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Initial differences in lipid processing leading to pig- and beef-derived mature adipocyte dedifferentiation

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Abstract

Clonal cultures of pig-derived mature adipocytes are capable of dedifferentiating and forming proliferative-competent progeny cells in vitro. Initial lipid processing, is different to that observed in cultures of beef-derived adipocytes. Mature pig adipocytes extrude lipid before proliferation, whereas beef-derived adipocytes symmetrically, or asymmetrically, divide without expelling lipid. These observations suggest that dedifferentiation of mature adipocytes relies on species-specific mechanisms, or that different culture conditions are required for pig-derived adipocytes to dedifferentiate in a manner similar to beef adipocytes. This in vitro system will aid in our understanding of lipid metabolism, regulation of single cells, processes involved in dedifferentiation of cells, and/or characteristics of putative stem cells residing in adipose tissue.
Key Words: adipocyte; dedifferentiation; lipid extrusion; pig; beef

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Numerous reports exist to demonstrate that populations of mature, lipid-filled adipocytes may revert to form proliferative-competent progeny cells [1,5,6,14,17,18]. A primary use of populations of these cells is in clinical applications leading to tissue regeneration or engineering [11]. However, this in vitro system may provide much more information about cell dedifferentiation (especially at the single cell level), lipid processing, and the potential of progeny cells as stem cells [3,4,8,11,12]. As such, our research involves clonal cultures of mature adipocytes, without any fibroblast-like cell contaminants [8].

We have previously shown that purified cultures of beef-derived mature (lipid-filled) adipocytes from numerous adipose depots (including the intramuscular depot) possess ability to dedifferentiate and form proliferative-competent progeny cells [3,4, 6-9, 12]. Dedifferentiation of beef-derived adipocytes, in a purified cell culture system, was shown to produce daughter cells which possessed cellular lipid in varying proportions (asymmetric or symmetric cell division) [6-8]. In all cases, however, the original content of cytosolic lipid was maintained or reserved by one or both of the daughter cells [7,8]. Subsequently, the progeny produced by this process re-differentiated into lipid-assimilating adipocytes, but in a manner different to that of available adipogenic cell lines [10,12] or to the stromal vascular cell fraction commonly used for adipogenesis studies [6]. As such, this system appears to be quite attractive for use in elucidating the regulation [5,10] of individual adipocyte dedifferentiation and redifferentiation of resultant progeny cells. Indeed, Taniguchi et al [15,16] demonstrated that progeny adipofibroblasts expressed a slightly different gene pattern during differentiation to form lipid-accumulating adipocytes in vitro than did other adipogenic cell models. For the present project, we tested the hypothesis that mature, lipid-filled, pig-derived adipocytes dedifferentiated in a manner similar to cells in the beef-derived system we had previously established.

Materials and Methods

Mature (lipid-filled) pig-derived adipocytes were obtained from the perirenal adipose depot during routine slaughter at the Washington State University (WSU) meats laboratory. The WSU Animal Care and Use Committee screened the use of animals in this research, and the animal use met the standards imposed by both the United Stated Department of Agriculture and Public Health Service. The method of initial isolation and cell culture of pig mature adipocytes was as described previously by Fernyhough et al. [8] with some modifications [2]. The isolated mature adipocytes were purified early on by using serial differential plating methods to prevent any contamination of mature adipocyte cultures by fibroblast-like cells [8]. In this procedure, mature adipocytes were transferred to a 12.5 cm2 cell culture flask which was filled completely with a 1:1 mix of DMEM/F12 + 10% FBS. The flask was inverted and incubated at 37 ° C in a 5% CO2 incubator. After 24 h, the loosely attached mature adipocytes were aspirated away from any potential fibroblast contaminants (held strongly to the cultureware) and transferred to a new ceiling culture. After another 24 h, the process was repeated, and the

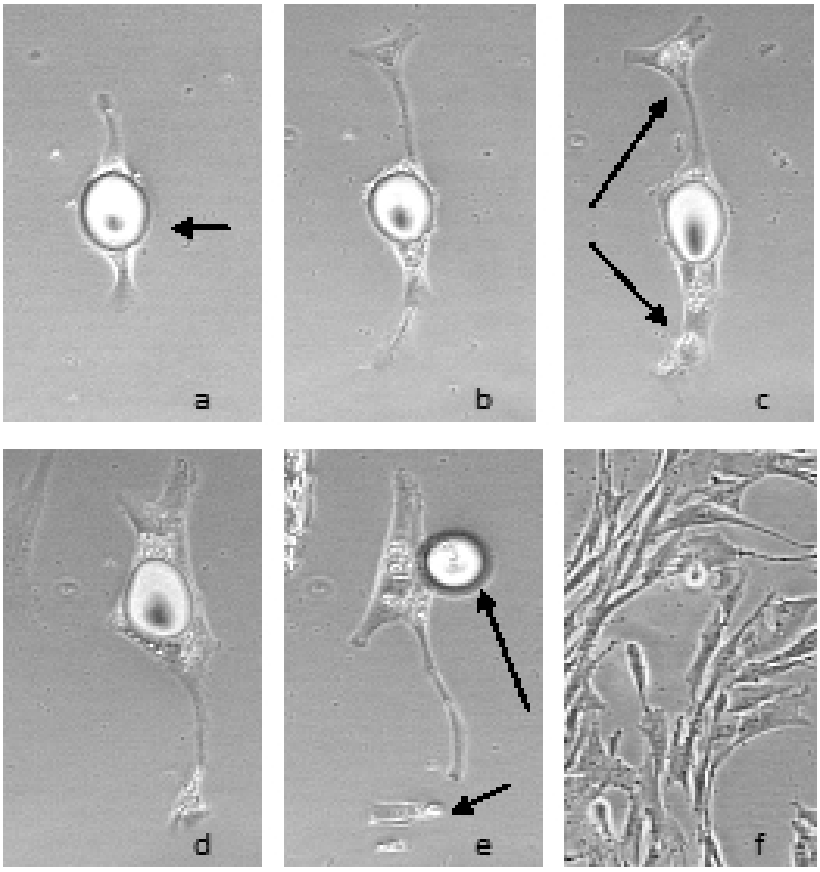

Fig. 1 *Dedifferentiation of pig-derived adipocytes in vitro. By applying the serial differential plating procedures after the isolation of the mature adipocytes, we eliminated any potential contamination of fibroblast-like cells. (a) By d 3 mature (lipid-filled) adipocytes attached firmly to the ceiling surface and displayed a characteristic "football-like" morphology. (b-d) The cells began to increase their cytosolic volume for the next few d in vitro. (c-e) During this time, numerous fat droplets were observed moving from the central part to the distal (terminal) end of the cell. Note the one large droplet was extruded directly from the cell (e). This mobilization process, could last ~1 wk before most of the lipid droplet migrated/was extruded. (d, e) The terminal end of the cell eventually formed a storage site for smaller lipid droplets. After much of the smaller lipid droplets moved to the distal end, the central portion of the cell separated from this storage site (e; bottom arrow). (f) Progeny cells eventually began to assume the appearance of fibroblasts or preadipocytes. All photographs were captured at 20× magnification with a NIKON inverted Diaphot microscope, equipped with Sony RGB (0.6 in chip) camera and OPTIX image analysis system.*

flask was thoroughly inspected for any contaminating (fibroblast or preadipocyte) cells. This differential plating method was repeated one more time to insure that only mature adipocytes were present in the cell culture. These (pure) ceiling cultures were left undisturbed for 3 days. This allowed the lipid-laden adipocytes to attach firmly, after which the flask was re-inverted to a normal position. Clonal mature adipocytes were marked using a fine-tip pen on the bottom of the flask, and photomicrographs of cell transitions were then captured for these marked adipocytes during dedifferentiation.

Results and Discussion

In this study, the serial morphological change from a lipid-laden (pig) mature adipocyte to dedifferentiated cells is presented in Fig. 1. The original portion of the cell that contained the nucleus (being depleted of substantial lipid) started to proliferate by 48 h after riding itself of the lipid storage site. It should be noted that the prevalence of this sequence of cellular events, in vitro, does not appear to be a random event. Also, for each tissue preparation and cell isolation sufficient cell clones may be generated in order to design studies for definition of the regulation of this entire scenario at the single cell level. What makes this observation interesting to us is that the extrusion of lipid from mature pig-derived adipocytes prior to proliferation does not seem to be required in other cell systems. Beef-derived adipocytes divide both symmetrically, or asymmetrically, into daughter cells without expelling cellular lipid [6,7].

Based upon these observations with two different adipocyte dedifferentiation systems, it is likely that mature adipocyte dedifferentiation depends on a species-specific mechanism, or that different culture conditions may be required for mature adipocytes from different animal species to respond similarly.

In the present case, pig-derived mature adipocytes must expel lipid from the cell prior to proliferation, whereas beef-derived adipocytes do not need to alter lipid levels prior to cell division.

A generally accepted concept of adipogenesis is that once the cells have accumulated intracellular lipid, the adipocytes are “terminally differentiated” [6]. This study, as well as our previous communications [3-13]; suggest otherwise. That is—the accumulation of lipid may not be the terminal differentiation event for adipocytes. Other alternative explanations have been offered [3,4,6-9].

The mechanism underlying the regulation of the complete process(es) of adipogenesis is of considerable interest. Our study presented a specific pattern of lipid processing during the dedifferentiation of individual mature adipocytes in pigs, which was different from that observed in similarly cultured cells derived from beef cattle. Further study of the mechanism underlying this process will provide a new understanding of adipogenesis. Future biochemical and molecular comparisons of the dynamics of lipid processing in both cell systems will reveal much information about the process of adipocyte dedifferentiation.

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References

[1] Cancello R, Pietri-Rouxel F, Clement K: Adipocytes 2005; 1: 73-78.
[2] Chen J, Guridi M, Fernyhough ME, Jiang Z, Guan LL, Hausman GJ, Dodson MV: Inter J Stem Cells 2009; 2: 76-79.
[3] Dodson MV, Fernyhough ME, Vierck JL, Hausman GJ: Anim Sci 2005; 80: 239-240.
[4] Dodson MV, Fernyhough ME: Tissue Cell 2008; 40: 307-308.
[5] Hausman GJ, Dodson MV, Ajuwon K, Azain M, Barnes KM, Guan LL, Jiang Z, Poulos SP, Sainz RD, Smith S, Spurlock M, Novakofski J, Fernyhough ME, Bergen WG: J Anim Sci 2009; 87: 1218-1246.
[6] Fernyhough ME, Helterline DI, Vierck JL, Hausman GJ, Hill RA, Dodson MV: Adipocytes 2005; 1: 17-24.
[7] Fernyhough ME, Bucci L, Hausman GJ, Antonio J, Vierck JL, Dodson MV: Tissue Cell 2005; 37: 335-338.
[8] Fernyhough ME, Vierck JL, Hausman GJ, Mir PS, Okine E, Dodson MV: Cytotechnology 2005; 46: 163-172.
[9] Fernyhough ME, Vierck JL, Dodson MV: Cells Tissues Organs 2006; 182: 226-228
[10] Fernyhough ME, Okine E, Hausman GJ, Vierck JL, Dodson MV: Dom Anim Endocrinol 2007; 33: 367-378.
[11] Fernyhough ME, Hausman GJ, Guan LL, Okine E, Moore SS, Dodson MV: Biochem Biophysl Res Commun 2008; 368: 455-457.
[12] Fernyhough ME, Hausman GJ, Dodson MV: Cells Tissues Organs 2008; 188: 359-372.

[13] Kokta T, Dodson MV, Gertler A, Hill RA: Dom Anim Endocrinol 2004; 27: 303-331.
[14] Matsumoto T, Kano K, Kondo D, Fukuda N, Iribe Y, Tanaka N, Matsubara Y, Sakuma T, Satomi A, Otaki M, Ryu J, Mugishima H: J Cell Physiol 2007; 215: 212-222.
[15] Taniguchi M, Guan LL, Zhang B, Dodson MV, Okine E, Moore SS: Biochem Biophysl Res Commun 2008; 366: 54-59.
[16] Taniguchi M, Guan LL, Zhang B, Dodson MV, Okine E, Moore SS: Biochem Biophys Res Commun 2008; 366: 346-351.
[17] Tholpady SS, Aojanepong C, Llull R, Jeong JH, Mason AC, Futrell JW, Ogle RC, Katz AJ: Ann Plast Surg 2005; 54: 651-656.
[18] Zhang HH, Kumar S, Barnett AH, Eggo MC: J Endocrinol 2000. 164: 119-128.