ESTABLISHMENT OF SUBCUTANEOUS PREADIPOCYTE CLONAL LINE FROM CHINESE MEISHAN PIG

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Keywords: pigs, backfat, cell culture, preadipocyte, differentiation

Introduction

Chinese pigs such as the Meishan breed provide a particularly interesting genetic model to study. The Meishan can be characterised by its wrinkled face and skin (Figure 1a,1b), and is highly known as one of the most prolific breeds of pig where females begin puberty at only 60 days of age, and have a large litter size of 15-16 piglets. Meishan pigs also have the characteristics of slow growth and higher fat deposits than conventional breeds of pig (Legault, 1985; White *et al.*, 1995). Also, their backfat can become quite thick (Figure 1c).

Since 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 nutritional controls. On the other hand, approach from the comparative study among greatly different genotypes such as generating crosses between Meishan and Western pigs, have been set up to identify the quantitative trait loci associated with backfat thickness (Rohrer, 2000; Sato *et al.*, 2003). 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.

We have already established a preadipocyte clonal line from Western crossbred subcutaneous tissue (PSPA) (Nakajima *et al.*, 2003) for the study of pig fat. The present study was designed to establish another pig preadipocyte clonal line from the Meishan pigs (MSPA).

Figure 1: (a) Adult Meishan pig. (b) Meishan piglets. (c) Meishan meat. Note the thickness of the subcutaneous adipose tissue.

Materials and Methods

Isolation and cloning of Meishan pig preadipocytes.

Meishan pig preadipocytes were obtained according to Nakajima *et al.* (2003). Dorsal subcutaneous tissue was dissected from fetuses (85 days gestation) that were produced 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-mm 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 serum (FBS), 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 MSPA clone was selected upon the basis of its high growth rate and frequency of adipose conversion.

Differentiation of preadipocyte to adipocytes.

In order to produce mature adipocytes, MSPA cells were plated at 2.1×10^4 cells/cm^2 and grown for 3 days to obtain confluency. After reaching confluence (0 day), adipose conversion was induced in high glucose (4.5 g/l) DMEM containing 10% FBS 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 MSPA cells derived from Meishan subcutaneous tissue resembled fibroblasts in their typical spindle shape (Figure 2). The cells were passed every 4 days in non-adipogenic growth medium at a constant density of inoculation of 1×10^4 cells/cm^2. As shown in Figure 3, the ratio N/N_0 (N, cell number after 4 days over N_0, the initial number of inoculated cells) of 15-20th passage was nearly 1 which means the cells were quiescent. This phase was overcome after 20 passages, and then constantly, the cell numbers were doubly increased after 4 days culture (N/N_0>2). After achieving confluence and in the presence of insulin, dexamethasone, biotin, pantothenate, and octanoate, MSPA cells exhibited large amounts of TG at 10 days (data not shown). While confluent cells were cultured in growth medium, they did not accumulate any intracellular lipid droplets. Furthermore, adipose conversion was not induced upon exposure of MSPA cells to a standard hormonal cocktail of 1-methyl-3-isobutylxanthine, dexamethasone, insulin and fetal bovine serum, which is the medium to differentiate one of the most popular preadipocyte cell lines, the mouse 3T3-L1 cells (Student *et al.*, 1980). This reaction of MSPA cells was consistent with the result of PSPA cells, which strongly supported the idea that there is species specificity in response to adipocyte inducers between pig and mouse.

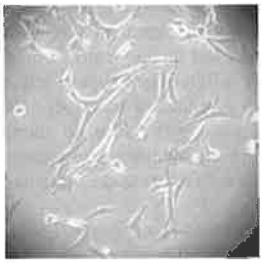

Figure 2: Exponentially growing MSPA cells with a fibroblastic appearance after 28 passages. (Original magnification ×100).

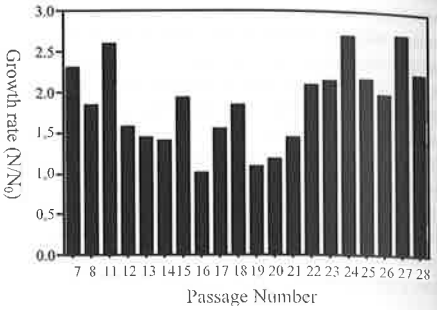

Figure 3: Growth rate of MSPA cells in 4days passage. N, cell number after 4 days; N_0, the initial number of inoculation cells.

Conclusions

We have established a clonal subcutaneous preadipocyte cell line derived from the Meishan pig. So far, MSPA cells have undergone at least 28 passages with no detectable loss of phenotypic properties or the ability to proliferate and develop into mature adipocytes. The study of comparing MSPA cells with PSPA cells may give us an answer as to why Chinese breeds deposit so much fat, and this will give us a clue to develop methods for manipulating the backfat content of pigs in the near future.

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