Human Hypertrophic Nonunion Tissue Contains Progenitor Cells With Multi-lineage Capacity in vitro

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Introduction: Traditionally, aseptic nonunions are classified as atrophic and hypertrophic. Atrophic nonunion usually reflects poor vascularity, and often requires resection of the nonviable bone and intervening tissue at the nonunion site (nonunion tissue) and bone grafting in addition to the stabilization of the nonunion site. On the other hand, hypertrophic nonunion typically reflects instability and shows hypertrophic callus formation which indicates biologically active. In such situations, hypertrophic nonunion simply requires the stabilization of the nonunion site, and debridement of the nonunion site, bone grafting or decortication is not necessarily required for healing. However, why union occurs simply by the stabilization of the nonunion site without treating the nonunion site directly is not well understood.

Recently, we demonstrated fracture haematomas contain multilineage mesenchymal progenitor cells and indicated haematomas play an important role in bone healing (1). In hypertrophic nonunion, the healing process is inhibited by a lack of stability and the nonunion tissue fails to be transformed into an osseous bridge. Therefore, we hypothesized that nonunion tissues play an important role during the healing process of hypertrophic nonunion. In this in vitro study, we investigated whether the cells in nonunion tissue (NCs) have the capacity for multi-lineage mesenchymal differentiation.

Materials and Methods: Patient characteristics
Nonunion tissue was obtained from 7 hypertrophic nonunion patients (age range, 37-74 years; 6 males and 1 female) during the operation for nonunion. The samples were derived MSCs (BM-MSCs), BM samples were obtained with informed consent using a FACSaria instrument. To compare the data with that of bone marrow-genes, PPARγ differentiation was evaluated by Oil Red-O staining. Expression of adipocyte-specific genes, PPARγ, LPL, and BMP-6. Chondrogenic differentiation was evaluated by Alizarin Red staining, alkaline phosphatase (ALP) activity. Expression of osteoblast-specific genes, ALP, osteocalcin (OC), bone sialoprotein (BSP) and a housekeeping gene: glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was also measured by reverse transcription polymerase chain reaction (RT-PCR).

Chondrogenesis
2.5×105 cells were pelleted and cultured for 21 days in chondrogenic medium (Ch+), DMEM-high glucose supplemented with dexamethasone, β-glycerophosphate, and ascorbic acid. Osteogenic differentiation was evaluated by Alizarin Red staining, alkaline phosphatase (ALP) activity. Expression of osteoblast-specific genes, ALP, osteocalcin (OC), bone sialoprotein (BSP) and a housekeeping gene: glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was also measured by reverse transcription polymerase chain reaction (RT-PCR).

Adipogenesis
In Ad+, many of the cells had formed large lipid vacuoles, as shown by Oil red-O staining (Fig. 3). RT-PCR revealed that expression of PPARγ and LPL was induced by Ad+ (Fig. 4).

Eptipo Profile
The cultured cells were consistently positive for MSC-related markers, CD13, CD29, CD44, CD90, CD105, and CD166, while negative for hematopoietic markers, CD14, CD34, CD45, and CD133. (Tab.1).

References: