Evaluation of Correlation Between Pfirrmann Grade and BMP Signaling in Degenerative Disc Disease

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Introduction

Degenerative disc disease (DDD) is a major burden to society.[1] Back pain and neck pain can be caused by DDD and up to 80% of the population will have low back pain at a certain point as a result and 10% may become chronically disabled .[2] The intervertebral disc is composed of the two cartilage endplates, nucleus pulposus, and annulus fibrosus. All three of these components can contribute to DDD and subsequent pain. There have been great strides in our understanding of the biology of DDD, however, few translational treatments have arisen. In the surgical arena, a variety of implants are being innovated however the biologic management of DDD continues to evolve. Bone morphogenetic proteins (BMPs) are critical in embryogenesis and the role of BMP-2 as an anabolic agent in the etiology or prevention of DDD is being actively investigated. The BMP-2 receptor activin receptor like-kinase 3 (ALK3) is critical for the initiation of the SMAD signaling cascade (Fig. 1).[5] However, to date no group has evaluated the role of BMP receptor Type I or ALK3 overexpression in the BMP-SMAD anabolic signaling pathway within the intervertebral disc as a therapeutic strategy for DDD. Pfirrmann grade is a validated MRI based classification to grade the extent of degenerative disc disease (Table 1). We aimed in this study to evaluate the correlation if any between pfirmann grade and BMP anabolic signaling pathway in intervertebral disc degeneration.

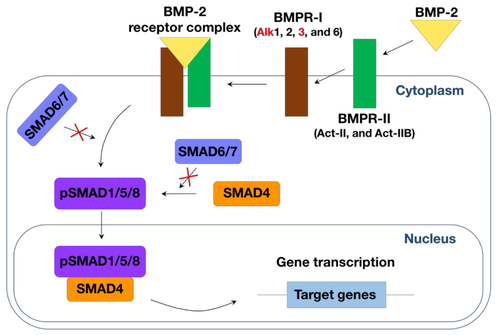

Figure 1. SMAD signaling cascade: BMP signal transduction is via two cell surface serine/threonine kinase Type I and Type II receptors.[6] When both Type I and Type II receptors are present their binding affinity increases significantly.[7] Once BMP is bound, the Type II receptor phosphorylates the Type I ALK3 receptor, and the ALK3 receptor in turn initiates the downstream signaling via the SMAD pathway.

Materials and Methods

- **Study subjects: 37** (10 cervical herniations, 27 lumbar herniations) degenerative disc specimen from patients undergoing cervical and lumbar spine surgery and **13** control disc specimen from patients undergoing surgery for spine fractures, scoliosis or spine tumors were managed during the study period.
- There were 20 females and 17 males, with an average age of 43.7 (19-82).
- Using the Pfirrmann MRI classifications (Fig. 2.), We rated the degenerative discs from Grade III to V, and assigned grades 1 and 2 to our non degenerated control group.
- Using immunohistochemistry we assessed expression of the following markers of anabolic activity in the SMAD-1,-5,-8, signaling pathway: ALK3, BMP-2, and pSMAD
- A Mann Whitney U test was used for both the evolution of the results of the definitive statistical methods (mean, standard deviation, median and the comparison of quantitative data parameters between groups. Significance was defined as $p < .05$.

Immunohistochemistry: Parrafin sections of dissected intervertebral disc from the two groups was made. The discs were fixed in 4% paraformaldehyde, decalcified in 10% ethylene-diaminetetraacetic acid and sectioned at 3 um. We used goat polyclonal anti-ALK3 (BMPR-1A) (Santa Cruz Biotechnology, Santa Cruz, CA; optimum dilution 1:50) and goat polyclonal anti-BMP-2. We also used rabbit polyclonal antiphospho-SMAD-1,-5,-8 (Cell Signaling Technology, Beverly, MA) to assess for activity in the SMAD pathway.

Table 1. Pfirrmann MRI Classification. Disc degeneration can be graded on MRI T2 spin-echo weighted images using a grading system proposed by Pfirrmann.

Grade	Structure	Distinction of Nucleus and Anulus	Signal Intensity	Height Of Intervertebral Disc
I	Homogenous, bright white	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
II	Inhomogenous with or without horizontal bands	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
III	Inhomogenous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogenous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogenous, black	Lost	Hypointense	Collapsed disc space

Results

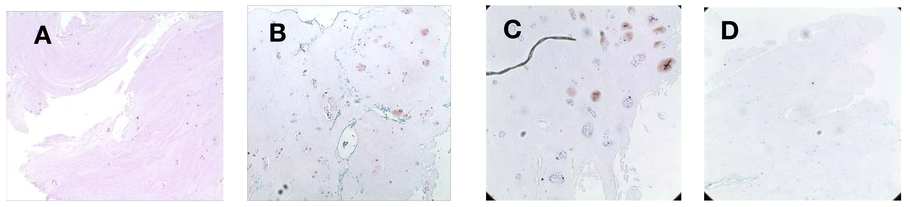

Figure 2. Immunohistochemical staining: Intervertebral disc cells showed cluster formations in degenerated discs and these cells stained positive for BMP-2 (B) and active (phosphorylated) SMAD-1,-5,-8 (pSMAD-1,-5,-8) (C), which cannot be detected in the normal disc (D).

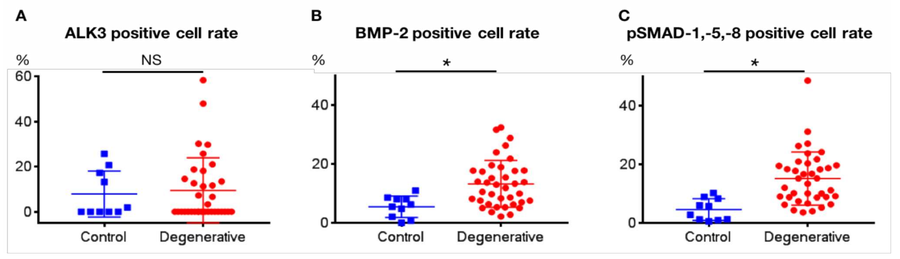

Figure 3. Positive cell rates in immunohistochemical staining: A) ALK3 B) BMP-2 and C) pSMAD-1,-5,-8. *p<0.05 Control vs. Degenerative by Mann-Whitney test. The comparative analysis of expression levels failed to show significant differences in ALK3 expression ($p = 0.98$); however, we found a significant BMP-2 and pSMAD-1,-5,-8 overexpression ($p < 0.05$, respectively) in the Degenerative group compared to the Control group.

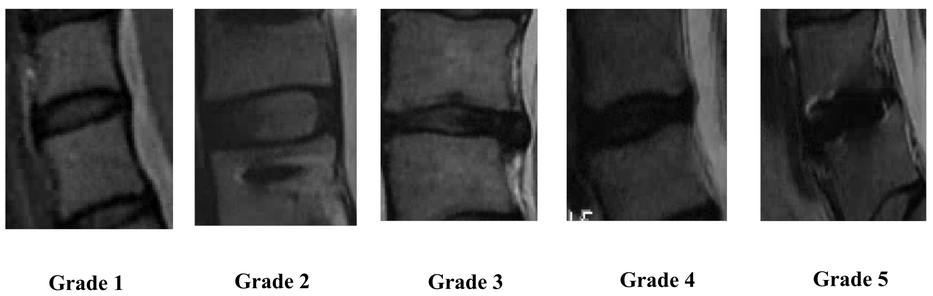

Figure 4. Representative MRI- Sagittal T2 Images: Graded according to Pfirmann Classification. Higher grade corresponds to a higher degree of disc degeneration.

Grade 1 and 2 patients were assigned to our Control group and Grade 3-5 were assigned to our Degenerative group

		Grade			
		Grade 3 (n=9)	Grade 4 (n=24)	Grade 5 (n=4)	[a]p
BMP2	Mean ± SD	11.1% ± 0.041003084	15.0% ± 0.089919695	8.4% ± 0.0655796	**<.001***
	Median	10.8%	14.5%	6.9%	
pSMAD	Mean ± SD	14.3% ± 0.136200557	15.3% ± 0.074608288	15.7% ± 0.06463722	**<.001***
	Median				
ALK3	Mean ± SD	5.4% ± 0.092830184	7.7% ± 0.113622925	16.4% ± 0.21120133	**.107**
	Median	0.0%	0.0%	10.7%	

Table 2. Correlation of Pfirrmann grades in intervertebral disc degeneration with expression of BMP, pSMAD, and ALK3. Mann Whitney U test p <.05

Discussion

Pffirmann developed a 5 step MRI grading classification system based on signal intensity, homogeneity in structure, distinction of the nucleus and annulus fibrosis, and height of the intervertebral disc. It is traditionally thought that intervertebral disc degeneration is owed to catabolism in the ECM. However, there are anabolic processes that are active concurrently. We have demonstrated BMP-2 is increased in patients with disc degeneration. We have also shown that pSMAD is increased in disc degeneration. However, our study showed that ALK3 expression was not significantly increased in degenerated discs. This BMP-2 and pSMAD 1/5/8 upregulation suggests BMP/SMAD signaling is activated in the degenerative disc disease process but perhaps not through ALK-3 binding. Based on our data we can now conclude that there is a significant relationship between the expression levels of anabolic proteins BMP/ pSMAD, and degree of degradation in MRI grading based on the Pfirmann classification. In terms of pfirrman IVD degeneration grade, there was an overall increase in expression of all proteins observed in grade 4 compared to grade 3. With the exception of BMP, expressions of the proteins in the BMP signaling pathway were also increased in grade 5 compared to grade 4. However the Mann Whitney U test failed to show significance in demonstrating if higher grade correlated with higher SMAD BMP levels.

Clinical Significance

The role of BMP-2 as an anabolic agent in the intervertebral disc is known. However, to date no group has evaluated the correlation between the levels of expression of ALK3 in the BMP-SMAD signaling pathway and the validated Pffirmann MRI grading scores in intervertebral disc degeneration.

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