STATIN UPREGULATES ENDOGENOUS BMP-2 EXPRESSION TO STIMULATE CHONDROGENIC PHENOTYPE OF INTERVERTEBRAL DISC CELLS

+*Lin, C-Y; **Zhang, H
Spine Research Laboratory, +*Department of Neurosurgery, **Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI
lincy@med.umich.edu

Introduction
Resembling a variety of previous and ongoing observations of BMPs on cartilage repair, more and more investigations using BMPs to facilitate the regeneration of intervertebral disc (IVD) have been conducted. It has been shown that exogenously supplemented recombinant human BMP-2 simulates the biosynthesis of proteoglycan and collagen type II in both chondrocytes [1] and IVD cells [2]. These molecules can be also increased in cartilaginous matrices when endogenous BMP-2 expression is up-regulated. The cholesterol-lowering drug statin that inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase has been observed to increase expression of BMP-2 and in turn stimulate bone formation [3] and chondrogenic phenotype [4]. However, the effects of statins on IVD cells have not been elucidated. This study is aimed to investigate whether statins are anabolic to chondrogenic expression of IVD cells via up-regulating BMP-2 and evaluate the potential of statins as alternative growth factors for IVD regeneration.

Materials and Methods

Disc cell isolation: Nucleus pulposus and inner anulus fibrosus cells of lumbar intervertebral discs from Sprague-Dawley rats were harvested by the enzymatic digestion and grown in monolayer until reached confluence.

Monolayer and Three-dimensional cell culture: Cells were trypsinized and subcultured in 12-well plate or embedded and cultured in alginate beads supplemented with complete DMEM/F12 medium after primary culture. When the cells got 80% confluent, simvastatin were added to the medium to reach the final concentration at 0.3-3 uM.

Quantitative real-time PCR: Total RNAs were extracted and cDNAs were synthesized using invitrogen superscript™ first-strand synthesis kit. Aggrecan, type II collagen and BMP-2 mRNA expression were quantified using ABI PRISM® 7700 Sequence Detection System.

Proteoglycan Production Assay: 1,9-Dimethylmethylene blue (DMMB) staining for sGAG was used to measure proteoglycan production. Total s-GAGs in the media or alginate beads were normalized according to counted cell number.

Statistical analysis: The data were expressed as mean ± Standard deviation. One-way ANOVA and Dunnett post-hoc test were used to determine the significant difference.

Results
The effect of various concentration of simvastatin on IVD cell proliferation was investigated prior to the treatment to determine the range of cell tolerance. Simvastatin inhibited the IVD cells proliferation when the dose was over 3 uM. We therefore chose 0.3-3 uM of simvastatin to conduct the main observations. In monolayer culture, 3 uM simvastatin began to increase BMP-2 mRNA expression at day 2 and reached maximal at day 3 by 2-fold. However, both aggrecan and collagen II mRNA expression in the treated group did not show difference compared with the control one. In 3-D culture, our data showed that simvastatin with the concentration of 3 uM continuously elevated BMP-2 mRNA expression from day 3 up to day 7, when the peak level was present (5-fold). As the consequence responsiveness to the elevating BMP-2, aggrecan was expressed increasingly from day 3 to day 21 by 11-fold (Fig. 1), while collagen type II showed slight postpone of the increase after day 7 and the increase sustained to day 21 by 16 fold (Fig. 2).

Discussion
The susceptibility of IVD cells to simvastatin significantly differed based upon the culturing systems. Three-dimensional resembled the favorable environment for IVD cells treated with statin to retain chondrogenic phenotype for longer duration compared to monolayer culture. The mRNA level of BMP-2 responded positively to the given doses of simvastatin and presented the largest increase at 3 uM. The consequence of the responsiveness of aggrecan and collagen type II suggested that the endogenous BMP-2 expression induced by simvastatin activated the cascade of chondrogetic pathway of IVD cells and stimulated the synthesis of key molecules that are critical for maintaining IVD functions. Retaining these molecules may indicate the potential to retard disc degeneration and also give the hope for disc regeneration. However, further characterizations are necessarily required to elucidate whether the anabolic effect of statin proceeds solely along with the BMP-2 pathway or there exists a synergistic mechanism with induced BMP-2 expression to facilitate IVD chondrogenic expression.


Figure 1: Aggrecan mRNA expression

![Figure 1: Aggrecan mRNA expression](image1)

Figure 2: Collagen type II mRNA expression

![Figure 2: Collagen type II mRNA expression](image2)

53rd Annual Meeting of the Orthopaedic Research Society
Poster No: 1096