Regulation Of Adipogenesis By Bone Morphogenetic Protein -3b (bmp-3b)in Human Rotator Cuff Derived Stem Cells

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Introductions: Fatty degeneration is often seen in rotator cuff muscles with torn tendons. Fatty degeneration of supraspinatus muscle is related to poor shoulder function and re-tear after rotator cuff repair. Mesenchymal stem cell (MSC)s are also known to reside in the tissue such as rotator cuff.(1) Regulating the differentiation fate of MSCs in torn tendon is one of the ultimate goals for rotator cuff regeneration.

Bone morphogenetic protein (BMP) family is a subgroup of the transforming growth factor-β (TGF-β) superfamily. BMPs are considered to regulate not only bone or cartilage formation but also signal transduction throughout the body. BMP-3b (also known as growth/differentiation factor-10) was firstly isolated from rat femur and related osteoblast differentiation. BMP-3b is also highly expressed in adipose tissues and inhibits preadipocyte differentiation in murine cells.(2)

The aim of this study is to analyze regulation of adipogenesis by BMP-3b in human rotator cuff derived cell and investigate mechanism of fatty degeneration on human torn rotator cuff edge.

Methods:
Recombinant BMP-3b
To prepare the recombinant BMP-3b protein, CHO cells which express BMP-3b were generated by the dihydrofolate reductase-coupled method. Parental wild-type CHO cells were used as controls. The CHO conditioned medium was collected and filtered through a 0.45-mm Milliex Filter Unit. The concentration of BMP-3b was estimated by Western immunoblots using a BMP-3b specific antibody.

Cell Culture
Human rotator cuff derived cells were isolated from torn edges of human supraspinatus tendons, which were obtained during arthroscopic rotator cuff repair with informed consents from the patients. The tissues (weighing about 0.3 g each) were cut into small pieces under sterile conditions, followed by a 4 hours digestion in Dulbecco’s modification of Eagle’s Medium (DMEM) supplemented with 30 mg/mL collagenase II. After digestion, the cells washed and subsequently cultured in 75-cm2 cell culture flasks with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) (regular medium). All experiments were performed with 1 or 2 passaged cells, and the same passage of cells was used for each experiment. For adipogenic differentiation, StemMACS AdipoDiff Media (Miltenel Biothech, Auburn CA, USA) was used after the cells reached to confluent. The medium was replaced twice a week. To analyze the role of BMP-3b in adipogenesis, 15% v/v BMP-3b conditioned medium or control medium was added to adipogenic medium. The samples were subjected to analyze at determined time.

Oil Red-O Staining
Torn edge of human rotator cuff (N=6) was fixed with 4% paraformaldehyde (PFA) and sectioned serially at a thickness of 6 μm, mounted on silane-coated glass slides (Sigma), air dried for 1 h and stained immediately. The cells were fixed in 4% PFA for 10 minutes. The sections or the cells are stained with Oil red-O solution in 60% isopropanol for 40 minutes. Excess stain was removed by washing with water. For quantitative analysis, the stain was eluted with determined volume of 100% isopropanol. The absorbance of the elution was analyzed by spectrophotometer.

Real-time PCR
Total RNA was extracted from the cell cultures using an RNaseasy mini kit (Qiagen, Valencia, CA). Total RNA was reverse transcribed into single-strand cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed in triplicate on the cDNA with an Applied Biosystems 7900HT Fast Real-Time PCR System and SYBR Green regents (Applied Biosystems). Results were normalized to housekeeping gene expression levels and expressed relative to the control (untreated) culture levels using the 2-ΔΔCt method.

Results: Oil-red-O staining of torn human rotator cuff
3 samples out of 6 samples (50%) demonstrated positive staining of Oil-red-O which indicated adipogenic change can occur on human rotator cuff (Fig 1).

Expression of BMP-3b was decreased during adipogenesis
Human rotator cuff derived cells showed adipogenic potential after 3 weeks treatment with adipogenic medium. The expression of BMP-3b mRNA was significantly lower in adipogenic group than that of the control, while the expression of peroxisome proliferator-activated receptor γ (PPAR-γ) and lipoprotein lipase (LPL) was significantly higher (Fig 2a). The expression of BMP-3b gene deceased in time dependent manner (Fig 2b).

Administration of BMP-3b inhibited adipogenesis

At 3 weeks after adipogenic treatment, the expression of PPAR-γ and LPL significantly decreased in BMP-3b treatment group (Fig 3a). Quantitative analysis of Oil-red-O staining showed inhibition of adipogenesis in BMP-3b treatment group (Fig 3b).

Discussion: We have been reported that the cells from torn human rotator cuff had a potential to differentiate into bone, cartilage and adipose tissues.(1) That indicated the rotator cuff derived cells had a MSC potential. Histological examination showed fatty degeneration on torn edges of human rotator cuff. Therefore, to control the adipogenic change is one of the strategies to achieve better clinical outcome of the rotator cuff repair. It has been reported that BMP-3b is highly expressed in preadipocytes and inhibits adipogenesis in murine derived cells.(2) Present study showed expression of BMP-3b gene decreased during adipogenesis and treatment with BMP-3b had an inhibitory effect on adipogenesis of human rotator cuff derived cells. The result might help understanding mechanism of fatty degeneration and improving clinical outcome of rotator cuff repair.

Significance: Treatment with BMP-3b had an inhibitory effect on adipogenesis of human rotator cuff derived cells. The result might help improving clinical outcome of rotator cuff repair.

Acknowledgments: None

References: (1)Nagura et al; ORS 2012
(2)Hino et al; Int J Obes