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Bone Morphogenic Protein-2 Signaling in Human Disc Degeneration and Correlation to the Pfirrmann MRI Grading System

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Abstract

Background Context: Back and neck pain secondary to disc degeneration is a major public health burden. There is a need for therapeutic treatments to restore intervertebral disc (IVD) composition and function.

Purpose: To quantify ALK3, BMP-2, pSMAD1/5/8 and MMP-13 expression in IVD specimens collected from patients undergoing surgery for disc degeneration, to correlate ALK3, BMP-2, pSMAD1/5/8 and MMP-13 expression in IVD specimens to the 5-level Pfirrmann MRI grading system, and to compare ALK3, BMP-2, pSMAD1/5/8 and MMP-13 expression between cervical and lumbar degenerative disc specimens.

Study Design: An immunohistochemical study assessing ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression levels in human control and degenerative IVD specimens.

Methods: Human IVD specimens were collected from surgical patients who underwent discectomy and interbody fusion at our institution between 1/2015 and 8/2017. Each patient underwent MRI prior to surgery. The degree of disc degeneration was measured according to the 5-level Pfirrmann MRI grading system. Patients were categorized into either the 1) control group (Pfirrmann grades I-II) or 2) degenerative group (Pfirrmann grades III-V). Histology slides of the collected IVD specimens were prepared and immunohistochemical staining was performed to assess ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression levels in the control

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and degenerative specimens. Expression levels were also correlated to the Pfirrmann criteria. Lastly, the degenerative specimens were stratified according to their vertebral level and expression levels between the degenerative lumbar and cervical discs were compared.

Results: Fifty-two patients were enrolled; however, 2 control and 2 degenerative patients were excluded due to incomplete data sets. Of the remaining 48 patients, there were 12 control and 36 degenerative specimens. Degenerative specimens had increased expression levels of BMP-2 ($p = 0.0006$) and pSMAD1/5/8 ($p < 0.0001$). Pfirrmann grade 3 ($p = 0.0365$) and grade 4 ($p = 0.0008$) discs had significantly higher BMP-2 expression as compared to grade 2 discs. Pfirrmann grade 4 discs had higher pSMAD1/5/8 expression as compared to grade 2 discs ($p < 0.0001$). There were no differences in ALK3 or MMP-13 expression between the control and degenerative discs ($p > 0.05$). Stratifying the degenerative specimens according to their vertebral level showed no significant differences in expression levels between the lumbar and cervical discs ($p > 0.05$).

Conclusions: BMP-2 and pSMAD1/5/8 signaling activity was significantly upregulated in the human degenerative specimens, while ALK3 and MMP-13 expression were not significantly changed. The expression levels of BMP-2 and pSMAD1/5/8 correlate positively with the degree of disc degeneration measured according to the Pfirrmann MRI grading system.

Clinical Significance: BMP-SMAD signaling represents a promising therapeutic target to restore IVD composition and function in the setting of disc degeneration.

Keywords

Bone morphogenic protein; BMP-2; pSMAD1/5/8; ALK3; MMP-13; disc degeneration; Pfirrmann; MRI; proteoglycan; disc herniation

INTRODUCTION

Back and neck pain as a result of disc degeneration is a major public health burden [1, 2]. Up to 80% of the population will be affected with back pain at some point in their lifetime and 10% may become chronically disabled [3, 4]. In 2005, an estimated \$86 billion was spent in the United States for the management of neck and back problems [5]. Etiology of disc degeneration has been attributed to a multitude of factors including environment, atherosclerosis, genetics, nicotine use, obesity, occupation, and low vitamin D levels [6, 7]. Clinically, the management of axial neck and back pain is with physical therapy and non-steroidal anti-inflammatory medications [8]. There is a limited role for spinal fusion for axial symptoms [9–13]. Thus, there is currently a need for therapeutic treatments to restore intervertebral disc (IVD) composition and function in the setting of disc degeneration.

The IVD, which functions as a shock absorber between adjacent vertebral bodies and allows for motion of the spine, is composed of an annulus fibrosus, nucleus pulposus (NP), and two cartilaginous endplates [6]. The annulus fibrosus has an inner and outer layer and is predominantly composed of type I collagen. It is responsible for tensile strength. The NP is composed mainly of NP cells and proteoglycan aggrecans enmeshed in a loose type II collagen matrix that provides compressive strength to the disc [14, 15]. Throughout development and aging, the extracellular matrix (ECM) within the IVD undergoes extensive remodeling via anabolic and catabolic processes. A disruption in ECM

homeostasis leading to increased catabolic activity (mediated by matrix metalloproteinases [MMPs], disintegrins, metalloproteinases with thrombospondin motifs [ADAMTS], and positively regulated by inflammatory cytokines such as interleukin 1- β [IL-1 β], IL-6, IL-8, prostaglandin E2 and nitric oxide) and decreased anabolic activity (mediated by bone morphogenetic proteins [BMPs], transforming growth factor- β [TGF- β] and insulin-like growth factor [IGF]) ultimately results in disc degeneration characterized by a loss of proteoglycans and dehydration of the NP [16–18]. As disc degeneration progresses, nociceptive nerve fibers and blood vessels can grow on the annulus fibrosus resulting in back pain [19, 20]. Fissures can progress through the annulus fibrosus, leading to disc herniation and subsequent radiculopathy.

BMPs are members of the TGF- β superfamily and are critical in the growth and differentiation of osteoblasts, chondroblasts, neurons, and epithelial cells [21]. BMP signal transduction occurs through two cell surface serine/threonine kinase type I and type II receptors (Fig. 1) [22–25]. Of the type I receptors, Activin receptor like-kinase 3 (ALK3) is responsible for binding BMP-2 [25]. There are three type II receptors (BMPRII, ActRII, ActRIIB). Once BMP-2 binds, the type II receptor phosphorylates the type I ALK3 receptor. The ALK3 receptor in turn initiates downstream signaling via the SMAD pathway. pSMAD1/5/8 binds to SMAD4 and together translocate to the nucleus where transcription of downstream target genes is regulated [26].

MMPs are members of the zinc-dependent endopeptidase family and are believed to be a primary driver of catabolic activity during disc degeneration [27]. MMPs can be categorized into 6 groups: 1) collagenases (MMP-1, -8, -13, and -18), 2) gelatinases (MMP-2 and -9), 3) stromelysins (MMP-3, -10, and -11), 4) matrilysins (MMP-7 and -26), 5) membrane-type MMPs (MMP-14, -17, -24, and -25), and 6) other non-classified MMPs (MMP-12, -19, -20, -21, -23, -27, and -28). They are secreted in an inactive form and require extracellular activation by regulatory proteins to have physiologic effects. In particular, MMP-13 is secreted by cells as a 60-kDA precursor form (ProMMP-13), and then activated to a 48-kDA form by plasmin and other MMPs [28, 29]. MMP-13 has demonstrated the ability to degrade a variety of ECM proteins, including type I and type II collagen, aggrecan, fibronectin, tenascin, osteonectin, laminin, perlecan, and gelatin [30].

The roles of BMP-2 and MMP-13 in the etiology or prevention of disc degeneration are areas of active investigation. *In-vitro* and *in-vivo* studies have used adenoviruses expressing BMP-2 to evaluate the anabolic reaction on IVDs [7, 21, 31–34]. These studies have shown that BMP-2 has anabolic effects and represent a potential agent for treating disc degeneration. Alternatively, other studies have proposed inhibiting MMP-13 activity as a strategy to slow the progression of disc degeneration [27]. Therefore, the objective of this study was threefold: 1) To quantify ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression in IVD specimens collected from patients undergoing surgery for disc degeneration; 2) To correlate ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression in IVD specimens to the Pfirrmann MRI grading system; and 3) To compare ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression between cervical and lumbar degenerative disc specimens.

MATERIAL & METHODS

Patients and Intervertebral Disc Specimens

This study was approved by our institution's Institutional Review Board, STUDY00000577. Human IVD specimens (including annulus fibrosus and NP) were collected from patients who underwent discectomy and interbody fusion at our institution between 1/2015 and 8/2017. The dissected discs were placed into a sterile container and then transferred into a separate container with 4% paraformaldehyde. Patients were categorized into one of two groups based on their clinical presentation: 1) patients with disc degeneration presenting with cervical or lumbar disc herniation, spinal stenosis, or spondylolisthesis (degenerative group) or 2) patients without disc degeneration being treated for spinal fracture, tumor, or scoliosis (control group). Exclusion criteria for both groups included patients with a history of infection of the spine. Patient characteristics were collected. Magnetic resonance imaging (MRI) was taken of all patients prior to surgery.

Pfirrmann MRI Grading System

The degree of disc degeneration was assessed for each patient using the 5-level Pfirrmann MRI grading system on the T-2 weighted sagittal MRI sequences. The Pfirrmann grades are summarized in Table 1 [35]. Patients with grades I and II, which indicate non-degenerative discs on T2-weighted MRI, were assigned to the control group. Patients with grades III, IV, and V, which indicate degenerative discs, were assigned to the degenerative group. Immunohistochemical expression levels of ALK3, BMP-2, pSMAD1/5/8, and MMP-13 in the control and degenerative specimens were compared. The expression levels were also compared across the Pfirrmann grades. This was done to evaluate whether the degree of disc degeneration that can be assessed on T2-weighted MRI correlates with BMP-2 receptor signaling or MMP-13 activity. In addition, the degenerative specimens were stratified according to their vertebral level and expression levels between the degenerative lumbar and cervical discs were compared. This analysis was done to determine whether BMP-2 receptor signaling or MMP-13 activity in the degenerative specimens vary significantly based on the vertebral level.

Histology and Immunohistochemistry

After collection, the human IVD specimens were fixed in 4% paraformaldehyde, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), and embedded in paraffin. Three- μ m sections were prepared and immunohistochemistry was performed using goat polyclonal anti-ALK3 (BMPR-1A) (Santa Cruz Biotechnology, Dallas, TX), goat polyclonal anti-BMP-2 (Abcam, Cambridge, MA), rabbit polyclonal anti-phosphorylated-SMAD1/5/8 (Cell Signaling Technology, Danvers, MA), and human MMP-13 antibody (R&D Systems, Minneapolis, MN). Permeabilization was done with 0.1% Triton in a TRIS-citrate retrieval buffer at 95°C for 15 min. To block endogenous peroxidase activity, sections were treated with methanol/1% H₂O₂ for 30 min. After the wash, sections were blocked with TBS/0.1% triton/10% normal serum for 1 hr, followed by incubation with the primary antibody for 1 hr and with the corresponding HRP-conjugated secondary antibody. Signal was developed with DAB substrate and slides counterstained with hematoxylin. Slides were photographed. ALK3, BMP-2, and pSMAD1/5/8 slides were analyzed for positive and total

cell count using OsteoMeasure software (Osteometrics, Decatur, Georgia). Expression levels are expressed as a percent ratio of positive cell count to total cell count. MMP-13 slides were analyzed for total signal intensity per area using ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical Analysis—All statistical analyses were performed in GraphPad Prism 9.0 (GraphPad Software, San Diego, CA). A Mann-Whitney U test was used to analyze patient characteristic data and to compare expression levels of ALK3, BMP-2, pSMAD1/5/8, and MMP-13. A Kruskal-Wallis test and Dunn's multiple comparison was used to correlate the Pfirrmann grades with IVD expression levels of ALK3, BMP-2, pSMAD1/5/8, and MMP-13. Significance level was set at $p < 0.05$.

RESULTS

Patient Characteristics

Fifty-two patients were enrolled in the study; however, 2 control and 2 degenerative patients were excluded due to incomplete data sets. Of the 48 remaining patients, 36 IVD specimens were collected from patients with disc degeneration (15 males and 21 females; range 19–82 years) and 12 IVD specimens were collected from control patients (6 males and 6 females; range 14–66 years). There were no significant differences in age, weight, height, body mass index, or vitamin D levels between the two groups ($p > 0.05$). Patient characteristics are summarized in Table 2.

Increased Expression of BMP-2 and pSMAD1/5/8 in Human Degenerated Discs

After surgical collection of the IVD specimens, immunohistochemical staining for ALK3, BMP-2, pSMAD1/5/8, and MMP-13 was performed (Fig. 2a). Staining revealed positive cell cluster formation in the degenerative specimens for BMP-2 (Fig. 2b). BMP-2 expression was significantly elevated in the degenerative specimens as compared to the control specimens ($p = 0.0006$) (Fig. 2c). Staining also revealed positive cell cluster formation in the degenerative specimens for pSMAD1/5/8 (Fig. 3a), which was significantly elevated as compared to the control specimens ($p < 0.0001$) (Fig. 3b). However, no significant difference in ALK3 expression was observed between the degenerative and control specimens ($p = 0.7903$) (Fig. 4a-b). Similarly, no significant difference in MMP-13 expression was present between the degenerative and control specimens ($p = 0.5805$) (Fig. 5a-b). Altogether, these results suggest that BMP-SMAD signal activity is upregulated during disc degeneration, however, catabolic activity, measured by MMP-13 expression levels, was not significantly changed in the degenerative specimens.

Expression of BMP-2 and pSMAD1/5/8 Correlate with Pfirrmann MRI Grades

Next, we used the Pfirrmann MRI grading system to correlate the degree of disc degeneration to expression levels of ALK3, BMP-2, pSMAD1/5/8, and MMP-13. We found that Pfirrmann grade 3 discs ($n = 9$) had significantly upregulated BMP-2 expression as compared to grade 2 discs ($n = 9$) ($p = 0.0365$) (Fig. 6a). In addition, Pfirrmann grade 4 discs ($n = 23$) had significantly upregulated expression of BMP-2 as compared to grade 2 discs ($p = 0.0008$). Grade 4 discs had increased BMP-2 expression as compared to

grade 3 discs, though this was nonsignificant ($p = 0.6288$). In regard to pSMAD1/5/8, Pfirrmann grade 4 degenerated discs had significantly higher expression levels as compared to grade 2 discs ($p < 0.0001$) (Fig. 6b). Though nonsignificant, Pfirrmann grade 3 discs had increased pSMAD1/5/8 expression as compared to grade 2 discs ($p = 0.2430$). Also, there were no significant differences in ALK3 or MMP-13 expression across any of the Pfirrmann grades ($p > 0.05$) (Fig. 6c-d). Altogether, these findings suggest that BMP-2 and pSMAD1/5/8 expression correlate positively with the degree of disc degeneration, while ALK3 and MMP-13 activity do not change depending on the severity of disc degeneration, measured according to the Pfirrmann MRI grading system.

Expression Among Degenerated Discs Do Not Differ Based on Vertebral Level

Previous studies have suggested that degenerative disc pathology differs according to the vertebral level [36]. As such, we sought to determine whether there was differential expression of ALK3, BMP-2, pSMAD1/5/8, and MMP-13 between the degenerative lumbar and cervical discs. The degenerative specimens were stratified into lumbar ($n = 27$) and cervical ($n = 9$) discs, and expression levels were compared. We found no significant difference in expression between the degenerative lumbar and cervical discs for BMP-2 ($p = 0.7536$), pSMAD1/5/8 ($p = 0.6994$), ALK3 ($p = 0.0765$), or MMP-13 ($p = 0.0794$) (Fig. 7a-d). These findings suggest that the BMP-SMAD signal and MMP-13 activity during disc degeneration do not significantly differ based on the vertebral level.

DISCUSSION

Disc degeneration is characterized by a loss of proteoglycans from the ECM of the IVD, changing the compressive load on the vertebral bodies and causing injurious changes to the spine [37, 38]. It has been proposed that as IVDs age and degenerate, there is a physiologic compensatory upregulation of BMPs and their downstream signaling receptors as an anabolic response. This notion is supported by two studies comparing IVD gene expression between young and old New Zealand white (NZW) rabbits and reporting significantly higher mRNA levels of BMP-2 in the older rabbits [39, 40]. Similarly, an immunohistochemical study of rodent cervical spines found a differential expression of BMP-2 between young and old mice [41]. Furthermore, investigations using an external fixation device to induce lumbar disc degeneration in NZW rabbits found upregulated BMP-2 gene expression [42, 43].

In our study, we compared BMP-2 receptor signaling expression between human degenerative and non-degenerative IVDs. Our results indicate a higher expression level of both BMP-2 and pSMAD1/5/8 in human degenerative discs as compared to control specimens. Consistent with previous studies, we believe that this increased expression represents a protective and anabolic response to disc injury and degeneration. Of significant note, we found no upregulated ALK3 expression in the degenerative specimens. Though ALK3 represents the type IA receptor that binds BMP-2, it is possible that the upregulated BMP-SMAD signal activity in response to disc degeneration is mediated via a type IB receptor or another yet to be determined pathway. It is also possible that in these conditions, ALK3 gene simply does not transcriptionally respond to increased BMP-SMAD signaling.

In addition to quantifying expression levels in IVDs of control and degenerative patients, we also attempted to correlate ALK3, BMP-2, pSMAD1/5/8, and MMP-13 expression to the Pfirrmann MRI grading system. The Pfirrmann grading system is a validated tool to evaluate degenerative abnormalities on T2-weighted sagittal MRI. It classifies IVD degeneration based on signal intensity, disc structure, distinction between nucleus and annulus, and disc height [44]. In our study, Pfirrmann grade 3 IVD specimens had significantly higher BMP-2 expression as compared to the healthier grade 2 discs. Also, Pfirrmann grade 4 IVD specimens showed significantly higher BMP-2 and pSMAD1/5/8 expression as compared to grade 2 discs. No significant differences were observed across the other Pfirrmann grades. Our results demonstrate a positive correlation between BMP-SMAD activity and the degree of disc degeneration measured on MRI according to the Pfirrmann criteria. Interestingly, it has been reported that MRI signal change correlates directly with proteoglycan concentration within the IVD [45]. While degenerated discs are known to have proteoglycan loss, the upregulated BMP-SMAD signaling that we observed in the degenerative discs likely represents an insufficient anabolic response to injury. The control discs, on the other hand, had lower BMP-2 and pSMAD1/5/8 expression likely because there was less inciting injury prompting a compensatory anabolic response.

The anabolic role of BMP-2 in the IVD has been well documented in animal models. In a study of NZW rabbits that received stab injuries to their lumbar spine, there was a reported delayed yet upregulated expression of BMP-2, suggesting an inability of the IVDs to mount an early anabolic response to disc injury [46]. The authors proposed that early use of exogenous BMPs in the setting of disc degeneration may be beneficial in promoting reparative processes. Several studies have shown *in vitro* that human IVD cells transfected with recombinant human BMP-2 promote a chondrocyte-like phenotype with increased production of aggrecan and type II collagen, as well as an increase in proteoglycan synthesis [32, 47]. Similarly, a study utilizing simvastatin containing polymer gels injected into a rat tail degenerative disc model to upregulate BMP-2 expression found an increase in aggrecan expression, as well as a significant improvement in NP weight, T2 signal intensity, and MRI index [33]. In addition to promoting cellular proliferation within the IVD [48], BMP-2 has also been shown to have anti-catabolic effects in human NP cells by antagonizing IL-18 mediated disc degeneration [49]. Accordingly, these studies in conjunction with our results demonstrate the potential utility of upregulating BMP-SMAD signaling as a therapeutic strategy for treatment of disc degeneration. Given that we found no significant difference in upregulated BMP-SMAD activity between the degenerative lumbar and cervical discs, we propose that promoting BMP-SMAD signaling would be beneficial in both lumbar and cervical degenerative disc pathology.

Our study also found no significant difference in MMP-13 expression when comparing between the control and degenerative specimens, as well as when comparing across the Pfirrmann grades. However, the grade 1 discs had noticeably lower expression levels compared to the grade 2–5 discs. While the low sample size for grade 1 discs precluded our ability to infer statistical significance, our results may suggest that the mildest degree of disc degeneration is associated with increased catabolic activity, though beyond Pfirrmann grade 2 discs, MMP-13 activity is not significantly upregulated. These results are supported by Le Maitre et al. [50] who reported increased MMP-13 expression in discs with early

degeneration, however in discs with higher grades of disc degeneration, MMP-13 levels did not significantly change. Similarly, Klawitter et al. [51] reported increased MMP-28 expression in cases with disc degeneration, but found no significant correlation between the severity of disc degeneration and MMP-28 expression. One possible explanation for these and our results is that upregulated BMP-2-mediated anabolism counteracts the catabolic signals during disc degeneration. Several studies have described the inhibitory effects of BMPs on MMPs [52–54]. In fact, in a study examining the effect of BMP-2 on the human carcinoma cell line TSU-Pr1, Kumagai et al. [55] showed that BMP-2 inhibits the activity, migration, and secretion of MMP-9. It should be noted though that other studies have reported significant correlations between MMP expression and the degree of disc degeneration [56–58], and as such, future work will be needed to further elucidate the interactions of BMPs and MMPs in the setting of disc degeneration. In addition, understanding the temporal pattern of change in anabolic and catabolic markers during the development and progression of disc degeneration will be crucial for the discovery of therapeutic treatments.

Our study contributes to the growing literature describing the anabolic role of BMP-2 in response to disc degeneration. One limitation to our study is the small sample size for Pfirrmann grade 1 and grade 5 discs. In addition, while we report upregulated expression of both BMP-2 and pSMAD1/5/8 using immunohistochemistry, we cannot draw any conclusions regarding the mechanism underlying this anabolic response. Due to the practicality of intraoperative discectomy, we also did not discern between the annulus fibrosus and NP. Lastly, while we used MMP-13 as a catabolic marker, others exist which are also known to contribute to the development of disc degeneration.

To our knowledge, this is the first study to evaluate the expression of BMP-2 type I receptor ALK3 in human degenerative discs. While several studies have utilized the Pfirrmann grading system to investigate expression of genes associated with disc degeneration [59, 60] and their response to BMP-2 treatment [61], here we report that BMP-2 and pSMAD1/5/8 have significantly upregulated expression in human degenerative discs as compared to the non-degenerative control discs. Interestingly, we did not find any difference in ALK3 expression between the control and degenerative specimens, suggesting that an alternative receptor or pathway was activated to increase BMP-SMAD signaling or that ALK3 gene simply does not transcriptionally respond to BMP-2 signaling in these conditions. Pfirrmann grade 3 and grade 4 IVD specimens were associated with higher levels of BMP-2 and pSMAD1/5/8 expression as compared to Pfirrmann grade 2 discs. In addition, MMP-13 expression levels were not significantly different between the control and degenerative specimens. This is potentially related to upregulated BMP-2 activity counteracting MMP-13 signal. In future work, we plan to elucidate the mechanism of upregulated BMP-2 signaling in the setting of disc degeneration, as well as evaluate the protective effects of gain-of-function BMP signaling in a mouse model of disc degeneration.

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Abbreviations:

IVD	intervertebral disc
NP	nucleus pulposus
ECM	extracellular matrix
IL	interleukin
BMP	bone morphogenic protein
TGF-β	transforming growth factor- β
ALK3	Activin receptor like-kinase 3
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
NZW	New Zealand white

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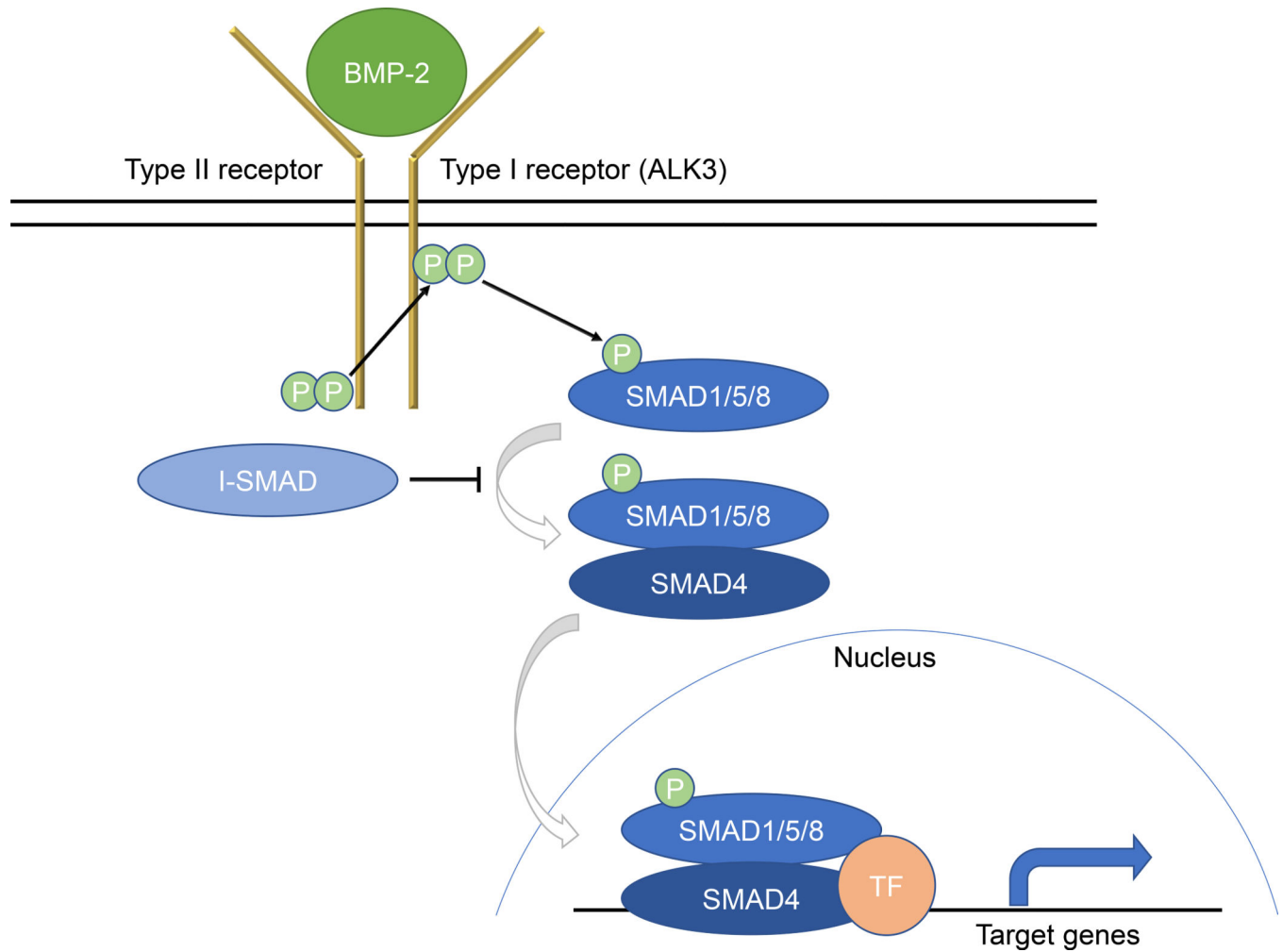
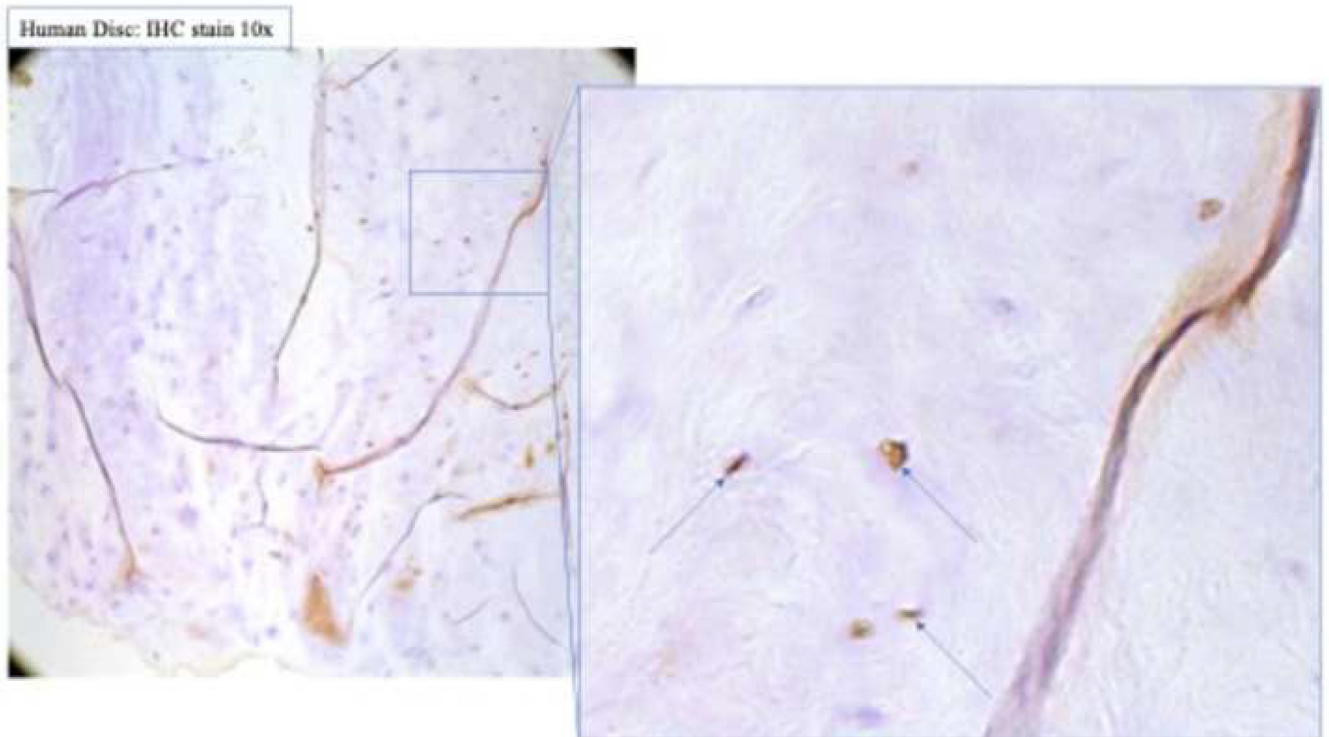
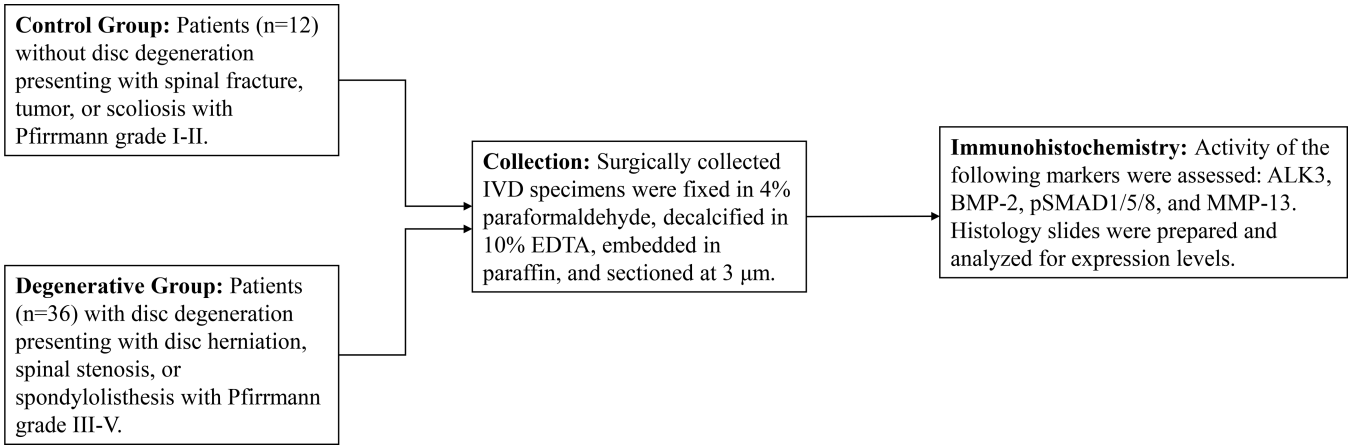
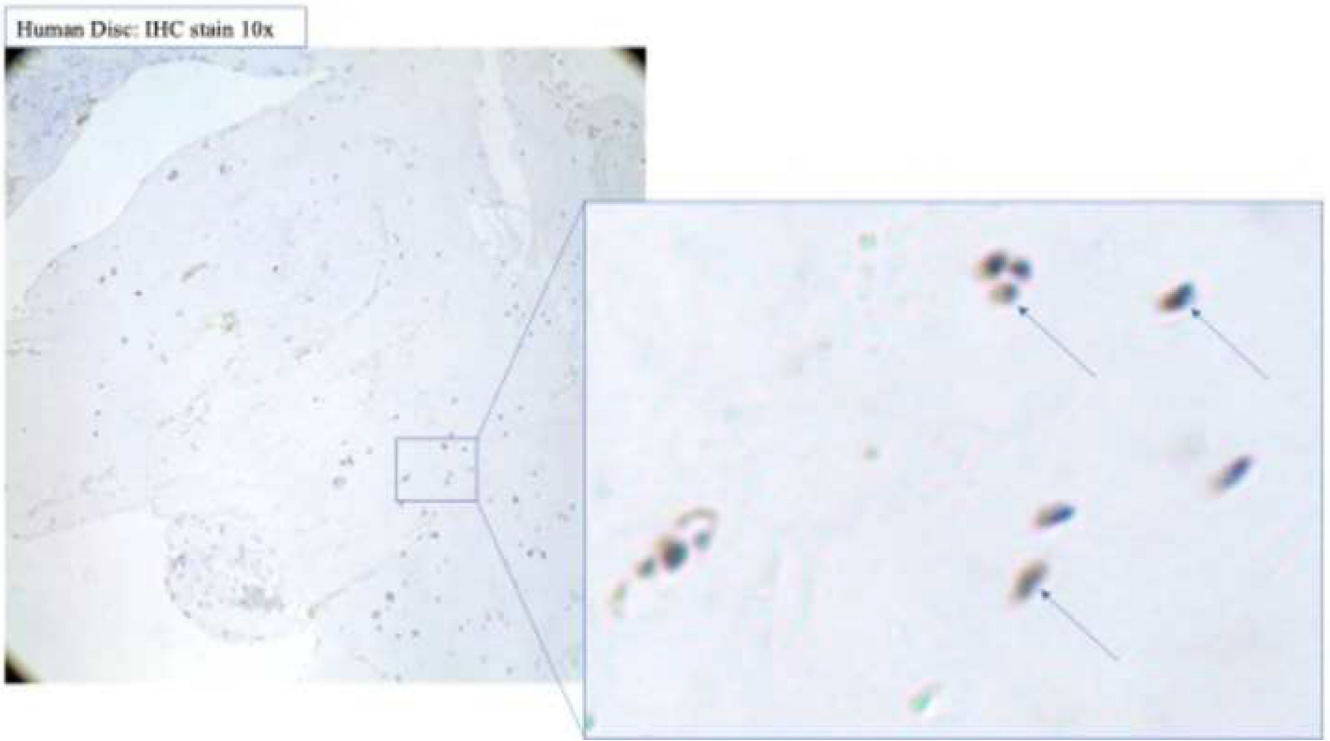


Figure 1. Schematic of the BMP-2 signaling pathway.

Upon BMP-2 binding to two serine/threonine kinase receptors (type I and type II), the type II receptor transphosphorylates the type I receptor (e.g., ALK3). After becoming active, the type I receptor phosphorylates SMAD1/5/8 to propagate the signal into the cell. pSMAD1/5/8 then forms a hetero-oligomeric complex with SMAD4, where together they translocate to the nucleus to interact with specific transcription factors (TF) to regulate target gene expression.





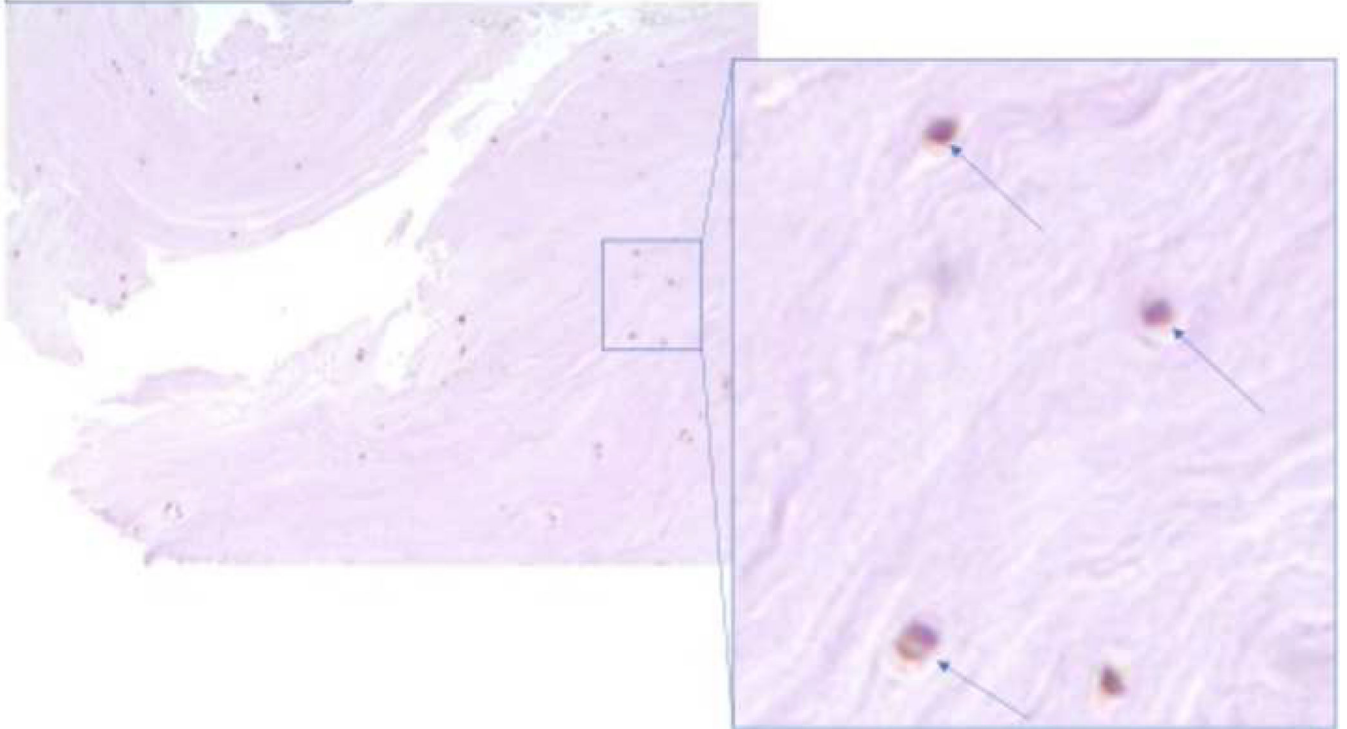
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Human Disc: IHC stain 10x

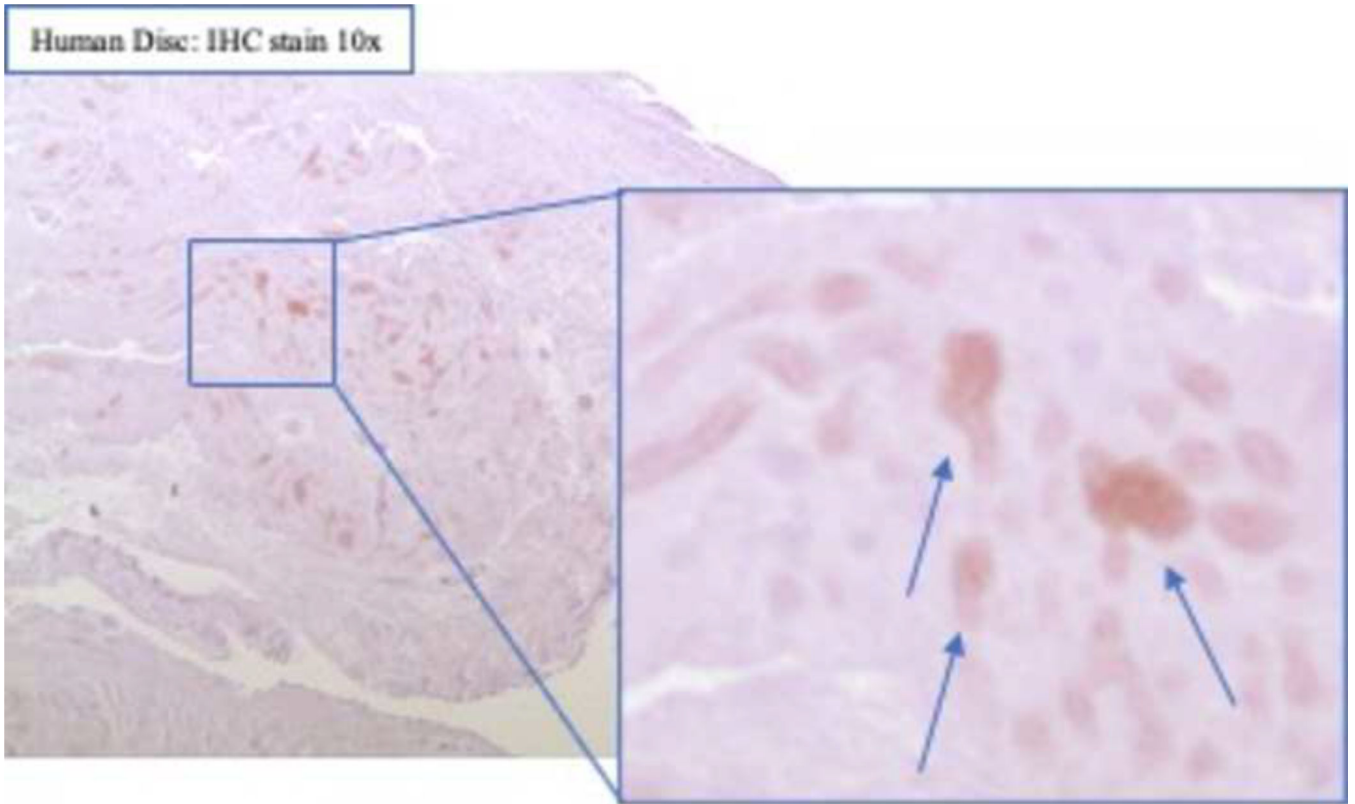


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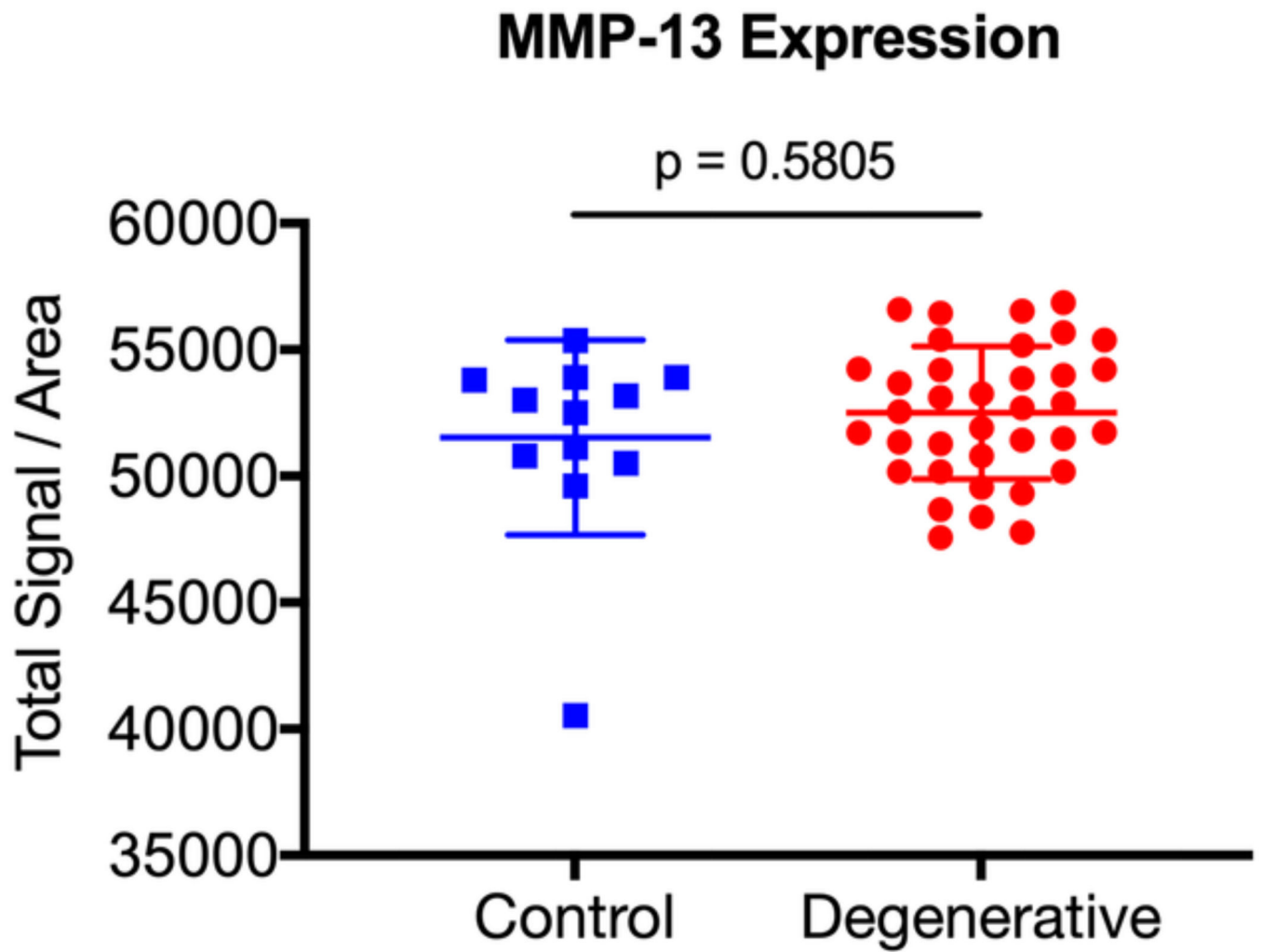
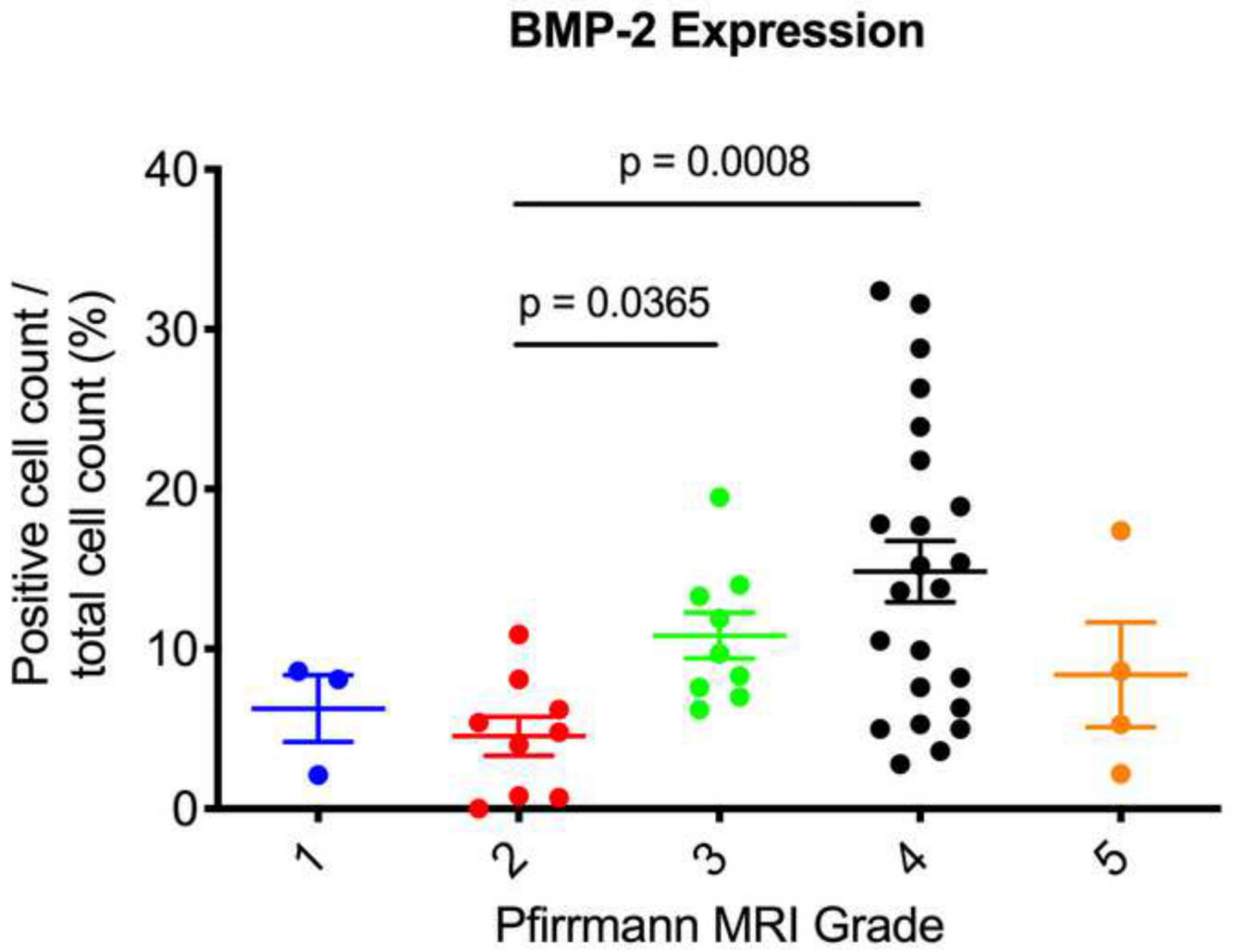


Figure 5. MMP-13 expression does not significantly change in human degenerated discs. (a) Staining revealed positive signal intensity in the degenerative specimens. (b) MMP-13 expression was not significantly different between the degenerative discs and the control specimens.

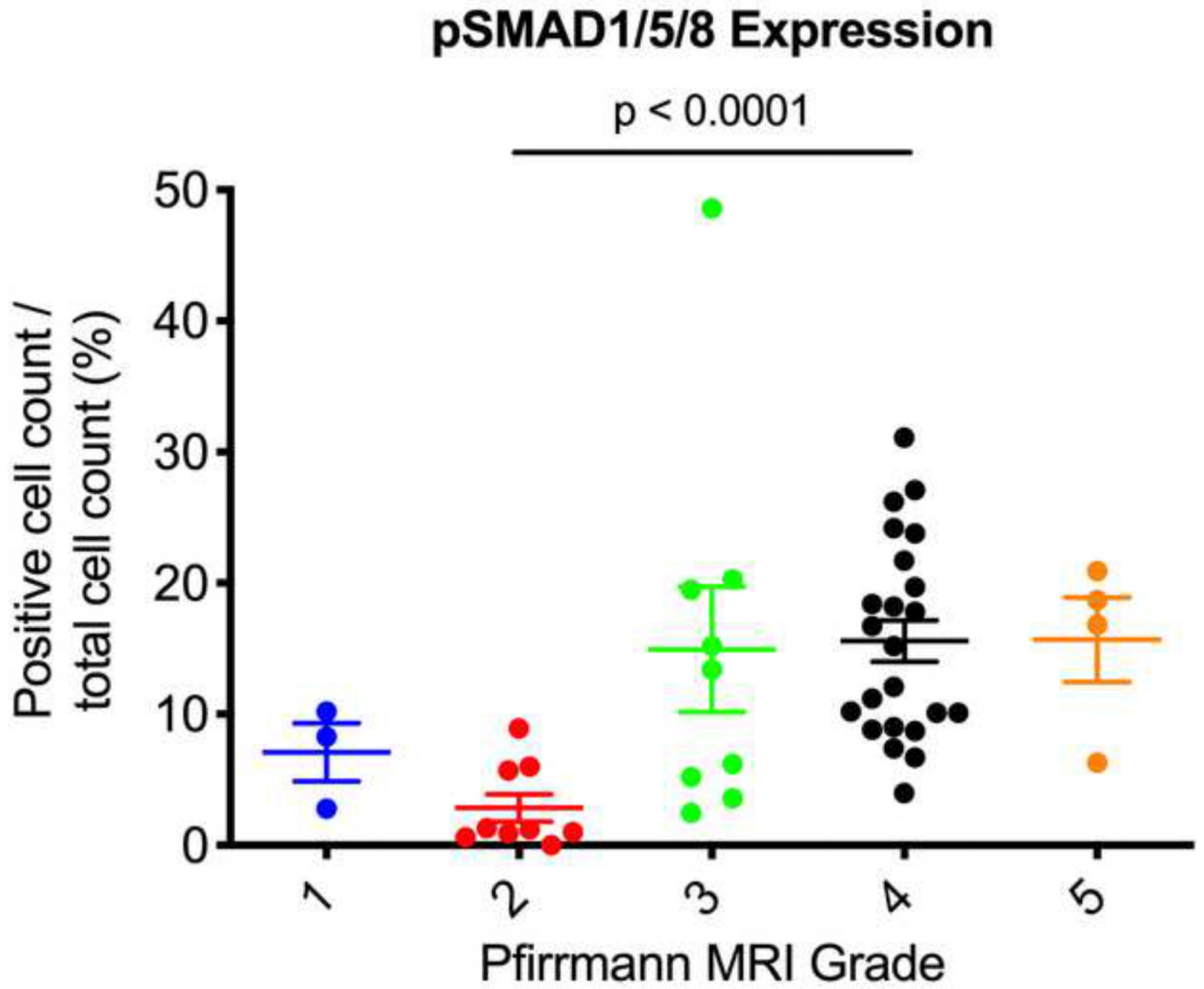


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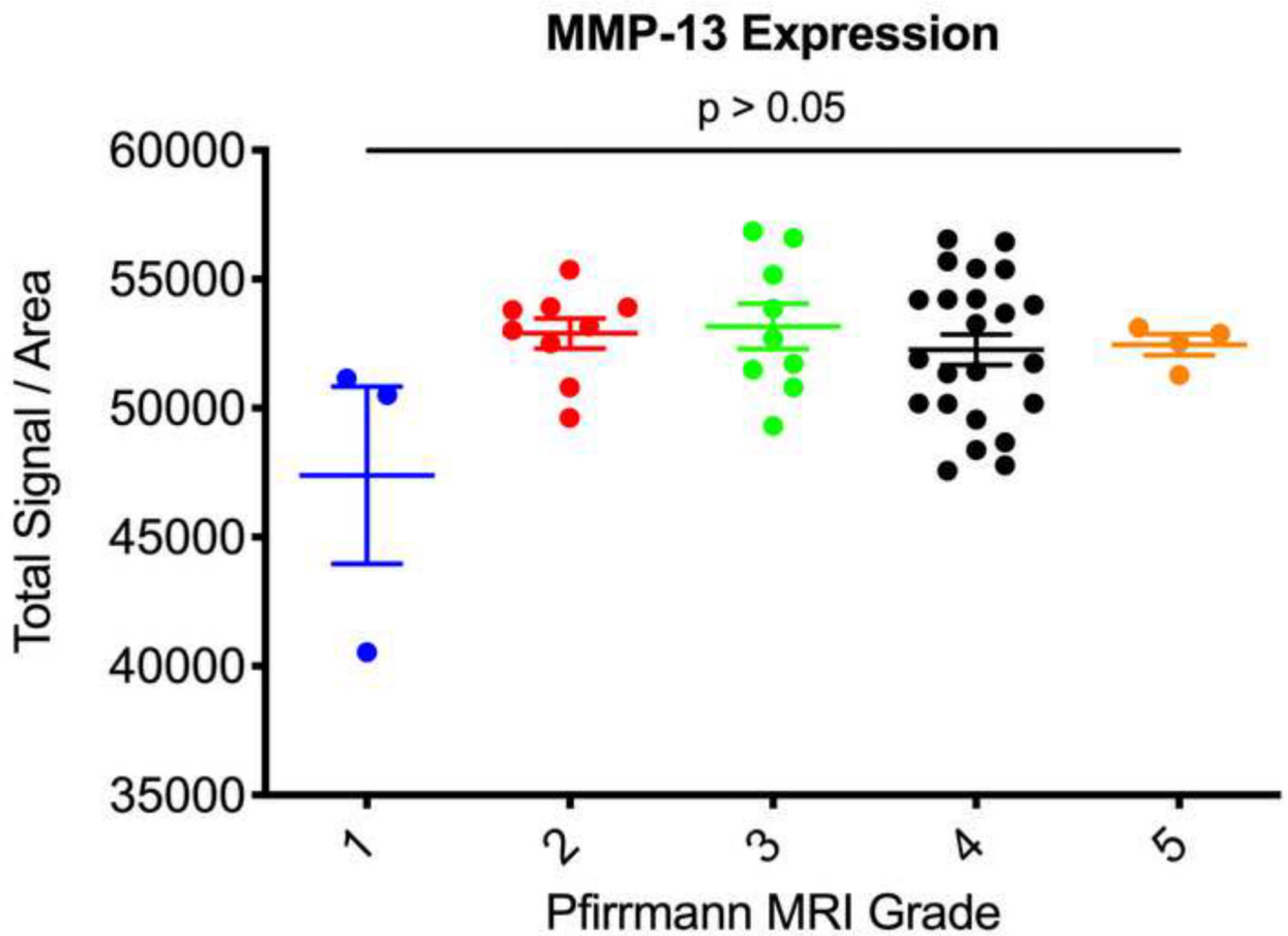
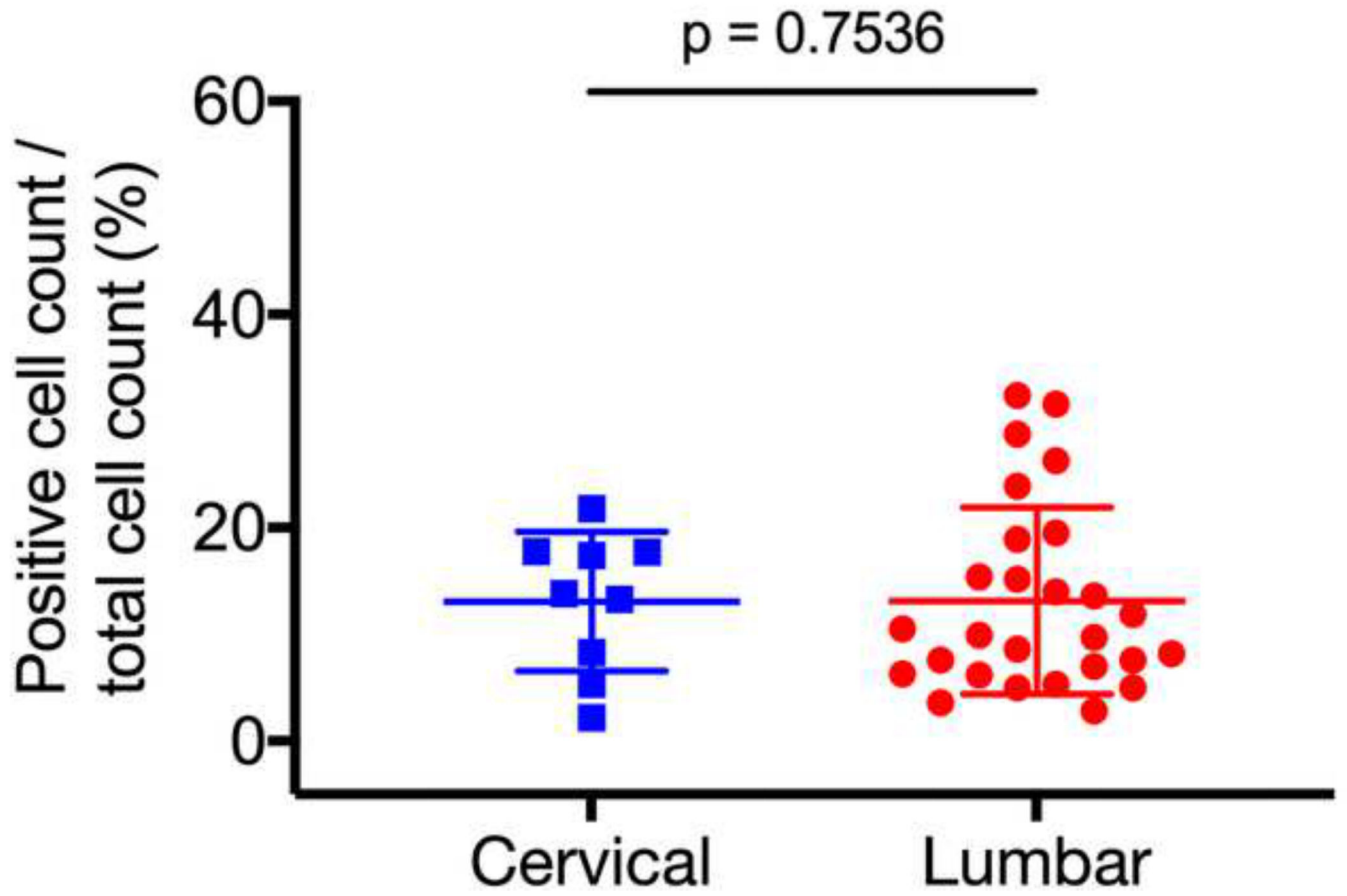


Figure 6. BMP-2 and pSMAD1/5/8 expression correlate with Pfirrmann MRI grades.

(a) Pfirrmann grade 3 and grade 4 discs had significantly higher BMP-2 expression as compared to Pfirrmann grade 2 discs. (b) Pfirrmann grade 4 discs had significantly higher pSMAD1/5/8 expression as compared to Pfirrmann grade 2 discs. (c) No significant differences in ALK3 expression were observed across the Pfirrmann grades. (d) No significant differences in MMP-13 expression were observed across the Pfirrmann grades.

BMP-2 Expression



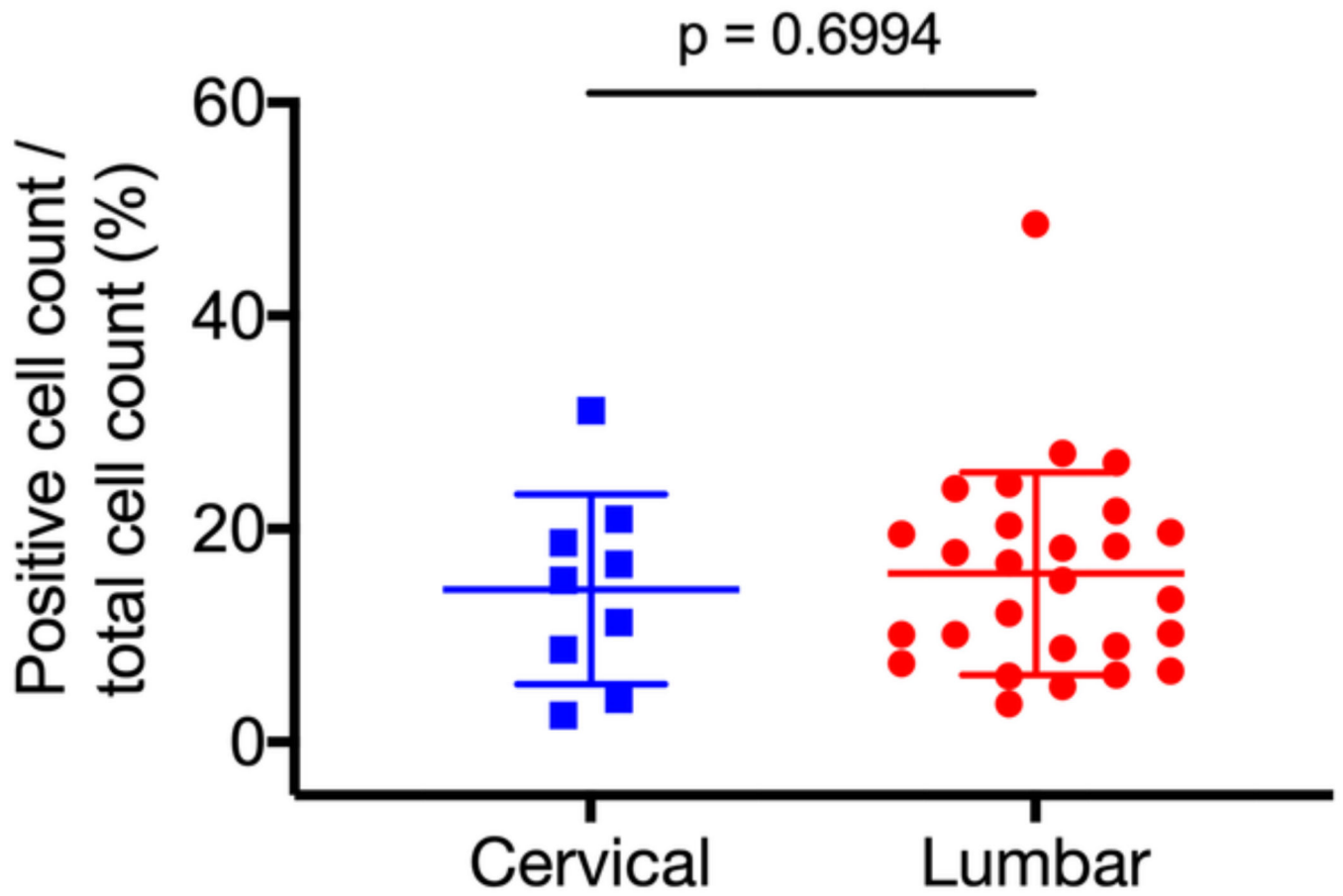
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pSMAD1/5/8 Expression



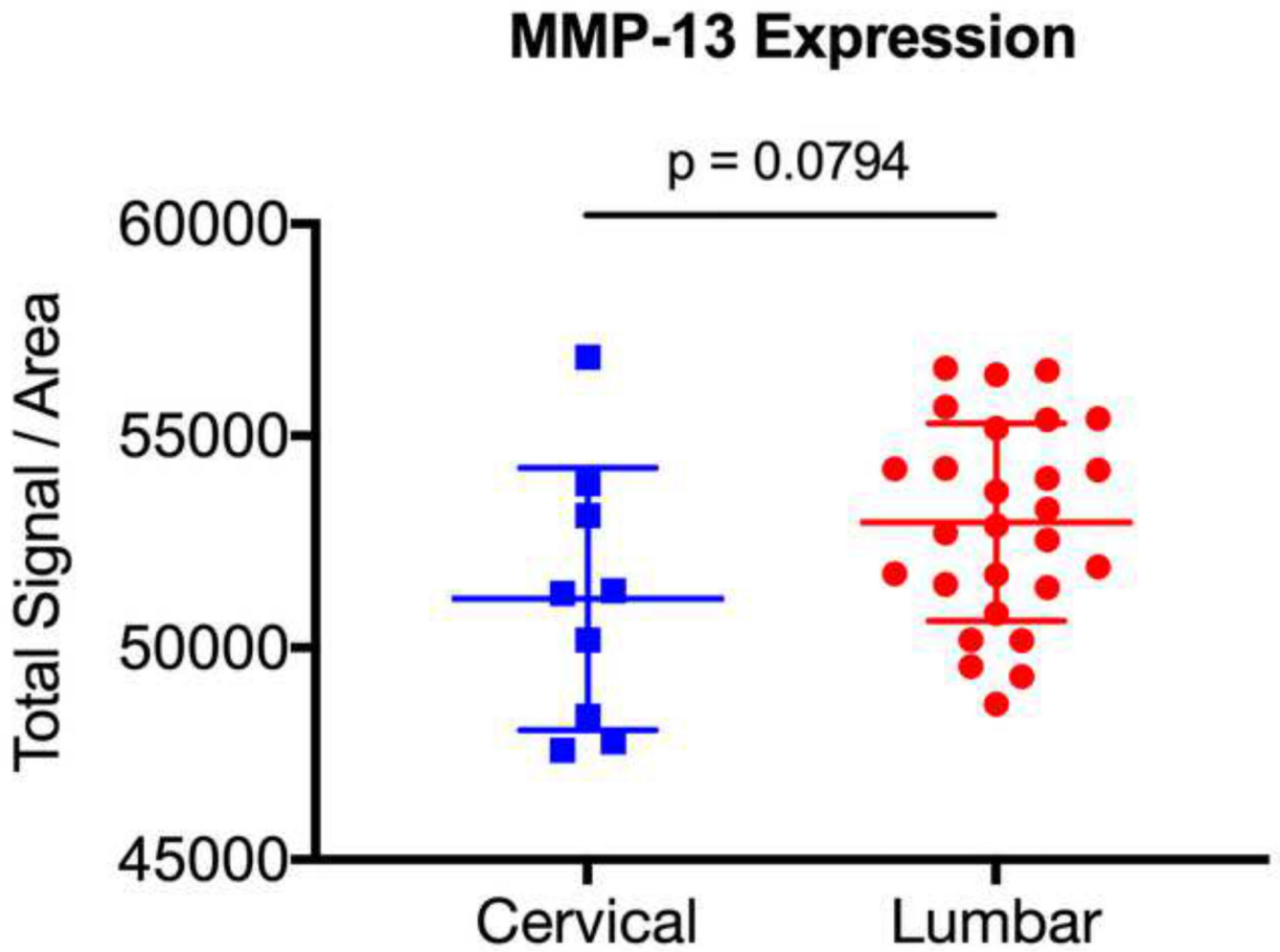


Figure 7. BMP-SMAD expression in degenerated discs do not vary based on the vertebral level. The degenerative specimens were stratified into lumbar (n = 27) and cervical (n = 9) discs, and expression levels were compared. No significant differences in expression were observed between the degenerative lumbar and cervical discs for (a) BMP-2, (b) pSMAD1/5/8, (c) ALK3, and (d) MMP-13.

Table 1:

Pfirrmann Classification of Intervertebral Disc Degeneration

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

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Table 2:

Patient Characteristics

	Control (n=12)	Degenerative (n=36)	P-value
Age, years (mean \pm SD)	39.8 \pm 17.6	44.2 \pm 15.2	0.4763
Male:Female	6:6	15:21	
Race			
Caucasian	12	28	
African American	0	6	
Asian American	0	1	
Other	0	1	
Weight, kg (mean \pm SD)	81.9 \pm 28.5	88.9 \pm 20.7	0.1066
BMI, kg/m ² (mean \pm SD)	27.3 \pm 7.5	31.2 \pm 7.5	0.0984
Height, m (mean \pm SD)	1.7 \pm 0.1	1.7 \pm 0.1	0.2620
Vitamin D, ng/mL (mean \pm SD)	34.9 \pm 14.1	25.3 \pm 9.7	0.2657
Smoking			
Current	2	9	
Former	3	7	
Never	7	20	
EBL	384.1 \pm 526.3	163.5 \pm 370.6	0.0989
Pfirrmann grade	1.75 \pm 0.5	3.9 \pm 0.6	< 0.0001
Diagnosis			
Facet slip/fracture	3	0	
Vertebral body fracture	4	0	
Tumor	3	0	
Scoliosis	1	0	
Other	1	0	
Disc herniation	0	28	
Spondylolisthesis	0	6	
Stenosis	0	2	
Procedure			
Anterior cervical fusion	11	9	
Lumbar discectomy	0	21	
Lumbar posterior spinal fusion	1	6	
Immunohistochemical Staining (mean \pm SD)			
BMP-2	5.0 \pm 0.03	13.1 \pm 0.08	0.0006
SMAD1/5/8	3.9 \pm 0.04	15.4 \pm 0.09	< 0.0001
ALK3	7.5 \pm 0.1	8.5 \pm 0.1	0.7903
MMP-13	51526.3 \pm 3855	52509.6 \pm 2617	0.5805

Abbreviations: BMI, body mass index; SD, standard deviation; BMP-2, bone morphogenic protein-2; ALK3, Activin receptor like-kinase 3; MMP-13, matrix metalloproteinase-13.