Introduction: Cartilage intermediate layer protein (CILP) has been reported to be associated with lumbar disc disease (LDD) a process that results in disc herniation, sciatica, and the need for spinal surgery (discectomy). In osteoarthritis, CILP is among the few cartilage matrix proteins whose expression is up-regulated in early and late stages of the disease. Genetic analysis has shown significant association between a single nucleotide polymorphism (SNP) in CILP that resulted in higher affinity of CILP to TGF-beta. At this time, the CILP SNP is the only genetic marker for indicaive of a higher risk for spinal surgery. However, regulation of the CILP gene remains largely unknown. In this study we investigated the regulation of the CILP gene in intervertebral disc as a function of age and growth factors BMP-2 and TGF-beta. One objective was to determine whether cells from older disc would show higher CILP promoter activity. For this purpose, we used tissue from rabbits (young and old) to determine age related changes in CILP promoter activity. Another objective of the study was to determine how the relative potency of BMP-2 and TGF-beta regulation of CILP promoter activity.

Materials and Methods: Human anulus fibrosus (AF) and nucleus pulposus (NP) cells were isolated from intervertebral disc tissues from six patients during surgical procedures performed by one author (a surgeon). Rabbit anulus fibrosus (AF) and nucleus pulposus (NP) cells were isolated from lumbar discs of four six-month old New Zealand White rabbits (young rabbits) and four five-year-old New Zealand White rabbits (old rabbits). The cells from the AF and NP tissues of both human disc tissue and rabbit disc tissue were cultured in DMEM/F-12 medium. Cells were treated for 2 days with rhBMP-2(200ng/ml), TGF-b1(10ng/ml), and IL-1b (10ng/ml), respectively. DMSO treated cells served as mock control. After treatment for 2 days, the cells were harvested to isolate total RNA. Real time PCR was used to determine CILP mRNA levels.

The promoter region of human CILP was amplified from the human disc genomic DNA, and was cloned into pGL3-Basic. The CILP promoter luciferase reporters were cotransfected into the indicated cells with a pR TK-Luc vector, which expresses the Renilla Luciferase (RLuc) to normalize transfection efficiency. Three days after transfection, Firefly luciferase (Luc) and RLuc activities were measured by using the Dual Luciferase Reporter Assay System, according to the manufacturer's instructions. Firefly luciferase was always normalized with RLuc activity.

Results: BMP-2 and TGF-β1 upregulates CILP mRNA expression and promoter activity in human disc cells. The results are shown Figure 1. Each bar on the graph represents the mean ± s.d. of three experiments performed in duplicate. Real-time -PCR demonstrated that CILP mRNA levels were dramatically increased in cells treated with BMP-2 and TGF-β1 relative to control. In addition, BMP-2 was found to induce substantially higher CILP mRNA expression than TGF-β1. In sharp contrast, we found that CILP mRNA levels were not significantly altered in cells treated with IL-1β. Figure 2 showed that BMP-2 and TGF-β1 induced significantly increased CILP promoter activity. Similarly, we found that BMP-2 induced much higher level of CILP promoter activity than TGF-β1. In contrast, IL-1β did not affect on CILP promoter activity.

The CILP promoter activity changes with ageing in the rabbit disc cells. As shown in figure 3, the CILP promoter possess higher activity in old lumbar rabbit disc cells than in young rabbit disc cells. BMP-2 and TGF-β1 induced substantially higher CILP promoter activity in the old rabbit disc cells than in the young rabbit disc cells. Figure 4 shows that BMP-2 and TGF-β1 induced much higher level of CILP promoter activity in the old rabbit disc cell and NP cells than in the young rabbit disc cells. Consistent with our real-time PCR analysis, BMP-2 induced much higher level of CILP promoter activity than TGF-β1 in the old and young rabbits disc AF and NP cells. IL-1β did not affect on CILP promoter activity. Taken together, these results demonstrated that the growth factors induced substantially higher CILP promoter activity in the old rabbit disc cells than in the young rabbit disc cells.

Discussion: Our major findings are as follows: 1) BMP-2 upregulated CILP promoter activity more highly than TGF-beta, 2) CILP promoter activity is higher in disc cells from old rabbits 3) CILP promoter activity is more highly upregulated by BMP-2 and TGF-beta in the older rabbit disc cells than younger rabbit disc cells. These results suggest that BMP-2 may be an important or more important regulator of CILP than TGF-β1 and that age is also important. The functional consequence this is not clear, however, one can speculate that the interaction between growth factors such as BMP-2 and TGF-beta may be important in the process of disc changes the disease conditions of herniation and sciatica. Further investigation between the interaction between age and growth factors and CILP and subsequent disease is warranted.

