

10593

A novel method to apply adipose-derived stem cells to bone and soft tissue defects after tumor excision.

Katsuhiro Hayashi, Norio Yamamoto, Toshiharu Shirai, Akihiko Takeuchi, Hiroaki Kimura, Shinji Miwa, Seigo Suganuma, Issei Nomura, Hiroyuki Inatani, Yu Aoki, Xiang Fang, Hiroyuki Tsuchiya

Department of Orthopaedics

Graduate School of Medical Science, Kanazawa University

13-1 Takara-machi, Kanazawa 920-8641, Japan

#### Introduction

We have investigated the applications of adipose derived stem cells (ADSCs), which are recognized as one of mesenchymal stem cells, in regenerative medicine. ADSCs have recently shown differentiation potential in multiple mesenchymal lineages in vitro and in vivo. These cells can be easily isolated in large amounts from autologous adipose tissue and, in some case, used without culturing or differentiation induction, which may make them relatively easy to use for clinical purposes. Regeneration of bone, cartilage and nerve defect after tumor resection is highly needed. When osteoarticular allograft is used for bone defect, bone regeneration and prevention of osteoarthritic change are needed. In our laboratory, ADSCs experiments are conducted as below.

1) ADSCs promote bone formation in distraction osteogenesis 2) ADSCs sheet accelerate and enhance bone 3) peripheral nerves can be regenerated using ADSCs 4) intra-articularly injected ADSCs prevent articular cartilage degeneration during osteoarthritis development.

#### Methods

In animal model, adipose tissue was harvested and the tissue was cut into strips over a period of 5 min. Collagenase was used to digest adipose tissue. Filtering and centrifuging created pellet of ADSCs.

1) Sixty rats were used for distraction osteogenesis with ADSCs model. Mixture of ADSCs and Type I collagen gel was injected into the distracted callus immediately after distraction termination. radiographic analysis, histological analysis , biomechanical testing and RT-PCR were examined.

- 2) Ascorbate-2-phosphate was used to to make the cell sheets of ADSCs in vitro. The osteogenic capacity of ADSCs sheet was evaluated by ALP staining, Alizarin Red staining and ALP activity after induction of osteogenesis.
- 3) Thirty rats were assigned for nerve regeneration. A 10 mm sciatic nerve defect was bridged using a silicon tube filled with ADSCs.
- 4) ADSCs with hyaluronic acid were intra-articularly injected into OA knee model of rabbit. Knees were compared macroscopically, histologically and immunohistochemically. DiI-labeled ADSCs were used for localization after intra-articular injection.

## Results

- 1) The bone density of the distracted callus in the ADSCs group increased by 46% ( $p = 0.003$ ) compared with the control group at 6 weeks after injection. RT-PCR of the distracted callus from the ADSCs group had higher levels of bone morphogenetic protein-2. Cell labeling in the newly formed bone showed the ADSCs differentiated into osseous tissue at 3 weeks after injection.
- 2) The ALP staining of ADSCs sheets showed positive results after 5 days osteo-induction and the Alizarin Red staining of ADSCs sheets showed positive results after 1 week osteo-induction. The ALP activity of ADSCs sheets groups showed higher value than that of separate ADSCs.
- 3) Continuity of nerve regenerated tissue was observed in ADSCs group. Protein gene product 9.5 staining confirmed significantly faster regeneration. The regenerated tissue in the ADSCs group had higher levels of Neu-1 and VEGFA expression than the control group.
- 4) Osteoarthritis progression was macroscopically milder in the ADSCs-treated knees than in the control knees eight weeks after anterior cruciate ligament transection ( $p < 0.05$ ). Immunohistochemically, MMP-13 expression was lesser in the ADSCs-treated cartilage than in the control.

## Discussion and Conclusion

Our study demonstrated that the ADSCs could be favorable resources for bone, cartilage and nerve regeneration. We have other promising results for tendon and meniscus repair as well. The availability of an easily accessible cell source may greatly facilitate the development of new cell-based therapies in bone and soft tissue defect.