Introduction: Intervertebral disc degeneration is associated with cellular and biochemical changes, which include decreased synthesis of cartilage specific gene products such as type II collagen and aggrecan. The search for cytokines that can stimulate intervertebral disc cells is an important part of the research focused on treating disc degeneration. While BMP-2 is a well-known osteoinductive cytokine that can induce chondrogenesis during new bone formation, its effects on intervertebral disc cells have not been characterized. We therefore decided to carry out an experiment using rat intervertebral disc cells to determine the effect of recombinant human BMP-2 (rhBMP-2) on cell proliferation, proteoglycan synthesis, the expression of chondrogenic genes (type II collagen, aggrecan, and Sox9), and non-chondrocytic genes (type I collagen and GAPDH).

Method: Cells were isolated from anulus fibrosus and transition zone of eleven-month-old Sprague-Dawley rat lumbar discs. Institutional Animal Care and Use Committee approval was obtained for usage of the rats. The cells were grown in monolayer and treated with rhBMP-2 (0, 10, 100, 1000 ng/ml) in DMEM/F-12 with 1% FBS (day 0). On day 2, 4, and 7 after rhBMP-2 treatment, sulfated-glycosaminoglycan (s-GAG) content in the media was quantified using 1,9-dimethylmethylene blue (DMMB) staining. The results were normalized according to culture duration and cell number. On day 7, mRNA was extracted for RT-PCR and real time PCR to quantitate mRNA of type I collagen, type II collagen, aggrecan, Sox9, and GAPDH. Cell number was determined with a hemocytometer.

Essential Results: RhBMP-2 changed cell morphology, increased cellular aggregation, and induced a swirling arrangement in culture that is characteristic of chondrocytic cells. RhBMP-2 at 100 and 1,000 ng/ml yielded a 17% and 42% increase in cell number on day 4, and a 59% and 79% on day 7, respectively (Figure 1). RhBMP-2 at 10 ng/ml had no effect on cell number. Media s-GAG increase was greatest at day 7, increasing by 1.3, 2.1 and 3.6 fold with rhBMP-2 treatments of 10, 100, 1,000 ng/ml, respectively (Figure 2). Increases in mRNA levels of type II collagen, aggrecan and Sox9 were observed with rhBMP-2 concentrations of 100 and 1,000 ng/ml by RT-PCR. No detectable increase in mRNA level of type I collagen was observed with any levels of rhBMP-2. Real-time PCR showed the greatest effect at 1,000 ng/ml rhBMP-2, leading to an 11.5 fold increase in aggrecan, a 4.6 increase in type II collagen (Figure 3) and a 5.3 fold increase in Sox9 mRNA above untreated controls at day 7.

Discussion: The results of our study show that rhBMP-2 promotes a chondrocytic phenotype and has a modest mitogenic effect on intervertebral disc cells. The change in cellular morphology and cellular arrangement is probably related a more chondrocytic differentiation state of the cells after exposure to rhBMP-2 and may also indicate expression of more or different adhesion molecules.

Our results show an increase in s-GAG production per cell with increasing concentrations of rhBMP-2 over the 7 days of culture. The cells behaved differently at day 7 than at day 4 in the sense that they produced far more s-GAG with a given concentration of rhBMP-2 and did not seem to have reached a plateau in response to increasing rhBMP-2 concentrations. This probably reflects a higher responsiveness of the cells to rhBMP-2 after prolonged exposure to rhBMP-2. One possible mechanistic explanation is that rhBMP-2 upregulates its own receptor. RhBMP-2 stimulated type II collagen, Sox9, and aggrecan genes, but not type I collagen gene expression. This indicates that rhBMP-2 specifically upregulates chondrocyte specific genes in intervertebral disc cells. However, rhBMP-2 did not stimulate type I collagen at any dose. This preferential stimulation of type II collagen and aggrecan is consistent with the response of other cell types to rhBMP-2.

We conclude that rhBMP-2 may be useful in enhancing disc matrix production of intervertebral disc cells.