Micro RNA Attenuates the Osteogenic Differentiation of Human Annulus Fibrosus Cells

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Introduction: During the process of disc degeneration, there is bone/fibrocartilage formation in the intervertebral area. As degeneration of the IVD and consequent chronic low back pain progresses, nerves and blood vessels are found increasingly in the inner part of the annulus fibrosus (AF) region of the intervertebral disc. Our previous study demonstrated that multipotent cells can be isolated from the inner AF tissue and, when cultured in vitro, will undergo differentiation into different lineage such chondrocytes, osteoblasts, and endothelial cells. The underlying cellular mechanism, however, has not studied yet. The objective of this study is to investigate not only the behavior but also the mechanisms of human AF cells from normal and degenerated IVDs.

Methods: Normal and degenerated human AF cells were isolated respectively from discarded tissue of patients (n=3) who have undergone sclerosis or spinal fusion, respectively. Cells at passage 2-4 were cultured in osteogenic medium (OIM) in vitro, and cellular differentiation was investigated using real time RT-PCR and histology at different time points. To measure the level of microRNA 221, total RNA was isolated with Trizol, reverse transcribed with Qiagen MicroRNA and amplified with universal master mix. Degenerated AF cells overexpressing miR221 were infected with lentiviral particles carrying miR221 and selected with puromycin. Total and phospho-Smad were detected with Western blotting with specific antibodies. Data are presented as mean ±standard deviation. Statistical analyses were performed by one-way ANOVA assuming equal variance using Microsoft Excel 2012 software. A p-value of less than 0.05 was considered statistically significant.

Results: Both normal AF (NAF) and degenerated AF (DAF) cells demonstrated their ability to undergo osteogenic differentiation as confirmed by mineralization (Fig. 1) and increased mRNA expression of BMP2, Runx2, ALP, and OCN (Fig. 2). DAF cells expressed increased osteogenic differentiation potential over the NAF cells. In contrast to the elevated phosphor- Smad1 and Smad 1/5/8 in DAF cells, the basal level of miR221 significantly decreased in DAF compared to NAF cells (Fig. 3 & 4). Osteogenic differentiation reduced miRNA221 expression in NAF cells, but there was no significant further reduction in DAF cells. Overexpression of miR221 with lentiviral particles markedly decreased the level of p-Smads and bone marker genes in DAF cells (Fig. 5). Consistently, the osteogenic potential of AF cells was diminished by miR221 expression (Fig. 6).

Discussion: We have demonstrated that human AF cells have the potential to differentiate into various cell lineages upon appropriate stimulation. Consistent with the observation in degenerated IVD, DAF cells showed a stronger potential for osteogenic differentiation than NAF cells. The osteogenic ability may be modulated by post-transcriptional regulation of Smads (1, 5, 8), which, are crucial regulators for signal transduction by BMPs during the process of osteogenesis through morphogenesis and development and in tissue repair. Moreover, the elevation of p-Smads is attenuated by overexpressing miR221 in the DAF cells. miR221 has an important role in cell proliferation and migration, and may serve as a regulator of the mecanotransduction pathway. While loss of function of miR221 is still under investigation, our results demonstrate that the osteogenic potential of degenerated AF cells is regulated by BMP-Smad pathways that are potentially modified by the expression of miR221.

Significance: Targeting microRNAs may provide a potential therapy for the management of disc degeneration.

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