4

Sakuma T[1], Matsumoto T[2], Sato K[1], Igarashi T[1], Sugimoto S[1], Kano K[3], Fukuda N[2], Yoshida T[1], Takimoto Y[1], Mugishima H[2]
1. The Department of Urology, Nihon University School of Medicine, 2. Division of Cell Regeneration and Transplantation, Nihon University School of Medicine, 3. Department of Animal Science and Resources College of Bioresource Science, Nihion University

DEDIFFERENTIATED FAT (DFAT) CELLS CAN DIFFERENTIATE INTO BLADDER SMOOTH MUSCLE CELLS IN VITRO AND IN VIVO

Hypothesis / aims of study
We have established a novel preadipocyte cell line from adult mature adipocytes. The mature adipocytes were isolated from fat tissues by taking only the floating population of mature fat cells and dedifferentiated into fibroblast-like cells. We named these cells DFAT cells to imply dedifferentiated from fat cells. In our previous studies we have found the plasticity of DFAT cells, the osteogenic, adipogenic, myogenic, and chondrogenic lineages as similar as mesenchymal stem cells. In the present study, we investigated whether DFAT cells could differentiate into smooth muscle cell lineage and could contribute to bladder smooth muscle regeneration in mouse bladder injury model.

Study design, materials and methods
DFAT cells derived from human subcutaneous fat tissue were cultured in media containing various concentrations of FCS (0-20%) and TGF-β1 (1-50 ng/ml). After one week of cultures, transdifferentiation of smooth muscle-like cells from DFAT cells was evaluated by immunohistochemical analysis using anti α-smooth muscle actin (αSMA) antibody. Continuously, expression of smooth muscle specific marker genes (SM22α, SM-myosin heavy chain, αSMA, caldesmon) and adipocyte specific marker genes (PPARγ and C/EBPβ) were also measured by real time RT-PCR. In order to make a mouse bladder injury model, right and left lateral bladder walls in C57BL/6 mice were injured by a frozen aluminum lot. One week after the injury, DFAT cells derived from a green fluorescent protein (GFP) transgenic mouse (1-1.5 × 10^6 cells) were injected into the injured bladder walls by a 10-μL Hamilton microsyringe. Bladder tissues were harvested at 7, 14 and 30 days after the transplantation. Differentiation of DFAT cells into bladder smooth muscle cells was evaluated by immunohistochemical analysis using anti αSMA and anti GFP antibodies.

Results
Transdifferentiation of DFAT cells into smooth muscle-like cells was efficiently (over 50%) induced when the cells were cultured in a medium containing 5% FCS and 5 ng/ml TGF-β1, although αSMA-positive DFAT cells were not found before induction. During the 1-week culture, expression of SM22α, αSMA, SM-myosin heavy chain, and caldesmon mRNA increased 1.2-, 4-, 1.5- and 1.2-fold, respectively. On the other hand, expression of PPARγ and C/EBPβ decreased. In a study of mouse bladder injury model, a large number of cells expressing both αSMA and GFP were observed in injured bladder walls at 14 and 30 days after the transplantation.

Interpretation of results
DFAT cells can differentiate into smooth muscle cells in vitro and in vivo, and they can contribute to bladder smooth muscle regeneration.

Concluding message
DFAT cell transplantation may be a new therapeutic strategy for bladder smooth muscle regeneration.