AN IN VITRO MODEL SYSTEM FOR THE STUDY OF PORCINE ADIPOSE DEVELOPMENT

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Background

Excess adipose tissue in pigs represents a major source of both cost inefficiency and consumer concerns. Investigation of the factors that regulate the development of adipose tissue will provide useful information for controlling adipose accretion. In vivo studies are difficult because of the interactions of numerous systems involved in adipocyte metabolism. Therefore, investigators have established in vitro cell culture systems to study adipose development. However, much of the knowledge about the cellular and molecular events accompanying adipose conversion has been based on murine preadipocyte cell lines. Studies based on cell lines from porcine adipose tissue have not been reported so far, though fat cells in short-term primary culture have been studied (3, 4). Because species-specific differences are observed in the molecular regulation of adipocyte differentiation (1), the use of preadipocyte culture models derived from the pig is now necessary for the livestock industry.

Objectives

The present study was designed to establish and to characterize the profiles of the preadipocyte clonal line from porcine backfat (PSPA).

Methods

Isolation and cloning of porcine preadipocytes.

Porcine preadipocytes were obtained according to Forest et al. (2). Dorsal subcutaneous tissue was dissected from crossbred fetuses (85 days gestation) that were reproduced at the National Institute of Livestock and Grassland Science. Briefly, the tissue was minced and digested in Dulbecco's modified Eagle's medium (DMEM, 1 g/l glucose) containing 1 mg/ml collagenase. The digestion proceeded for 1 hr in a sterile plastic tube at 37°C for 30 min with agitation. After filtration through a 75-μm mesh and centrifugation for 7 min at 300 g, the supernatant was discarded. The pellet fraction, composed primarily of stromal-vascular cells, was resuspended and seeded into a tissue culture flask in growth medium, DMEM supplemented with 10% fetal bovine calf serum (FCS), 1,000U/ml penicillin, and 1 mg/ml streptomycin. The cell monolayer was tripsinized, and cells were then cloned by a limiting dilution. Two to four weeks later, the different clones were grown separately, and the PSPA clone displaying the highest frequency of adipose conversion was selected.

Differentiation of preadipocytes to adipocytes.

In order to produce mature adipocytes, PSPA cells were plated at 2.1×10^4 cells/cm^2 and grown for 3 days to obtain confluency. After reaching confluence (day 0), adipose conversion was induced in high glucose (4.5 g/l) DMEM containing 10% FCS in addition to various combinations of 5 μg/ml insulin, 0.25 μM dexamethasone, 33 μM biotin, 17 μM pantothenate, and 5 mM octanoate. The medium was changed every other day and the cells were allowed to differentiate for 10 more days. Control cultures were grown after confluency in non-adipogenic growth medium as the preadipose state.

Triglyceride assay.

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

Results and discussion

Exponentially growing PSPA cells exhibited a fibroblastic appearance (Fig. 1a). The growth curves of PSPA cells at three different starting densities (0.5×10^4, 1×10^4 and 3×10^4 cells/cm^2) are shown in Fig. 2. These cells grew to confluence after 2, 4 and 6 days at high, middle and

low cell densities, respectively. On the basis of this result, we decided it was suitable to pass the cells every 4 days at a constant density of inoculation of $1x10^4$ cells/cm^2.

We then exposed confluent PSPA cells to various combinations of hormonal factors to determine the optimum adipogenic medium for the induction of PSPA adipocytes. The TG accumulation was markedly accelerated by treating the cells with medium containing all the agents, and thus this mixture was used the differentiation medium of PSPA cells (Fig. 3). When confluent cultures were shifted to this differentiation medium, growth was arrested and the cells exhibited a remarkable increase in lipogenesis. A phase-contrast image taken at 10 days of culture after stimulation indicated adipocytes occupied by numerous intracellular lipid droplets (Fig. 1b). Under non-adipogenic conditions in growth medium, PSPA preadipocytes continued to proliferate, though slightly, even after reaching confluency, but they never allowed for TG storage (Fig. 1c).

Conclusions

We have established a clonal porcine subcutaneous preadipocyte cell line. So far, PSPA cells have undergone at least 85 passages with no detectable loss of phenotypic properties. In addition, PSPA cells retain their ability to develop and differentiate to mature adipocytes after more than 60 passages. PSPA cells may be useful in the future study of adipose tissue metabolism and of the differentiation mechanism of adipocytes in pigs and, further, to develop methods for manipulating the carcass fat content of pigs.

Pertinent literature

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3. Hausman, G. J., Wright, J. T. and Richardson, R. L. (1996) J. Anim. Sci., 74, 2117-2128.
4. Suryawan, A., Swanson, L. V. and Hu, C. Y. (1997) J. Anim. Sci., 75, 105-111.

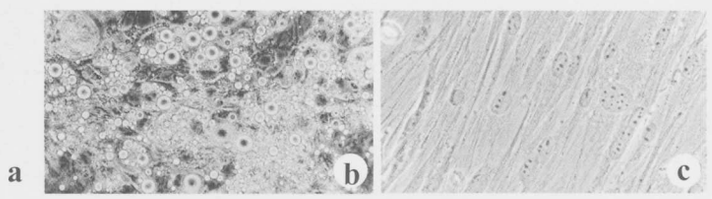

Fig.1. Morphology of PSPA cells. (x400)

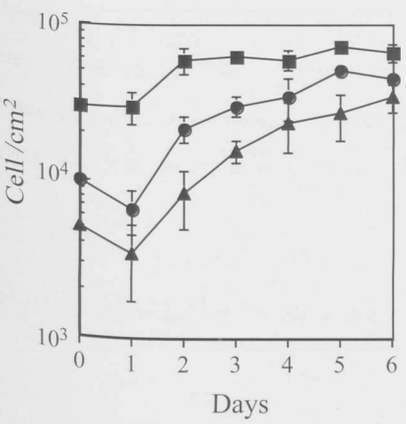

Fig.2. Growth curves of PSPA cells.

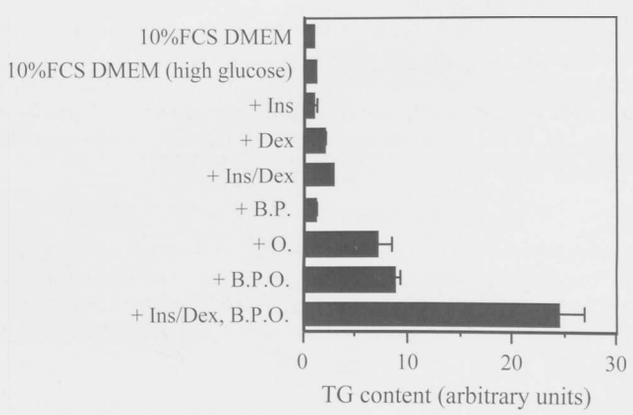

Fig.3. Stimulation of PSPA cells by various adipogenic factors. *Ins*: insulin, *Dex*: dexamethasone, *B.*: biotin, *P.*: pantothenate, *O.*: octanoate.