SPECIES-SPECIFIC DIFFERENCE IN INDUCING PIG ADIPOSE CONVERSION FROM THAT OF MOUSE

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Background

Subcutaneous adipose tissue in pigs represents a major source of both cost inefficiency and consumer concerns. Numerous investigations have been carried out to regulate the carcass fat for the meat quality traits through both nutritional controls and selective breeding. However, there are some difficulties to monitor adipose development in vivo studies, due to the complicated interactions of all kinds of neural and hormonal signals involved in adipocyte metabolism. Therefore, investigators have established in vitro cell culture systems as useful tools for studying adipose development.

Much of the knowledge about cellular and molecular events accompanying adipose conversion in recent years has been based on murine preadipocyte cell lines. Since no cell lines from porcine adipose tissue have been established so far, understanding the control of adipocyte differentiation in pigs is limited in either the approach of using these mouse cell lines or otherwise, primary culture systems. As a result, it can always come into question whether the data from mouse cell lines and pig primary stromal-vascular cells with cellular heterogeneity in its population, can represent the characteristics of pig adipocytes.

Objectives

We have established a preadipocyte clonal line from porcine subcutaneous tissue (PSPA) for the study of pig adipose development (Nakajima et al., 2003). The present study was designed to demonstrate whether there are species specificity in adipose conversion between mouse and pigs, by comparing the most widely studied mouse 3T3-L1 cell line and PSPA cells.

Materials and methods

Cell culture: A clonal porcine subcutaneous preadipocyte (PSPA) cell line was maintained in the preadipocyte condition by cultivation in Dulbecco's modified Eagle's medium (DMEM, 1 g/L glucose) supplemented with 10% fetal bovine serum, 1000 IU/ml penicillin, and 1 mg/ml streptomycin. Cells were passaged every 4 days, with the density of inoculation kept constant (1×10^4cells/cm^2).

For experiments, cells were plated at 2.1×10^4cells/cm^2 to obtain confluency within 3 days. After reaching confluence (day 0), adipose conversion was induced in high-glucose (4.5 g/L) DMEM containing 10% FBS, 5 μg/ml insulin, 0.25 μM dexamethasone, 33 μM biotin, 17 μM pantothenate, 5 mM octanoate. The medium was changed every other day and the cells were allowed to differentiate for 10 more days.

The mouse embryo 3T3-L1 cells were plated at a density of 0.5×10^4cells/cm^2 in growth medium for 3 days. At confluence, the medium was then shifted to high-glucose DMEM supplemented with 10% FBS, 0.5 mM of 1-methyl-3-isobutylxanthine (MIX), and 0.25 μM dexamethasone, to induce differentiation. Forty-eight hours later, this medium was replaced with 10% FBS high-glucose DMEM containing 5 μg/ml insulin for the remaining 8 days.

Triglyceride Assay: Triglyceride (TG) in the cell lysate was extracted with chloroform-methanol and quantified enzymatically using a Triglyceride G Test Wako Kit.

Extraction of mRNA and RT-PCR analysis: Messenger RNAs were isolated from PSPA cells using the QuickPrep Micro mRNA Purification Kit and then reverse transcribed using the First-Strand cDNA Synthesis Kit. The synthesized cDNA was amplified with AmpliTaq Gold by PCR using paired forward and reverse primers with the ribosomal protein L7 (RPL7) as the internal control (Venuti et al., 1995). Primer sequences were as follows: peroxisome proliferator-activated receptor (PPAR) γ2 (GGTGAAACTCTGGGA GATTCTCTTA, GGCTCTTCGTGAGGTTTGTTGTACAG); PPARγ1 (CCTTAAACGAAGAGTCATCTT TTAGCG, GGCTCTTCGTGAGGTTTGTTGTACAG); CCAAT element-binding protein α (C/EBPα, AAG TCGGTGGACAAGAACAGCAACGAGTA, ATTGTCACTGGTCAGCTCCAGCACCTT); adenovirus E2 promoter-binding factor 1 (E2F1, TGGACCTGGAAACTGACCATCAGTACCT, TCTTGGACTTCTTGGC

AATGAGCTGGATG); proliferating cell number antigen (PCNA, CTGGTGAATTTGCACGTATATGCCG AG, AGGGGTACATCTGCAGACATACTGAGTGT); thymidine kinase (TK, TCGGACCCATGTTCTCG GGAAAAAGT, ACTCCACGATGTCAGGGAAAAACTG); lipoprotein lipase (LPL, CATAGCAGCAAA ACCTTTGTGGTGATCC, TTGGTCAGACTTCCTGCAATGCCAGCA); adipocyte-specific fatty acid binding protein (aP2, TTTGCTACCAGGAAAGTGGCTGGCAT, GCAGTGACACCATTCATGACACAT TCC); stearoyl CoA desaturase 1 (SCD1, ACCGTGCCCACCACAAGTTTTCAGAAA, GCTCCAAGTGA AACCAGGATATTCTC); hormone-senstive lipase (HSL, CGCAGTGTGTCTGAAGCAGCACTGGC, AT GACCGAGTCGTCCAGCATGGGGTC); RPL7 (GCAGAACCCAAATTGGCGTTTGTCATCAG, GATG ATGCCGTATTTACCAAGAGATCGAGC).

Western blot analysis: Both nuclear proteins and cytoplasmic proteins were prepared by using the CelLytic NuCLEAR Extraction Kit. Twenty-five micrograms of nuclear protein and 50 μg of cytoplasmic protein were separated by electrophoresis through SDS-polyacrylamide 12.5% gels. After they were electrotransferred onto nitrocellulose membranes, blotting membranes were incubated with primary polyclonal antibodies specific to PPARγ, C/EBPα, PCNA, E2F1, and glycerol-3-phosphate dehydrogenase (GPDH). Secondary antibodies were horseradish perocidase-conjugated anti-rabbit, anti-mouse or anti-goat antibodies. Antigen-antibody complexes were visualized by the ECL detection system.

Results and discussion

When confluent PSPA cells were stimulated with insulin, dexamethasone biotin, pantothenate, and octanoate, growth was arrested, and the cells exhibited a marked increase in lipogenesis. However, adipose conversion was not induced upon exposure of PSPA cells to a standard hormonal mixture of 3T3-L1 cells (Student et al., 1980), and their cell numbers increased as did the preadipocytes in growth medium (Fig.1A). Also, some differences in cell behavior were observed between PSPA cells and 3T3-L1 cells (Fig.1A and B).

To investigate whether every component of the PSPA medium was equally essential to the induction of adipocytes, PSPA cells were exposed to differentiation media each of which was lacking one agent. Under these culture conditions, the absence of either octanoate or dexamethasone from the medium resulted in a marked decrease in lipid accumulation (data not shown). Furthermore, octanoate was the only factor able to induce growth arrest. Octanoate supplementation to 3T3-L1 medium dramatically improved TG accumulation of PSPA cells while accompanying growth arrest (data not shown). These data strongly suggested a correlation between the growth-inhibiting effect of octanoate and terminal differentiation. Additionally, based on the result of a dexamethasone-depleted medium in which the cell numbers were maintained as normal adipocytes, it appears that dexamethasone regulates PSPA preadipocyte differentiation through a different induction pathway from octanoate.

The expression of a number of adipogenic genes and proteins was studied by RT-PCR and Western blot analysis. Expression patterns of E2F1, PCNA and TK were consistent with the results indicated so far by means of cell numbers, and expression patterns of LPL, aP2, SCD1 and HSL consistent with TG content. The most interesting result was that the expression of those master regulators of adipocyte gene transcription, PPARγ2 and C/EBPα. The absence of octanoate from the differentiation medium expressed only PPARγ2 but not C/EBPα, and in contrast, removal of dexamethasone resulted in expression of C/EBPα but not PPARγ2 (Fig.2).

Conclusions

Species specificity in adipose conversion between mouse and pig preadipocytes has been shown as the responsiveness to inducers was not equal between these two. Hormonal cocktail of 3T3-L1 cells failed to induce adipose conversion of PSPA cells and kept on proliferating. Growth arrest by octanoate was required for PSPA cells to enter terminal differentiation. Our results can probably provide an answer as to why researchers often prefer serum-free conditions in porcine primary cultures instead of serum-containing medium (Hentges and Hausman, 1989; Surywan and Hu, 1993; Gerfault et al., 1999). This was because none of the agents added according to mouse reports were sufficient to make preadipocytes cease dividing, whereas serum-free medium readily did so. We propose that mouse is not suitable for the study of porcine adipose development.

References

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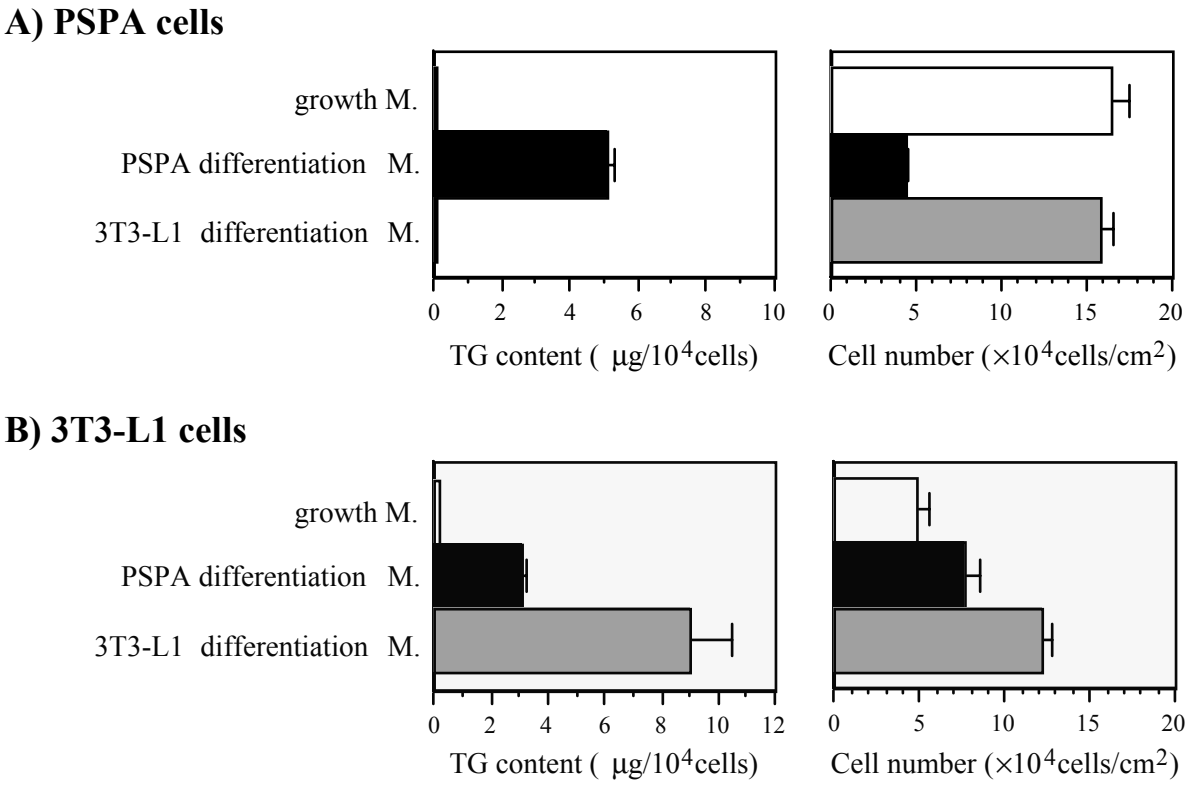

Fig. 1. TG accumulation and cell number of (A) PSPA cells and (B) 3T3-L1.

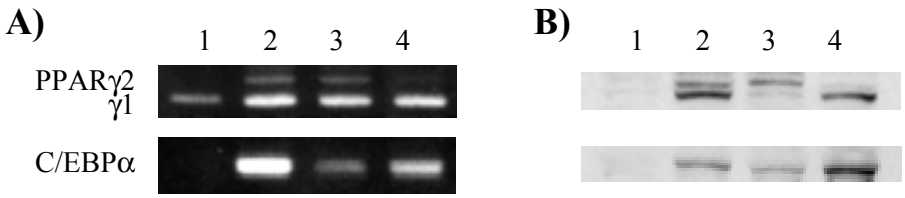

Fig. 2. Effect of octanoate and dexamethasone on the induction of adipocyte-specific transcription factors. (A) RT-PCR and (B) Western blot analysis. 1: growth medium, 2: PSPA differentiation medium, 3: octanoate-depleted medium, 4: dexamethasone-depleted medium.