, M. Tischkowitz, I. Nagel, R. Siebert, C. J. Stewart, J. Arseneau, W. G. McCluggage, B. A. Clarke, Y. Riazalhosseini, M. Hasselblatt, J. Majewski and W. D. Foulkes (2014). "Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type." Nat Genet **46**(5): 438-443.

Wong, S. H. M., C. M. Fang, L. H. Chuah, C. O. Leong and S. C. Ngai (2018). "E-cadherin: Its dysregulation in carcinogenesis and clinical implications." Crit Rev Oncol Hematol **121**: 11-22.

Wozney, J. M. and V. Rosen (1998). "Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair." Clin Orthop Relat Res(346): 26-37.

Wu, E. S., M. Shih Ie and T. P. Diaz-Montes (2013). "Dedifferentiated endometrioid adenocarcinoma: An under-recognized but aggressive tumor?" Gynecol Oncol Case Rep **5**: 25-27.

Wu, G., A. Broniscer, T. A. McEachron, C. Lu, B. S. Paugh, J. Becksfort, C. Qu, L. Ding, R. Huether, M. Parker, J. Zhang, A. Gajjar, M. A. Dyer, C. G. Mullighan, R. J. Gilbertson, E. R. Mardis, R. K. Wilson, J. R. Downing, D. W. Ellison, J. Zhang, S. J. Baker and P. St. Jude Children's Research Hospital-Washington University Pediatric Cancer Genome (2012). "Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas." Nat Genet **44**(3): 251-253.

Wu, J. I., J. Lessard and G. R. Crabtree (2009). "Understanding the words of chromatin regulation." Cell **136**(2): 200-206.

Wu, Q., P. Madany, J. Akech, J. R. Dobson, S. Douthwright, G. Browne, J. L. Colby, G. E. Winter, J. E. Bradner, J. Pratap, G. Sluder, R. Bhargava, S. I. Chiosea, A. J. van Wijnen, J. L. Stein, G. S. Stein, J. B. Lian, J. A. Nickerson and A. N. Imbalzano (2015). "The SWI/SNF ATPases Are Required for Triple Negative Breast Cancer Cell Proliferation." J Cell Physiol **230**(11): 2683-2694.

Wysocka, J., T. Swigut, T. A. Milne, Y. Dou, X. Zhang, A. L. Burlingame, R. G. Roeder, A. H. Brivanlou and C. D. Allis (2005). "WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development." Cell **121**(6): 859-872.

Xia, Y., Y. Wu, B. Liu, P. Wang and Y. Chen (2014). "Downregulation of miR-638 promotes invasion and proliferation by regulating SOX2 and induces EMT in NSCLC." FEBS Lett **588**(14): 2238-2245.

Xiang, R., D. Liao, T. Cheng, H. Zhou, Q. Shi, T. S. Chuang, D. Markowitz, R. A. Reisfeld and Y. Luo (2011). "Downregulation of transcription factor SOX2 in cancer stem cells suppresses growth and metastasis of lung cancer." Br J Cancer **104**(9): 1410-1417.

Xue, Y., Meehan, B., Fu, Z., Wang, XQD., Fiset, PO., Rieker, R., Levins, C., Kong, T., Zhu, X., Morin, G., Skerritt, L., Herpel, E., Venneti, S., Martinez, D., Judkins, AR., Jung, S., Camilleri-Broet, S., Gonzalez, AV., Guiot, M-C., Lockwood, WW., Spicer, JD., Agaimy, A., Pastor, WA., Dostie, J., Rak, J., Foulkes, WD., Huang, S. (2019). "SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer." Nature Communications **10**(557).

Xue, Y., Meehan, B., Macdonald, E., Venneti, S., Wang, XQD., Witokowski, L., Jelinic, P., Kong, T., Martinez, D., Morin, G., Firlit, M., Abedini, A., Johnson, RM., Cencic, R., Patibandla, J., Chen, H., Papadakis, AI., Auguste A., de Rink, I., Kerkhoven, RM., Bertos, N., Gotlieb, WH., Clarke, BA., Leary, A., Witcher, M., Guiot, M-C., Pelletier J., Dostie, J., Park, M., Judkins, AR., Hass, R., Levine, DA., Rak, J., Vanderhyden, B., Foulkes, WD., Huang, S. (2019). "CDK4/6 inhibitors target SMARCA4-determined cyclin D1 deficiency in hypercalcemic small cell carcinoma of the ovary." Nature Communications **10**(558).

Yan, H., D. W. Parsons, G. Jin, R. McLendon, B. A. Rasheed, W. Yuan, I. Kos, I. Batinic-Haberle, S. Jones, G. J. Riggins, H. Friedman, A. Friedman, D. Reardon, J. Herndon, K. W. Kinzler, V. E. Velculescu, B. Vogelstein and D. D. Bigner (2009). "IDH1 and IDH2 mutations in gliomas." N Engl J Med **360**(8): 765-773.

Yang, L., B. Rodriguez, A. Mayle, H. J. Park, X. Lin, M. Luo, M. Jeong, C. V. Curry, S. B. Kim, D. Ruau, X. Zhang, T. Zhou, M. Zhou, V. I. Rebel, G. A. Challen, B. Gottgens, J. S. Lee, R. Rau, W. Li and M. A. Goodell (2016). "DNMT3A Loss Drives Enhancer Hypomethylation in FLT3-ITD-Associated Leukemias." Cancer Cell **30**(2): 363-365.

Yang, Z., C. Li, Z. Fan, H. Liu, X. Zhang, Z. Cai, L. Xu, J. Luo, Y. Huang, L. He, C. Liu and S. Wu (2017). "Single-cell Sequencing Reveals Variants in ARID1A, GPRC5A and MLL2 Driving Self-renewal of Human Bladder Cancer Stem Cells." Eur Urol **71**(1): 8-12.

Yasuda, K., T. Torigoe, R. Morita, T. Kuroda, A. Takahashi, J. Matsuzaki, V. Kochin, H. Asanuma, T. Hasegawa, T. Saito, Y. Hirohashi and N. Sato (2013). "Ovarian cancer stem cells are enriched in side population and aldehyde dehydrogenase bright overlapping population." PLoS One **8**(8): e68187.

Yemelyanova, A., A. M. Gown, L. S. Wu, B. J. Holmes, B. M. Ronnett and R. Vang (2014). "PAX8 expression in uterine adenocarcinomas and mesonephric proliferations." Int J Gynecol Pathol **33**(5): 492-499.

Yin, D., Y. Jia, Y. Yu, M. V. Brock, J. G. Herman, C. Han, X. Su, Y. Liu and M. Guo (2012). "SOX17 methylation inhibits its antagonism of Wnt signaling pathway in lung cancer." Discov Med **14**(74): 33-40.

Yin, Y., E. Morgunova, A. Jolma, E. Kaasinen, B. Sahu, S. Khund-Sayeed, P. K. Das, T. Kivioja, K. Dave, F. Zhong, K. R. Nitta, M. Taipale, A. Popov, P. A. Ginno, S. Domcke, J. Yan, D. Schubeler, C. Vinson and J. Taipale (2017). "Impact of cytosine methylation on DNA binding specificities of human transcription factors." Science **356**(6337).

Yokomizo, R., K. Yamada, Y. Iida, T. Kiyokawa, K. Ueda, M. Saito, N. Yanaihara, M. Nakamura and A. Okamoto (2017). "Dedifferentiated endometrial carcinoma: A report of three cases and review of the literature." Mol Clin Oncol **7**(6): 1008-1012.

Yoshida, S., N. Furukawa, S. Haruta, Y. Tanase, S. Kanayama, T. Noguchi, M. Sakata, Y. Yamada, H. Oi and H. Kobayashi (2009). "Expression profiles of genes involved in poor prognosis of epithelial ovarian carcinoma: a review." Int J Gynecol Cancer **19**(6): 992-997.

You, J. S. and P. A. Jones (2012). "Cancer genetics and epigenetics: two sides of the same coin?" Cancer Cell **22**(1): 9-20.

Young, M. D., T. A. Willson, M. J. Wakefield, E. Trounson, D. J. Hilton, M. E. Blewitt, A. Oshlack and I. J. Majewski (2011). "ChIP-seq analysis reveals distinct H3K27me3 profiles that correlate with transcriptional activity." Nucleic Acids Res **39**(17): 7415-7427.

Yu, J., Chen, L., Bao, Z., Liu, Y., Liu, G., Liu, G., Li, F., Li, L. (2020). "BMI-1 Promotes Invasion and Metastasis in Endometrial Adenocarcinoma and Is a Poor Prognostic Factor." Oncol Rep **43**(5): 1630-1640.

Yu, W., Yang, L., Li, T., Zhang, Y. (2019). "Cadherin Signaling in Cancer: Its Functions and Role as a Therapeutic Target." Front Oncol.

Yuan, Y., J. S. Lee, S. E. Yost, P. H. Frankel, C. Ruel, C. A. Egelston, W. Guo, S. Padam, A. Tang, N. Martinez, D. Schmolze, C. Presant, B. Ebrahimi, C. Yeon, M. Sedrak, N. Patel, J. Portnow, P. Lee and J. Mortimer (2021). "Phase I/II trial of palbociclib,

pembrolizumab and letrozole in patients with hormone receptor-positive metastatic breast cancer." Eur J Cancer **154**: 11-20.

Zacarias-Fluck, M. F., B. Morancho, R. Vicario, A. Luque Garcia, M. Escorihuela, J. Villanueva, I. T. Rubio and J. Arribas (2015). "Effect of cellular senescence on the growth of HER2-positive breast cancers." J Natl Cancer Inst **107**(5).

Zhang, H., H. Li, S. Qi, Z. Liu, Y. Fu, M. Li and X. Zhao (2017). "Normal endometrial stromal cells regulate 17beta-estradiol-induced epithelial-mesenchymal transition via slug and E-cadherin in endometrial adenocarcinoma cells in vitro." Gynecol Endocrinol **33**(1): 82-86.

Zhang, H. H., R. Li, Y. J. Li, X. X. Yu, Q. N. Sun, A. Y. Li and Y. Kong (2020). "eIF4Erelated miR320a and miR3405p inhibit endometrial carcinoma cell metastatic capability by preventing TGFbeta1induced epithelialmesenchymal transition." Oncol Rep **43**(2): 447-460.

Zhang, J., D. Dominguez-Sola, S. Hussein, J. E. Lee, A. B. Holmes, M. Bansal, S. Vlasevska, T. Mo, H. Tang, K. Basso, K. Ge, R. Dalla-Favera and L. Pasqualucci (2015). "Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis." Nat Med **21**(10): 1190-1198.

Zhang, R., J. Erler and J. Langowski (2017). "Histone Acetylation Regulates Chromatin Accessibility: Role of H4K16 in Inter-nucleosome Interaction." Biophys J **112**(3): 450-459.

Zhang, S., T. T. Gong, F. H. Liu, Y. T. Jiang, H. Sun, X. X. Ma, Y. H. Zhao and Q. J. Wu (2019). "Global, Regional, and National Burden of Endometrial Cancer, 1990-2017: Results From the Global Burden of Disease Study, 2017." Front Oncol **9**: 1440.

Zhang, T., Cooper, S., Brockdorff, N. (2015). "The interplay of histone modifications – writers that read." Embo Reports **15**(11): 1467-1481.

Zhang, W., S. C. Glockner, M. Guo, E. O. Machida, D. H. Wang, H. Easwaran, L. Van Neste, J. G. Herman, K. E. Schuebel, D. N. Watkins, N. Ahuja and S. B. Baylin (2008). "Epigenetic inactivation of the canonical Wnt antagonist SRY-box containing gene 17 in colorectal cancer." Cancer Res **68**(8): 2764-2772.

Zhang, Y., Bao, W., Wang, K., Lu, W., Wang, H., Tong, H., Wan, X. (2016). "SOX17 is a tumor suppressor in endometrial cancer." Oncotarget **7**(46): 76036-76046.

Zhao, M., Fang, W., Wang, Y., Guo, S., Shu, L., Wang, L., Chen, Y., Fu, Q., Liu, Y., Hua, S., Fan, Y., Liu, Y., Deng, X., Luo, R., Mei, Z., Jiang, Q., Liu. (2015). "Enolase-1 Is a Therapeutic Target in Endometrial Carcinoma." Oncotarget **6**(17): 15610-15627.

Zhao, X., L. Dai, Q. Yue, H. Wang, X. U. Wang, Y. Li and R. Chen (2019). "MiR-195 inhibits migration, invasion and epithelial-mesenchymal transition (EMT) of endometrial carcinoma cells by targeting SOX4." J Biosci **44**(6).

Zheng, W., Q. Dai, P. Tao, A. Sun, Y. Wang, L. Bao and G. Zhang (2011). "Overexpression of tyrosine kinase receptor B promotes metastasis of ovarian serous adenocarcinoma by lymphangiogenesis." Tumori **97**(6): 756-761.

Zheng, Y., H. Zhang, Y. Wang, X. Li, P. Lu, F. Dong, Y. Pang, S. Ma, H. Cheng, S. Hao, F. Tang, W. Yuan, X. Zhang and T. Cheng (2016). "Loss of Dnmt3b accelerates MLL-AF9 leukemia progression." Leukemia **30**(12): 2373-2384.

Zhou, C., Y. L. Wu, G. Chen, J. Feng, X. Q. Liu, C. Wang, S. Zhang, J. Wang, S. Zhou, S. Ren, S. Lu, L. Zhang, C. Hu, C. Hu, Y. Luo, L. Chen, M. Ye, J. Huang, X. Zhi, Y. Zhang, Q. Xiu, J. Ma, L. Zhang and C. You (2011). "Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study." Lancet Oncol **12**(8): 735-742.

Zhou, J., Z. Wu, G. Wong, E. Pectasides, A. Nagaraja, M. Stachler, H. Zhang, T. Chen, H. Zhang, J. B. Liu, X. Xu, E. Sicinska, F. Sanchez-Vega, A. K. Rustgi, J. A. Diehl, K. K. Wong and A. J. Bass (2017). "CDK4/6 or MAPK blockade enhances efficacy of EGFR inhibition in oesophageal squamous cell carcinoma." Nat Commun **8**: 13897.

Zhou, L., Y. Zhang, S. Chen, M. Kmieciak, Y. Leng, H. Lin, K. A. Rizzo, C. I. Dumur, A. Ferreira-Gonzalez, Y. Dai and S. Grant (2015). "A regimen combining the Wee1 inhibitor AZD1775 with HDAC inhibitors targets human acute myeloid leukemia cells harboring various genetic mutations." Leukemia **29**(4): 807-818.

Zhu, B., Y. Zheng, A. D. Pham, S. S. Mandal, H. Erdjument-Bromage, P. Tempst and D. Reinberg (2005). "Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation." Mol Cell **20**(4): 601-611.

Zhu, H., Jin, Y-M., Lyu, X-M., Fan, L-M., Wu, F. (2019). "Long noncoding RNA H19 regulates HIF-1α/AXL signaling through inhibiting miR-20b-5p in endometrial cancer." Cell Cycle **18**(19): 2454-2464.

Zhu, P., Y. Wang, J. Wu, G. Huang, B. Liu, B. Ye, Y. Du, G. Gao, Y. Tian, L. He and Z. Fan (2016). "LncBRM initiates YAP1 signalling activation to drive self-renewal of liver cancer stem cells." Nat Commun **7**: 13608.

Zon, L. I. (2008). "Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal." Nature **453**(7193): 306-313.

Appendix

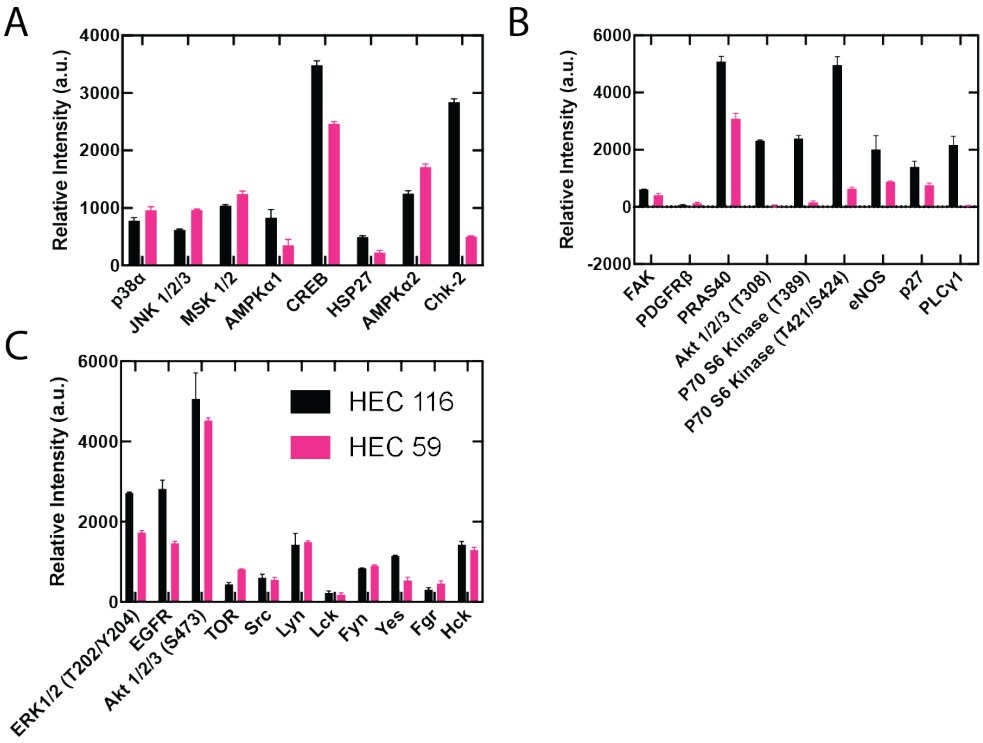
Supplemental Table 2.1. Summary of the genetic findings in 40 dedifferentiated carcinomas of the endometrium. * symbol indicates the % of sequenced DNA fragments or reads that possessed the specific mutations. Cases 1 to 15 on the light gray background belong to the SMARCA4 cohort, Cases 1 to 3 atop the medium gray background are considered part of the SMARCB1 cohort and the ARID1B cohort is made up of Cases 1 to 22 on the dark gray background.

Case number	Gene	Chromo-some number	Position (human hg 19)	Reference	Alternate	% of reads*	Functional consequence
1	*SMARCA4*	chr19	11097624	G	GC	44%	Frameshift
1	*SMARCA4*	chr19	11107032	GA	G	33%	Frameshift
2	*SMARCA4*	chr19	11096874	C	A	30%	Nonsense_S122X
2	*SMARCA4*	chr19	11098539	AC	A	32%	Frameshift
3	*ARID1A*	chr1	27107055	CT	C	25%	Frameshift
3	*SMARCA4*	chr19	11100063	C	T	28%	Nonsense_R397X
4	*ARID1A*	chr1	27023559	AC	A	50%	Frameshift
4	*ARID1A*	chr1	27105930	TG	T	49%	Frameshift
4	*SMARCA4*	chr19	11096026	AG	A	41%	Frameshift
5	*ARID1A*	chr1	27101212	G	A	45%	Nonsense_W1498X
5	*SMARCA4*	chr19	11097624	G	GC	37%	Frameshift
5	*SMARCA4*	chr19	11123698	T	TG	33%	Frameshift
6	*SMARCA4*	chr19	11118613	TC	T	26%	Frameshift
6	*SMARCA4*	chr19	11168965	C	T	21%	Nonsense_Q1487X
7	*ARID1A*	chr1	27106073	AG	A	35%	Frameshift
7	*ARID1A*	chr1	27106803	AC	A	37%	Frameshift
7	*SMARCA4*	chr19	11132638	GA	G	36%	Frameshift
8	*ARID1A*	chr1	27023685	C	A	41%	Nonsense_S264X
8	*ARID1A*	chr1	27101212	G	A	47%	Nonsense_W1498X
8	*SMARCA4*	chr19	11097624	G	GC	41%	Frameshift
8	*SMARCA4*	chr19	11121199	C	T	42%	Nonsense_Q756X
9	*ARID1A*	chr1	27024001	CG	C	58%	Frameshift
9	*SMARCA4*	chr19	11098416	TC	T	40%	Frameshift
9	*SMARCA4*	chr19	11113789	GC	G	35%	Frameshift
10	*SMARCA4*	chr19	11094889	GC	G	54%	Frameshift

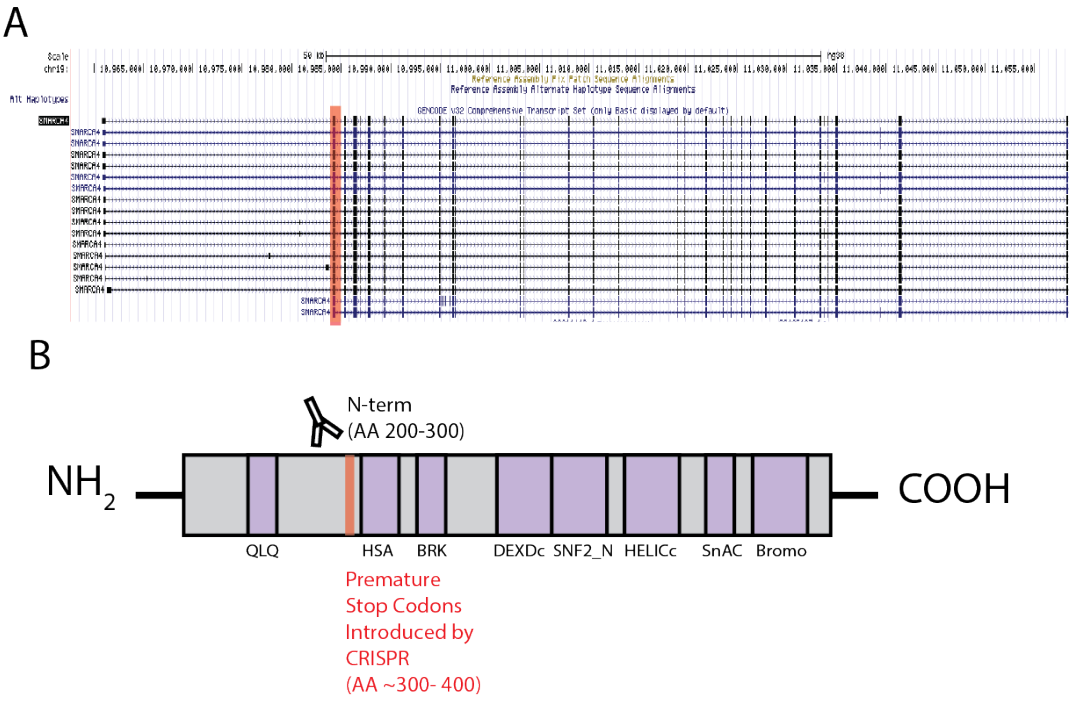
10	*SMARCA4*	chr19	11114037	TG	T	35%	Frameshift
11	*ARID1A*	chr1	27059175	TC	T	18%	Frameshift
11	*SMARCA4*	chr19	11101885	TG	T	44%	Frameshift
12	*ARID1A*	chr1	27106504	C	T	33%	Nonsense_Q1822X
12	*SMARCA4*	chr19	11121150	TGA	T	50%	Frameshift
13	*ARID1A*	chr1	27024001	C	CG	20%	Frameshift
13	*SMARCA4*	chr19	11097624	GC	G	27%	Frameshift
13	*SMARCA4*	chr19	11132493	AC	A	26%	Frameshift
14	*ARID1A*	chr1	27023768	A	AG	60%	Frameshift
14	*ARID1A*	chr1	27023774	A	AC	65%	Frameshift
14	*SMARCA4*	chr19	11107046	AG	A	50%	Frameshift
15	*No mutations identified*						
1	*SMARCB1*	chr22	24134006	C	T	84%	Nonsense_R53X
2	*ARID1A*	chr1	27099098	C	T	43%	Nonsense_Q1172X
2	*SMARCB1*	chr22	24133967	C	T	44%	Nonsense_R40X
2	*SMARCB1*	chr22	24145582	C	T	44%	Nonsense_R192X
3	*ARID1A*	chr1	27024001	C	CG	22%	Frameshift
3	*ARID1A*	chr1	27105930	TG	T	15%	Frameshift
3	*SMARCB1*	chr22	24135858	GC	G	24%	Frameshift
1	*ARID1A*	chr1	27105550	C	T	42%	Nonsense_R1721X
1	*ARID1A*	chr1	27105930	T	TG	35%	Frameshift
1	*ARID1B*	chr6	157469997	A	AT	39%	Frameshift
1	*ARID1B*	chr6	157528283	AG	A	55%	Frameshift
2	*ARID1A*	chr1	27058048	C	T	52%	Nonsense_Q586X
2	*ARID1A*	chr1	27089665	A	AT	46%	Frameshift
2	*ARID1B*	chr6	157519961	C	T	45%	Nonsense_Q1344X
3	*ARID1A*	chr1	27057919	C	T	21%	Nonsense_Q543X
3	*ARID1A*	chr1	27100183	C	T	49%	Nonsense_Q1327X

3	*ARID1B*	chr6	157522101	AG	A	42%	Frameshift
3	*ARID1B*	chr6	157528051	C	T	56%	Nonsense_R1926X
4	*ARID1A*	chr1	27089484	AACTATAAT	A	65%	Frameshift
4	*ARID1A*	chr1	27101054	C	T	49%	Nonsense_R1446X
4	*ARID1B*	chr6	157405953	CG	C	37%	Frameshift
4	*ARID1B*	chr6	157517382	G	T	48%	Nonsense_G1316X
4	*SMARCC2*	chr12	56559112	AG	A	50%	Frameshift
5	*ARID1A*	chr1	27100151	CG	C	23%	Frameshift
5	*ARID1A*	chr1	27105930	TG	T	24%	Frameshift
5	*ARID1B*	chr6	157405953	C	CG	34%	Frameshift
6	*ARID1A*	chr1	27097621	CA	C	30%	Frameshift
6	*ARID1A*	chr1	27099393	G	GTA	35%	Frameshift
6	*ARID1B*	chr6	157100576	AC	A	34%	Frameshift
7	*ARID1A*	chr1	27097621	C	CA	34%	Frameshift
7	*ARID1B*	chr6	157527355	AT	A	54%	Frameshift
8	*ARID1A*	chr1	27057919	C	T	43%	Nonsense_Q543X
8	*ARID1B*	chr6	157528243	C	T	45%	Nonsense_R1990X
8	*ARID1B*	chr6	157528283	AG	A	53%	Frameshift
9	*ARID1A*	chr1	27105964	TTC	T	32%	Frameshift
9	*ARID1B*	chr6	157522598	C	T	20%	Nonsense_R1624X
9	*ARID1B*	chr6	157528283	AG	A	20%	Frameshift
10	*ARID1A*	chr1	27105519	AG	A	42%	Frameshift
10	*ARID1A*	chr1	27105693	TCTAGGTCCTAAAC	T	56%	Frameshift
10	*ARID1B*	chr6	157256609	C	T	72%	Nonsense_Q646X
11	*ARID1A*	chr1	27057934	C	T	78%	Nonsense_Q548X
12	*ARID1A*	chr1	27105541	G	T	42%	Nonsense_E1718X
12	*ARID1A*	chr1	27106354	C	T	48%	Nonsense_R1989X
13	*ARID1A*	chr1	27106354	C	T	44%	Nonsense_R1989X

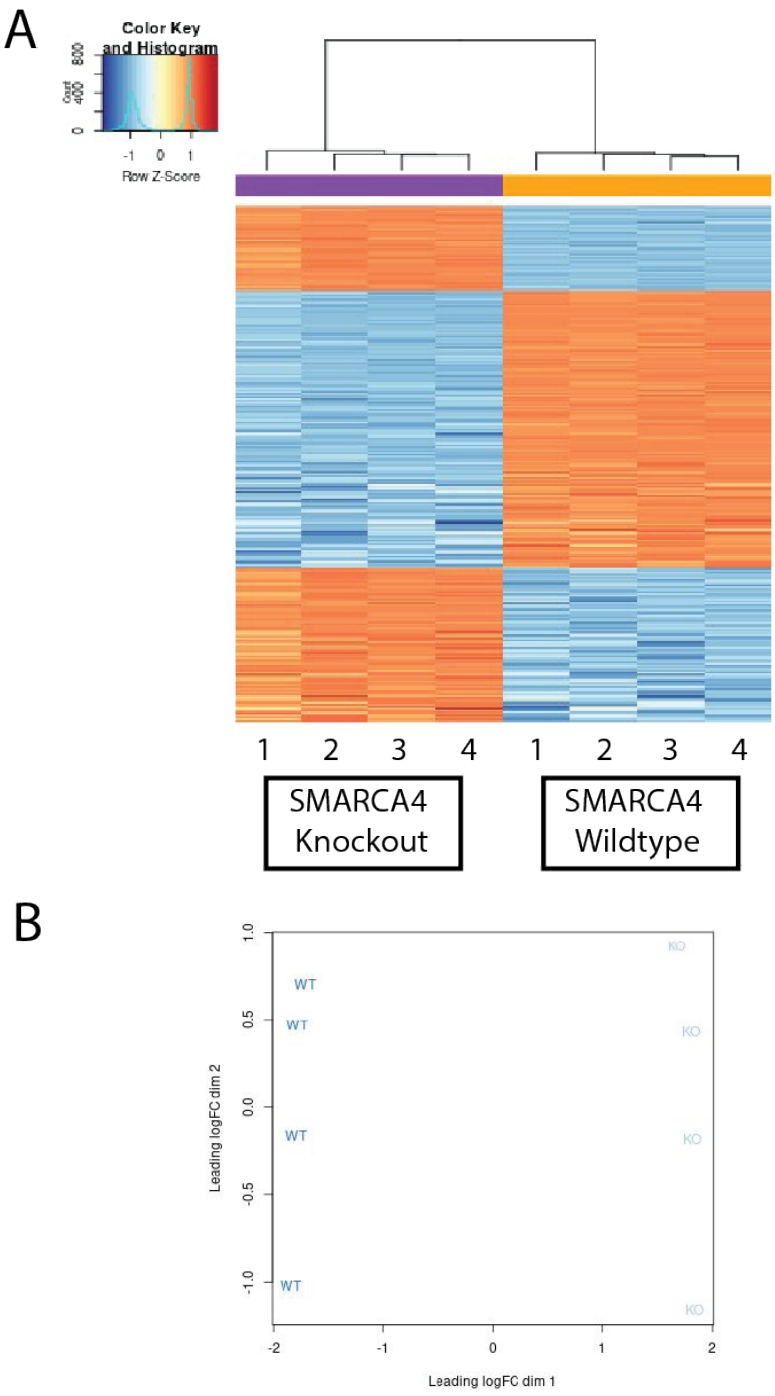
14	*No mutations identified*						
15	*No mutations identified*						
16	*No mutations identified*						
17	*ARID1A*	chr1	27087961	C	T	44%	Nonsense_R750X
18	*No mutations identified*						
19	*No mutations identified*						
20	*No mutations identified*						
21	*ARID1A*	chr1	27100175	AC	A	25%	Frameshift
22	*No mutations identified*						



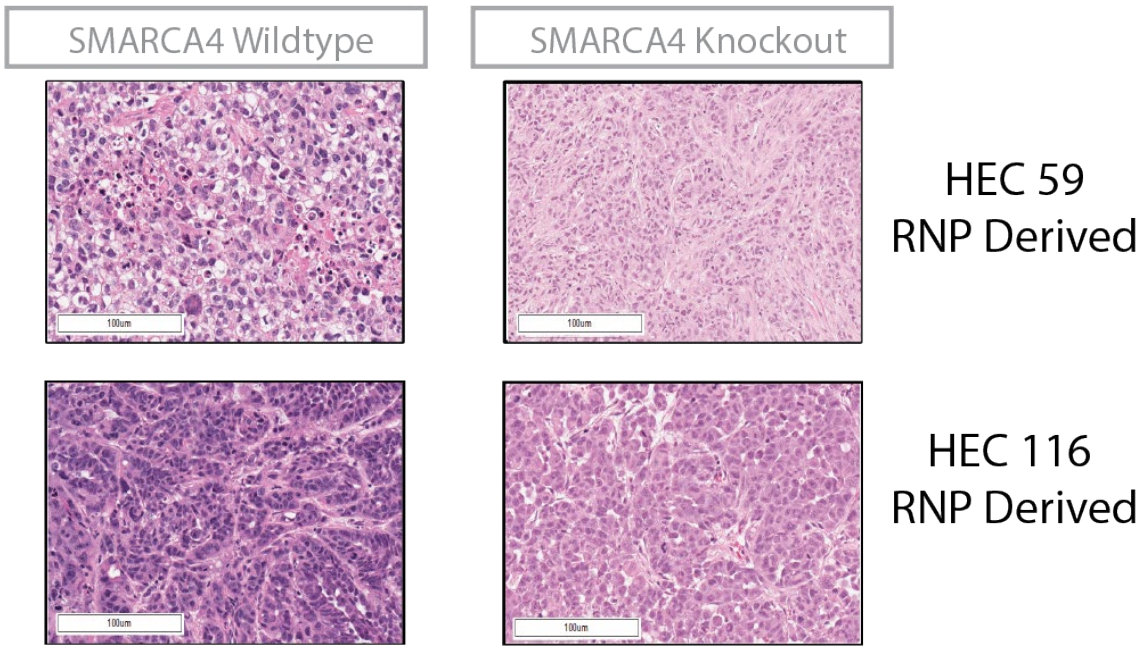
Supplemental Figure 3.1. Activation of CREB, AKT, ERK and AMPKα1 family kinases in mismatch repair protein deficient endometrial cancer cell lines HEC 116 and HEC 59. Quantitative analysis of phosphokinase antibody array blots using lysates of serum starved HEC 116 and HEC 59 EC cells. Data consists of two technical replicates. Activated proteins in these EC cell lines included **A)** CREB and AMPKα1 **B and C)** AKT and ERK related signaling molecules.



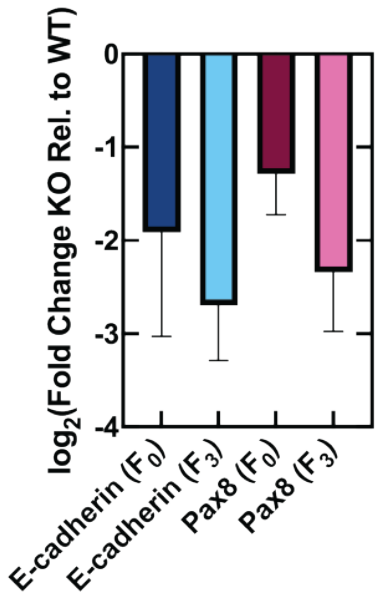
Supplemental Figure 3.2. Known isoforms and protein domains of SMARCA4. A) Track from the UCSC genome browser highlighting the numerous SMARCA4 transcript isoforms. gRNAs for CRISPR gene editing were designed within the exon highlighted red. **B)** Domain structure of SMARCA4. QLQ: Gln, Leu, Gln motif; HAS: helicase/SANT-associated domain; BRK: Brahma and Kismet domain; DEXDc: DEAD-like helicase superfamily domain; SNF2_N: SNF2 family N-terminal domain; HELICc: helicase superfamily C-terminal domain; SnAC: Snf2-ATP coupling, chromatin remodeling complex; Bromo: bromodomain. Within the N-terminus, the SMARCA4 antibody binding site and area wherein CRISPR gene editing introduced premature stop codons are indicated. Adapted from (25).



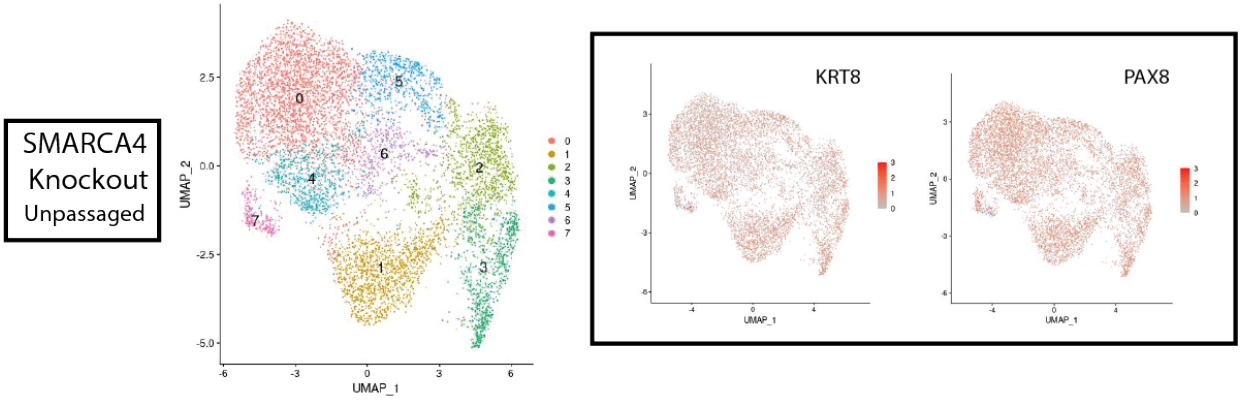
Supplemental Figure 3.3. Multiple visualizations of bulk gene expression data demonstrating grouping of samples based on SMARCA4 status. A) Heatmap and **B)** Multidimensional scaling (MDS) plot showing SMARCA4 KO and SMARCA4 WT biological replicates group together based on the similarity of their gene expression patterns.



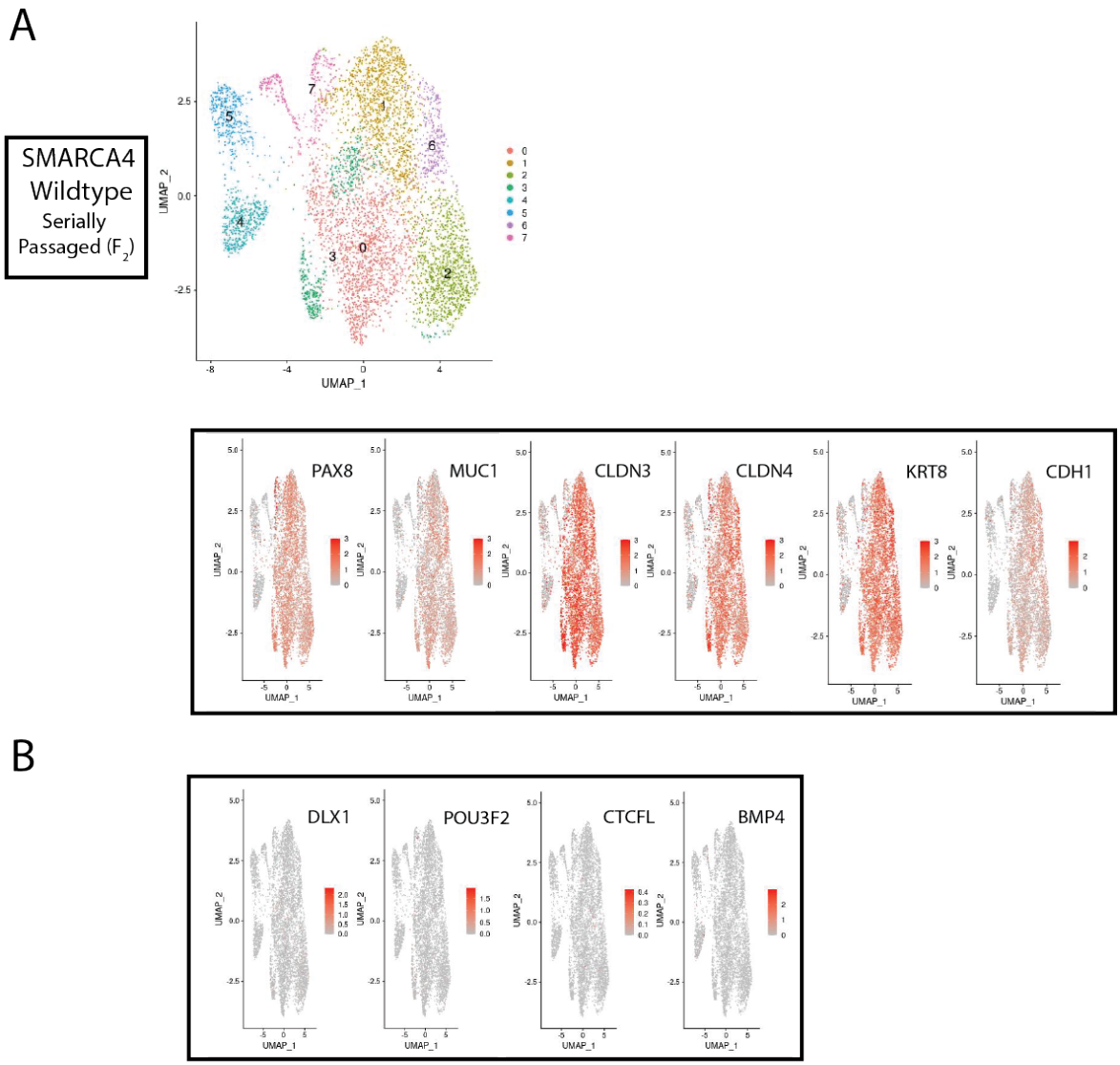
Supplemental Figure 3.4. Tumors formed from endometrial cancers lacking SMARCA4 by RNP based CRISPR gene editing lack noticeable dedifferentiation. H&E stained SMARCA4 KO EC tumors showing few remarkable features of well differentiated or undifferentiated endometrial carcinoma. Scale bar = 100 μM.



Supplemental Figure 3.5. HEC 116 EC cells lacking SMARCA4 expression exhibit a further reduction in gene expression of markers of epithelial cells and high-grade endometrial carcinoma. Quantitative real time PCR analysis of HEC 116 Plasmid Derived EC cells for E-cadherin and Pax8. Three SMARCA4 KO biological replicates were normalized to paired WT samples for each generation of serial passaging (F_0 and F_3) and represented as log2 fold changes.



Supplemental Figure 3.6. *In vitro* SMARCA4 deficient EC cells retain expression of markers of gynecological epithelial differentiation. UMAP plots depicting gene expression patterns for epithelial associated genes: *KRT8* and *PAX8* in SMARCA4 KO cells not subjected to *in vivo* selective pressure.



Supplemental Figure 3.7. *In vivo* SMARCA4 intact EC cells are characterized by gene expression programs associated with endometrial cellular dedifferentiation but no population of serially passaged SMARCA4 wildtype cells emerged displaying characteristics associated with phenotype switching. A) UMAP plots depicting gene expression patterns for select makers of undifferentiated regions in clinical DDEC tumors: *CLDN3, CLDN4, PAX8, MUC1, CDH1* and *KRT8* in SMARCA4 WT cells subjected to *in vivo* selective pressure. **B)** UMAP plots depicting gene expression patterns for select makers of phenotype switching: *CTCFL, DLX1, POU3F2,* and *BMP4* in SMARCA4 WT cells subjected to *in vivo* selective pressure.

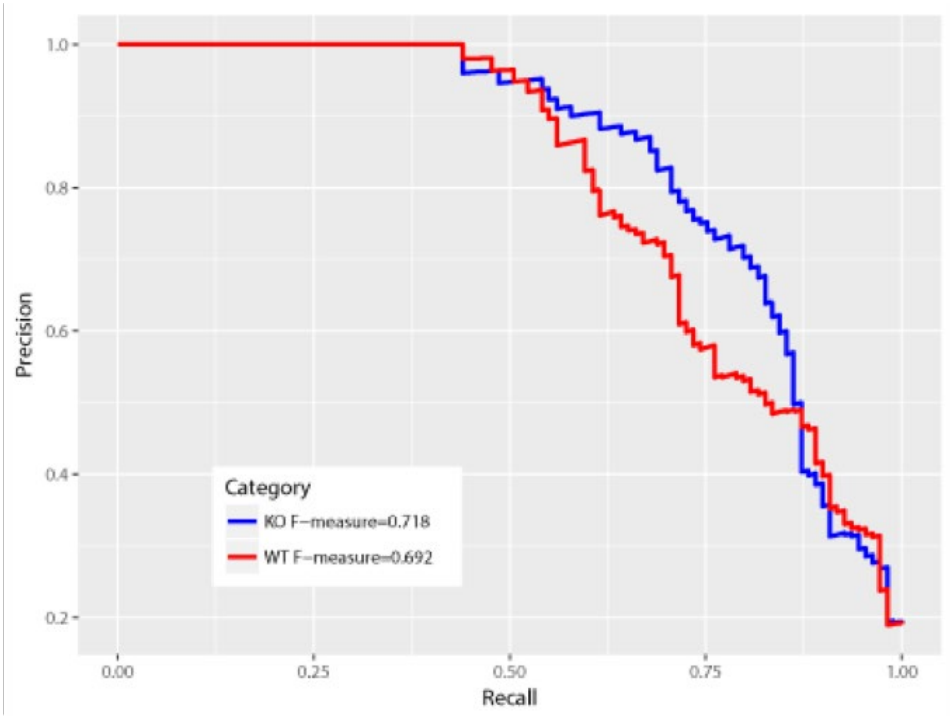
Supplemental Table 4.1. IC_{50} values determined for carboplatin against the various cell-line models of DDEC. A) HEC 116 Plasmid and RNP Derived SMARCA4 KO and WT EC cells in addition to the HEC 59 RNP Derived isogenic pair. **B)** HEC 116 Plasmid Derived serially passaged EC tumor cells cultured upon dissociation.

A

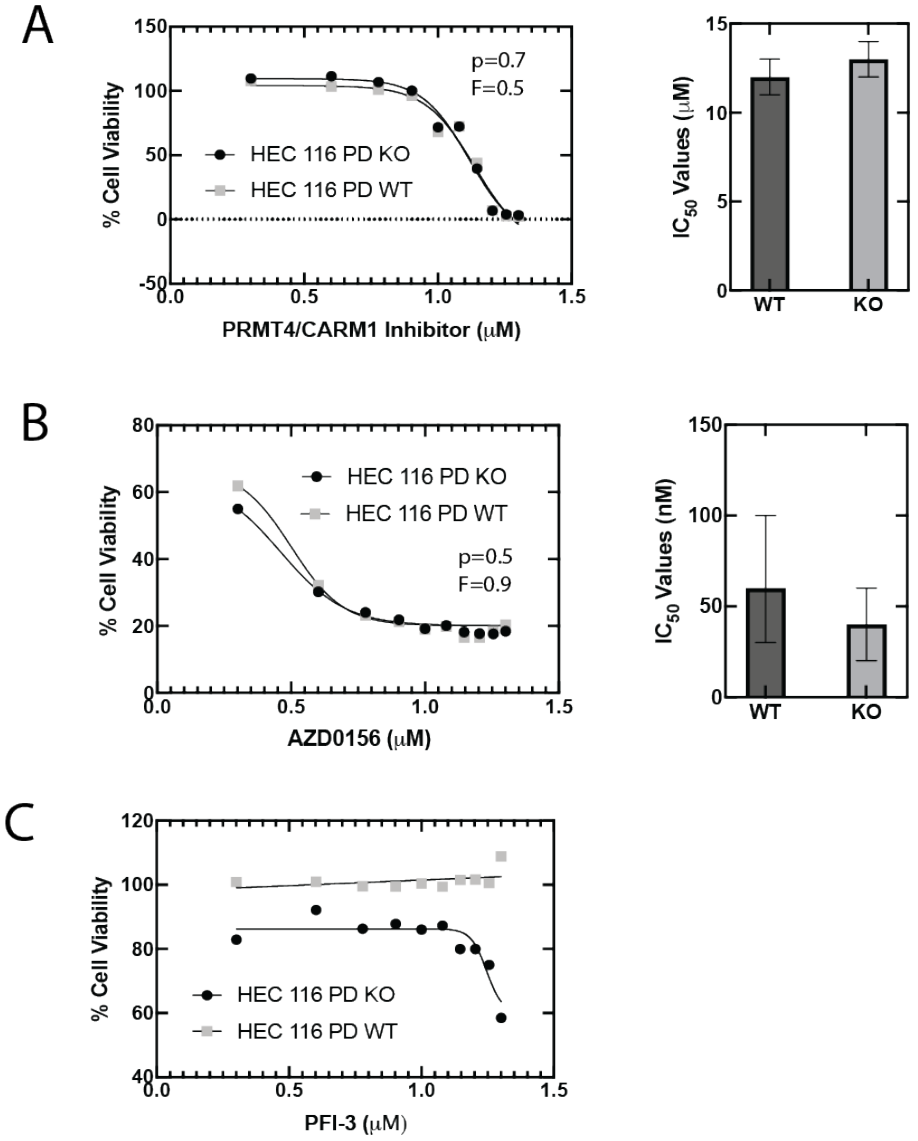
	Carboplatin **IC50 (μM)**
HEC 116 Plasmid Derived SMARCA4 Wildtype	11 (10-13)
HEC 116 Plasmid Derived SMARCA4 Knockout	11 (8-13)
HEC 116 RNP Derived SMARCA4 Wildtype	19 (18-19)
HEC 116 RNP Derived SMARCA4 Knockout	12 (10-14)
HEC 59 RNP Derived SMARCA4 Wildtype	11 (9-14)
HEC 59 RNP Derived SMARCA4 Knockout	9 (1-13)

B

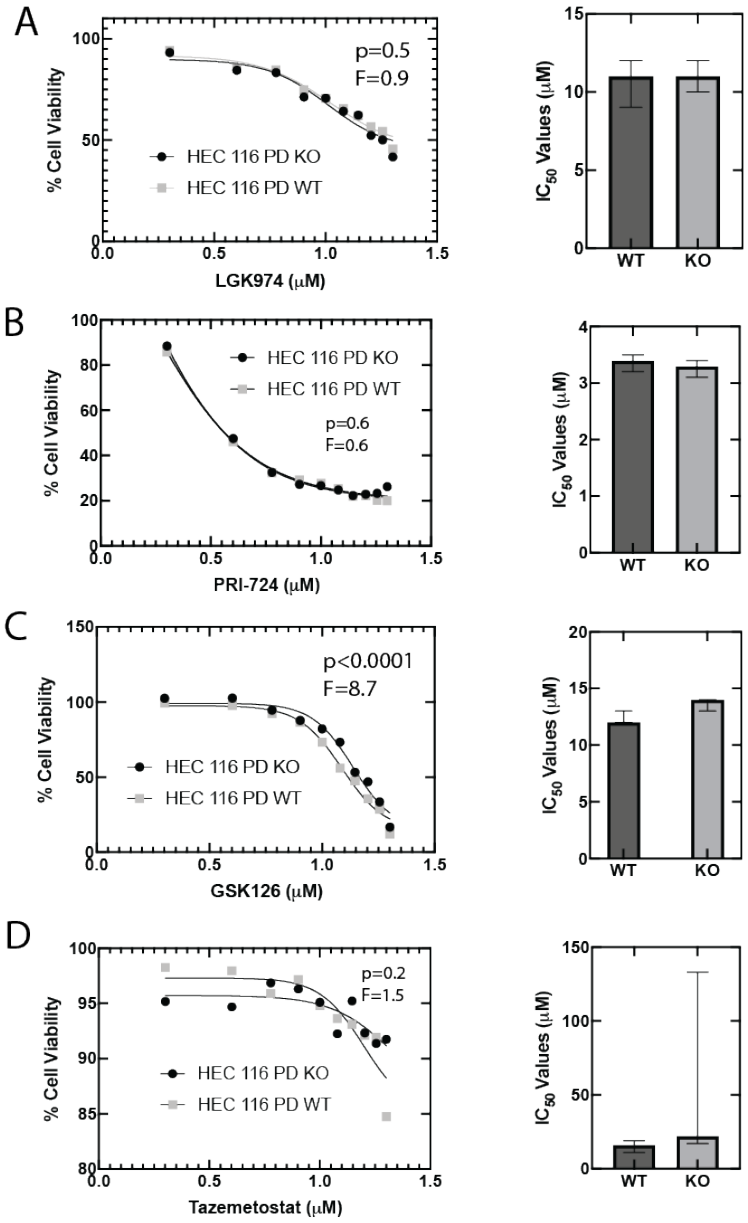
	Carboplatin **IC50 (μM)**
HEC 116 Plasmid Derived SMARCA4 Wildtype F3 Cell Derived Xenograft Tumor Cells	13 (13-14)
HEC 116 Plasmid Derived SMARCA4 Knockout F3 Cell Derived Xenograft Tumor Cells	9 (8-10)



Supplemental Figure 4.1. Precision recall curve measuring the core essential and non-essential genes from the pooled shRNA screen.



Supplemental Figure 4.2. Chemical inhibitors against synthetic lethality shRNA screen hits that were incapable of preferential suppression of SMARCA4 deficient HEC 116 Plasmid Derived EC cells. Cell viability assays with HEC 116 Plasmid Derived EC cell lines. SMARCA4 KO and WT cell lines were treated with increasing concentrations of **A)** CARM1 **B)** ATM **C)** SMARCA2 inhibitors (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.



Supplemental Figure 4.3. Additional chemical inhibitors against synthetic lethality shRNA screen hits that were incapable of preferential suppression of SMARCA4 deficient HEC 116 Plasmid Derived EC cells. Cell viability assays with HEC 116 Plasmid Derived EC cell lines. SMARCA4 KO and WT cell lines were treated with increasing concentrations of **A)** & **B)** β-catenin **C) & D)** EZH2 inhibitors (0 to 20 μM) for 72 hours prior to detection of luminescence (560 nm) with a microplate reader and Cell-Titer Glo reagents. Data consists of four technical replicates from three independent biological replicates. IC_{50} values with 95% confidence intervals were determined by fitting data with non-linear regression curve fits in GraphPad Prism. Statistical difference between the dose response curves was also calculated in GraphPad Prism using the extra sum-of-squares F test.

Supplemental Table 4.2. Functional enrichment analyses supporting HEC 116 EC dependency on A) CDK4 and B) EGFR signalling pathways in the absence of SMARCA4.

A

Molecular Function Pathway Enrichment	adj.Pval	nGenes
Phosphotransferase activity, alcohol group as acceptor	6.70E-13	73
Protein kinase activity	7.20E-13	66
Structural constituent of ribosome	3.20E-12	33
Kinase activity	6.70E-12	76
Transferase activity, transferring phosphorus-containing groups	3.50E-11	83
RNA binding	4.40E-11	120
Protein serine/threonine kinase activity	1.50E-10	50
Nucleotide binding	3.90E-10	140
Adenyl nucleotide binding	7.50E-10	111
ATP binding	1.30E-09	107
Purine nucleotide binding	1.30E-09	127
Small molecule binding	1.30E-09	155
Purine ribonucleoside triphosphate binding	3.40E-09	122
Adenyl ribonucleotide binding	3.50E-09	108

B

KEGG Pathway Enrichment	adj.Pval	nGenes
Ribosome	9.10E-12	27
Proteasome	1.40E-04	10
Chronic myeloid leukemia	1.40E-04	13
Pathways in cancer	1.80E-04	39
ErbB signaling pathway	2.10E-04	13
RNA transport	2.40E-04	18
Spliceosome	2.40E-04	16
FoxO signaling pathway	2.40E-04	16
TGF-beta signaling pathway	2.40E-04	13
Axon guidance	2.40E-04	19
Epstein-Barr virus infection	2.60E-04	20
Proteoglycans in cancer	7.10E-04	19
Human T-cell leukemia virus 1 infection	7.80E-04	20
Fluid shear stress and atherosclerosis	9.60E-04	15
Endocrine resistance	1.10E-03	12

Supplemental Table 4.3. IC_{50} values determined for CDK4 inhibitors and EGFR inhibitors against the various cell-line models of DDEC. A) HEC 116 Plasmid and **B)** RNP Derived SMARCA4 KO and WT EC cells in addition to **C)** the HEC 59 RNP Derived isogenic pair.

A

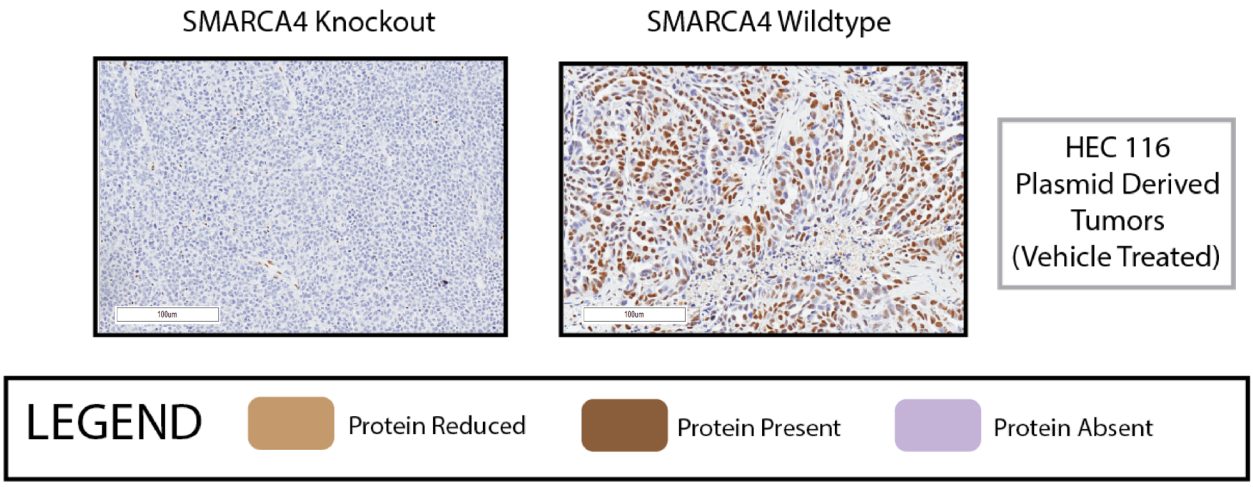
	HEC 116 Plasmid Derived SMARCA4 Knockout	HEC 116 Plasmid Derived SMARCA4 Wildtype
CDK4 Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Palbociclib	4 (3-5)	8 (7-8)
Ribociclib	6 (3-8)	15 (12-16)
EGFR Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Erlotinib	4 (3-5)	8 (7-10)
Gefitinib	10 (9-11)	15 (15-16)

B

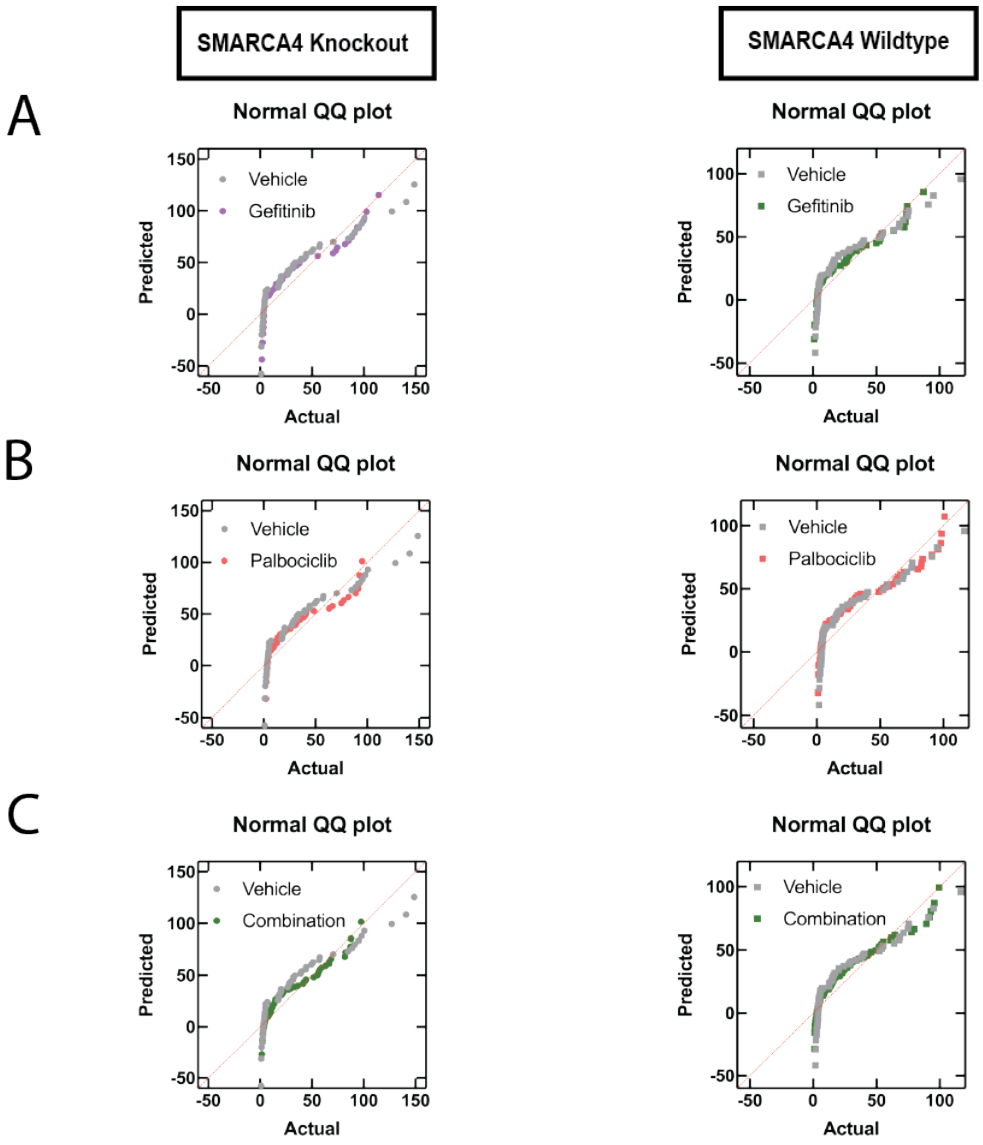
	HEC 116 RNP Derived SMARCA4 Knockout	HEC 116 RNP Derived SMARCA4 Wildtype
CDK4 Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Palbociclib	3 (1-5)	4 (2-6)
Ribociclib	11 (10-13)	16 (14-19)
EGFR Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Erlotinib	7 (5-8)	9 (8-10)
Gefitinib	9 (8-10)	10 (9-11)

C

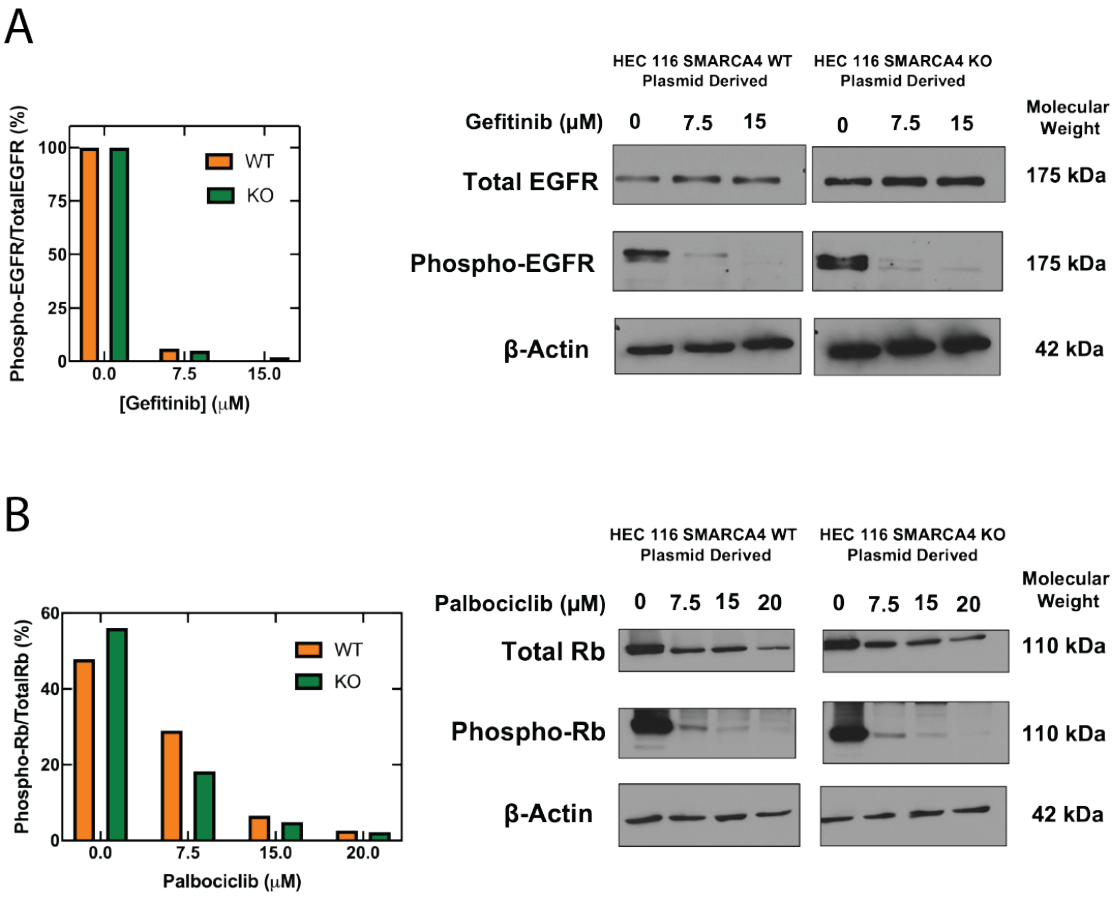
	HEC 59 RNP Derived SMARCA4 Knockout	HEC 59 RNP Derived SMARCA4 Wildtype
CDK4 Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Palbociclib	10 (9-11)	11 (10-13)
Ribociclib	16 (15-17)	7 (5-9)
EGFR Inhibitors	**IC50 (μM)**	**IC50 (μM)**
Erlotinib	5 (4-6)	10 (7-12)
Gefitinib	11 (10-11)	14 (14-15)



Supplemental Figure 4.4. Immunohistochemical verification of the SMARCA4 status of the cell line derived DDEC CDX sections utilized in preclinical animal studies. Scale bar = 100 μM.



Supplemental Figure 4.5. QQ-plots for assessing the distributions of palbociclib, gefinitib and combination treated tumor growth measurements. QQ-plots showing the quantiles expected from a normal distribution with the same mean and variance as the empirical distribution as a function of the empirically observed quantiles associated with tumor growth measurements.



Supplemental Figure 4.6. ***In vitro*** **administration of gefitinib and palbociclib to the HEC 116 Plasmid derived cell line model of DDEC results in less activation of EGFR and Rb, respectively. A)** Increasing concentrations of gefitinib led to reduced activation of EGFR when applied to adherent SMARCA4 WT and SMARCA4 KO EC cells. **B)** Drug treatment with palbociclib led to less Rb activation within cells not serially passaged regardless of their SMARCA4 status. Western blot represents a single experiment with β-Actin acting as a loading control. Densitometry allowed for the obtainment of ratios of band intensity relative to untreated samples of same SMARCA4 status.