Abstract of Outstanding Presentation (3)

Neural Induction of Adipose-Derived Stem Cells

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Introduction

Compared with embryonic stem cells, adult stem cells can be handled more easily with fewer ethical problems and can be used for auto transplantation. In the last few years, a large population of pluripotent stem cells, termed adipose-derived stem cells (ASCs), has been identified from a small amount of adipose tissue. Such ASCs can differentiate into various mesenchymal cell types, such as chondrocytes, adipocytes, osteoblasts, and myocytes, and into neural lineages.

However, there is a controversy over neural differentiation. In our previous report about neural induction of ASCs, we showed that ASCs after induction had microtubules, which were frequently observed with electron microscopy in neurocytes, and had neural markers which were revealed with immunocytochemical staining. To build upon our previous results, we attempted to observe directly using time-lapse microscopy the time course of changes in ASCs after neural induction.

Materials and Methods

Inguinal fat pads were harvested from a Wistar rat aged 4 weeks. After digestion with collagenase and centrifugation, the cell pellet was resuspended and cultured in the control medium: Dulbecco's modified Eagle's medium (DMEM), antibiotics, and 10% fetal bovine serum. The two-passaged rat ASCs were grown in 30-mm cell culture dishes and rat fibroblasts were used as a negative control. A neural induction medium (NIM) was prepared according to our previous report: DMEM, antibiotics, 5 μg/m*l* insulin, 200 μM indomethacin, and 0.5 mM isobutylmethylxanthine.

Time-lapse studies were performed using an inverted phase-contrast microscope equipped with a temperature-controlled stage with ×4 phase objective (Corefront, Tokyo). After the culture medium was changed to NIM and the dish was placed under the microscope, the cells were maintained in 5%CO_2/95% CO_2 at 37℃ and the images were obtained at 10-min intervals for 24 hr (total, 144 images).

Results

After neural induction, the morphology of more than 80% of ASCs had changed within 30 min. The cells developed characteristic round cell bodies with several processes. The processes were repeating expansion and contraction, which were consistent with neural cell morphologically. As a result of constant migration in random directions, none of the cells were the same as observed previously despite of the visual field remaining

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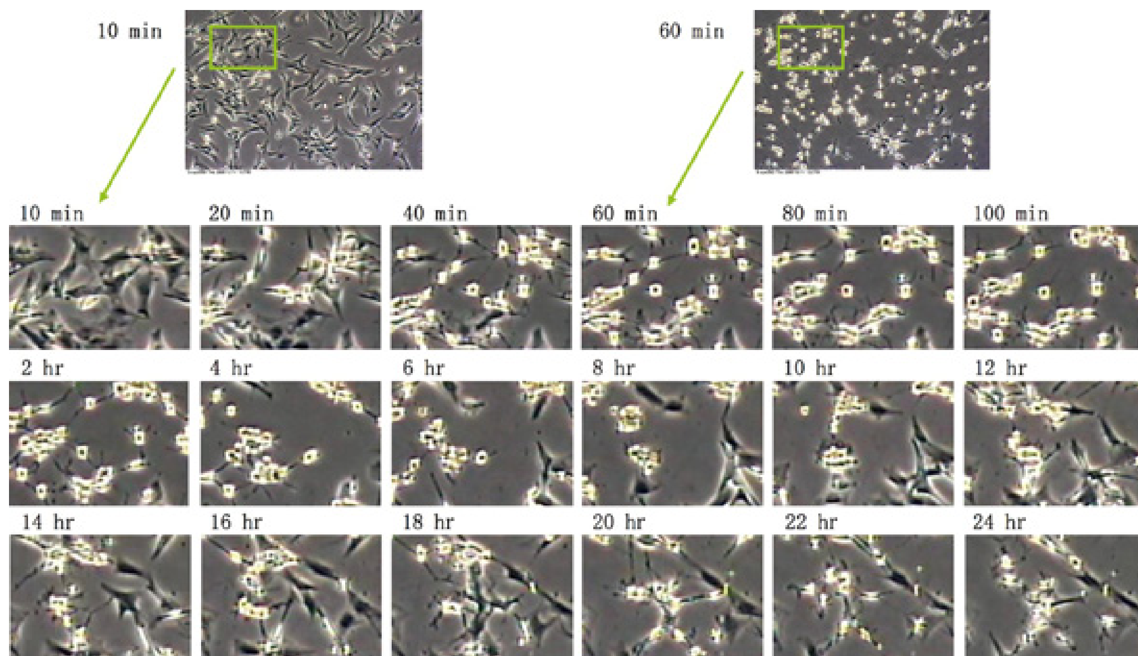

Fig. 1 Time-lapse microscopic images over the 24 hrs after exposure to NIM

unchanged. (Rat fibroblasts did not change after exposure to NIM.)

Discussion

We have demonstrated directly using time-lapse microscopy the neural induction of ASCs, and have shown that the morphological changes did not result from rapid disruption of the cytoskeleton due to the cytotoxic medium. ASCs may be an ideal source for further experiments on neural differentiation and transplantation in animal models of neurological diseases.