Research Article Open Access

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Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration

https://doi.org/10.1515/bmc-2018-0003
received January 22, 2017; accepted March 23, 2018.

Abstract: Chronic low back pain is a critical health problem and a leading cause of disability in aging populations. A major cause of low back pain is considered to be the degeneration of the intervertebral disc (IVD). Recent advances in therapeutics, particularly cell and tissue engineering, offer potential methods for inhibiting or reversing IVD degeneration, which have previously been impossible. The use of growth factors is under serious consideration as a potential therapy to enhance IVD tissue regeneration. We reviewed the role of chosen prototypical growth factors and growth factor combinations that have the capacity to improve IVD restoration. A number of growth factors have demonstrated potential to modulate the anabolic and anticatabolic effects in both in vitro and animal studies of IVD tissue engineering. Members of the transforming growth factor-β superfamily, IGF-1, GDF-5, BMP-2, BMP-7, and platelet-derived growth factor have all been investigated as possible therapeutic options for IVD regeneration. The role of growth factors in IVD tissue engineering appears promising; however, further extensive research is needed at both basic science and clinical levels before its application is appropriate for clinical use.

Keywords: growth factor; intervertebral disc; tissue engineering; intervertebral disc disease.

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Introduction

Chronic low back pain remains a prevalent and widespread complaint among patients seeking medical attention worldwide. Low back pain contends as one of the top chronic debilitating problems for many people, and continues to impose itself as a detriment to the growing healthcare concerns in terms of both direct medical costs and otherwise decreased productivity. While a wealth of variable statistics exists, the lifetime prevalence of low back pain is generally accepted to be in the 70–85% range, suggesting that most people will be afflicted by this ailment at some point in their lives[1]. When considering the annual prevalence, studies estimate a 15–45% annual occurrence rate and an average point prevalence of roughly 30%[1]. Low back pain is associated with huge economic and social burden in the United States. More than $100 billion per year is the estimated total cost of low back pain. 70% of this cost is a result of reduced productivity and lost wages[2]. Disability-adjusted life years burden of low back pain increased from 58.2 million in 1990 to 83 million in 2010[3].

With annual and lifetime prevalence this high, it stands to reason that the growing interest and research devoted to both the pathophysiology and potential treatment regimens for low back pain are well founded. The overwhelming culprit for the majority of chronic low back pain is degeneration of the intervertebral disc (IVD). Over a 10-year period from 1997–2007, the scientific and medical communities have witnessed a three-fold increase in the number of peer-reviewed journal articles devoted to various aspects of biology and biotherapeutics related to IVD disease[4]. Further, the documented impact on the hampered quality of life and even disability rates are formidable among low back pain sufferers. Among the United States population under 45-years old, back pain is the leading cause of activity limitation, second most common cause for physician visit, third for surgical intervention, and fifth most frequent cause for hospital admission[5-7]. These are impressive, yet appropriate, numbers that reflect the heightened awareness of low back pain as an area in need of more effective therapeutic treatment

options. Considering the potential sequalae of the costly and invasive surgical procedures, alternative treatment modalities via biogenetic modification is an area meriting investigation. Several biological based therapies have gained attention either as currently practiced options or as potential biologic and genetic manipulation methods. In addition to conventional surgical and pain management options, research communities are investigating disc or stem cell implantation, painful disk denervation, gene therapy, and injection of specific therapeutic proteins. While each of these approaches has its respective promise, for the purpose of this review we will primarily focus on the therapeutic possibilities revolving around prototypical growth factors involved in IVD disease.

With such an impact being made on the quality of life of so many patients, there has been an increase in efforts to find a minimally invasive, safe, and effective treatment through the injection of targeted proteins to delay or reverse the known pathology that comes with IVD disease. Much attention has been paid to prolotherapy as an effective way to provide some improvement to chronic low back pain. Prolotherapy is minimally-invasive injection of factors to promote proliferation of normal cells and tissues. Prolotherapy includes three main types, growth factor injection prolotherapy, growth factor stimulation prolotherapy, and inflammatory prolotherapy[8,9]. Growth factors injection prolotherapy has been heavily investigated recently to assess its feasibility, safety, and efficacy for IVD degeneration. Preliminary data have been generated over the past few years about the outcomes after the intradiscal administration of the following set of growth factors: platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), fibroblast growth factor-18 (FGF-18), growth and differentiation factor-5 (GDF-5), transforming growth factor beta-1 (TGF-β1), bone morphogenetic protein-2 (BMP-2), and bone morphogenetic protein-7 (BMP-7). The metabolic activity of IVD cells is modulated and regulated by several enzymes, cytokines, and growth factors via either autocrine or paracrine manner[10]. Any deviation from the homeostatic balance between the anabolic and catabolic factors in the normal, healthy disc will result in degenerative process of IVD. The definite role and mechanism of growth factors within the normal disc are poorly understood[10] .Our aim is to provide a brief evidence-based review about some of the previous and ongoing research concerning the prospect of the growth factors and their role in IVD tissue engineering; we do not aim to comprehensively cover all the existing literature, or all the suspected growth factors involved in degenerative disc disease (DDD).

Pathophysiology Of Intervertebral Disc Disease

Beginning with the anatomical makeup, three key components contribute to the biomechanics and pathophysiology of the IVD: the inner nucleus pulposus (NP), the outer annulus fibrosus (AF), and the adjacent vertebral end plates. The NP is surrounded by the AF in a circumferential manner, while the end plates provide separation from the disc superiorly and inferiorly. These structures are primarily composed of differing ratios of proteoglycans, collagen, and water. While the NP serves as more of a cushion due to its significant fractional content of water, the AF and end plates combine to dissipate the compression forces and shield the vertebral bodies from bony compromise.

At a cellular level, the NP is comprised of a roughly 1:20 ratio of type II collagen to the proteoglycan aggrecan[11]. The aggrecan creates an osmotic gradient that helps draw water into the IVD[12]. The hydrophilic nature of aggrecan and its abundance in the NP are primary contributors to the spongy and cushioning nature of the IVD. Further, these functionally desirable properties are compromised in degenerative disc disease to the extent that much of the type II collagen and aggrecan of the matrix composition is replaced by type I collagen and an associated decrease in aggrecan synthesis[13]. In turn, the IVD loses some of its hydrophilic nature and the accompanying space-retaining aspects of the disc, ultimately leading to abnormal loading, movement, and significant pain which ends up with constricted health-related quality of life.

In contrast to the NP, the AF has a composition heavily weighted with type I collagen yielding a more structurally sound matrix to circumferentially contain the inner NP. Like the NP, the composition of the AF gives insight to its functional properties. Roughly 60% of the AF is collagen, while another 25% is aggrecan, yielding the more stringent properties of this structural component compared to the NP[14,15]. The many lamellae provide rotational support and counterbalance the loading forces of adjacent vertebrae[4].

As the natural balance of the forces of the AF and NP is disturbed, shock loading is promoted at the enthesis during ordinary movement[4]. The potential result of this imbalance is painful movement and microtrauma. This microtrauma to the AF and surrounding bone allows angio- and neurogenesis to the IVD, where it is typically absent[16]. This angiogenesis has been shown to detrimentally affect patients' intensity of pain, postoperative pain improvement, mobility, and overall quality of life as a consequence of IVD herniation[17]. The aforementioned

Table 1: In vivo studies of Growth factors injection therapy for IVD degeneration.

Author	Animal model	Growth factor	Dose	Results
PDGF-BB				
Paglia et al.[20]	Rabbit [Annular puncture]	PDGF-BB	1 ng/μL/disc	↓ disc degeneration via preventing apoptosis and increasing collagen-3 matrix production
IGF-1				
Takayama B[61]	Rat [Compression]	IGF-1	-----	IGF-1 knockdown caused a reduction in mechanical allodynia
BMP-7 (OP-1)				
An HS[77]	Normal Rabbit	BMP-7	2 ng/10 μL	↑ disc height
Masuda K[64]	Rabbit [Puncture]	BMP-7	100 μg/10 μL	↑ disc height
Imai Y[63]	Rabbit [Chemonucleolysis]	BMP-7	100 μg/10 μL	↑ disc height and proteoglycans
Kei Miyamoto [78]	Rabbit [Puncture]	BMP-7	100 μg/10 μL	↑ disc height and proteoglycans
BMP-2				
Huang KY[78]	Rabbit [Annular tear]	BMP-2	100 μg/10 μL	↑ hypervascularity and fibroblast proliferation of the intervertebral disc after an annular tear.
GDF-5				
Walsh AJ[44]	Rat [Compression]	IGF-1, GDF-5, TGF-β, bFGF	IGF-1: 8 ng/8 μL GDF-5: 8 ng/8 μl TGF-β: 1.6 ng/8 μL bFGF: 8 ng/8 μL	GDF-5 and TGF-β ↑ expansion of inner annular chondrocytes into the nucleus
Chujo T[43]	Rabbit [Puncture]	GDF-5	100 μg/10 μL	↑ matrix synthesis and cell proliferation
Liang[45]	Mice [Puncture]	Adeno-GDF-5	1.8×1011pfu/mL	↑ disc height

microtraumatic effects in the NP can be seen in the AF and end plates resulting in fracture and facet compromise, resulting in an insult to structural integrity and presumed attenuation of degenerative changes. While a sufficient number of human studies that connect microtrauma with pain are still lacking, the link between in vitro microtrauma with the resulting angio-/neurogenesis and IVD pain yield a convincing juxtaposition that warrants ongoing consideration.

The previously described changes in the motion segment are related to the matrix changes of the IVD; however, the cellular biology of the NP, AF, and end plates serve to mitigate these motion segment variations[18]. It is also worth mentioning that the contentious issue of growth factor activation and the relentless cascade of degeneration continue to create end plate compromise and challenge disc integrity; these issues will surely be the focus of future research. Furthermore, much of the current literature focuses on the composition and alteration of the NP with less interest being paid to the AF cells; however, manipulating and potentially reversing some of the catabolic changes in the NP cells would also structurally benefit the AF. While the discussion has largely been based on the anatomic and structural aspects of IVD disease, these areas of focus are overshadowed by research in the field of biologic treatment options for degenerative disease. We will discuss several pertinent approaches to this area of study and potential treatment modalities.

The past two decades have seen a surge of research investigating the balance of anabolic and catabolic factors affecting IVD disease. Previously demonstrated in the literature, specific alterations in growth factor and cytokine expression alter matrix synthesis and accordingly adjust IVD homeostasis by shifting cellular metabolism to the anabolic state[10,18,19]. In turn, the anabolic state and cell proliferation are likely signs of tissue repair and hallmarks of IVD. We will briefly touch on each factor in the context of reviewing the tissue engineering approaches that are targeted for potential clinical application in the future [Table 1].

Platelet-derived Growth Factor (PDGF)

At a fundamental level, PDGF plays a role in angiogenesis and the growth of existing blood vessels. PDGF falls

into the category of anti-catabolic mitogens, which act to retard cellular breakdown and turnover. Paglia et al. (2016) demonstrated that when PDFG-BB is delivered in a thiol-modified hyaluronic acid hydrogel in rabbit preclinical DDD model, it significantly decreases disc degeneration via preventing apoptosis and increasing collagen-3 matrix production maintains the disc structure, and facilitates biomechanical functions[20]. Gruber et al. (2000) demonstrated that PDGF's influence significantly reduced the percentage of apoptotic AF cells induced by serum depletion in culture[21]. More recently, Pratsinis et al.[22] reported increased DNA synthesis of human IVD cells through the activation of the MEK/ERK and the PI-3K/Akt signal transduction pathways both in cell culture and by way of immunohistochemistry IHC results from human biopsies. Furthermore, Presciutti et al. (2014)[23] also reported similar findings that PDGF-BB inhibit IVD cell apoptosis and promote anabolic gene expression. Similar to other proteins we review, PDGF is a focus of research primarily because of its anti-catabolic properties and the possible implications of in vivo protein manipulation.

Insulin-like Growth Factor-1 (IGF-1)

Similar in structure to insulin, IGF-1 is vital in childhood growth and continues to play an important anabolic role in adults. The mitogenic IGF-1 levels have been shown to be down-regulated as IVD degeneration ensues and leads to decreased cell proliferation[24]. Conversely, Osada et al. (1996) demonstrated the stimulatory effect of IGF-1 on IVD cell proliferation and matrix synthesis[25]. Much of our discussion around PDGF can be applied to IGF-1, particularly in terms of pathway. The significant reduction in apoptotic AF cells mentioned in Gruber's study relating to PDGF was similarly demonstrated with IGF-1 (17).

In terms of patient-centered and therapy-focused research, IGF-1 has promising upside for future use. Takayama et al. (2011)[26] looked at IGF-1 localization and function in the dorsal root ganglion cells in a rat model of disc herniation. They were able to demonstrate that IGF-1 knockdown caused a reduction in mechanical allodynia, and later concluded that IGF-1 upregulation may play a key role in painful radiculopathy induced by mechanical factors. As was previously mentioned, one potential therapeutic approach would be the injection of growth factors to target upregulating anabolic growth factors while downregulating degradative factors influencing apoptotic and senescent cells. Gruber at al. (2008)[27] aimed at using IGF-1 exposure on human annulus cells to prevent or ameliorate senescence in vitro; they demonstrated a significant reduction following exposure to 500 ng/mL IGF-1 (control, 56.3% +/- 8.5 (9); mean +/ - SEM, (n) vs. treated, 39.6% +/ - 6.6 (9), p=0.0009). Recently, An et al. (2017)[28] reported that intraperitoneal injection of calcitriol in diabetic rat model protects the degeneration of IVD by upregulating IGF-1 and TGFβ expression. These studies provide a solid foundation for further investigation in the area in hopes of applying these principles in vivo to provide both symptomatic and pathophysiologic therapy.

Basic Fibroblast Growth Factor (bFGF) & Fibroblast Growth Factor-18 (FGF-18)

The family of fibroblast growth factors, bFGF and FGF-18 in particular, have been studied and implicated in the regulation of both articular and IVD cartilage homeostasis[29]. These proteins have been shown to bind heparin and heparan sulfate, and serve to modulate cell growth, differentiation, migration, and survival[30]. Along with PDGF and IGF-1, most consider FGF-18 to be anabolic in nature, while bFGF remains more controversial. FGF-18 has yet to be studied and explicitly proven to be anabolic in IVD disease in vivo. However, recent FGF-18 studies indicate a slightly more unified agreement among experts describing anabolic effects in human articular chondrocytes by activating FGFR3, increasing extracellular matrix (ECM) formation and cell differentiation while inhibiting cell proliferation, leading to dispersed cells surrounded by abundant ECM instead of clusters of cells seen after stimulation with bFGF[29]. Furthermore, there are reports that FGF helps mesenchymal stem cell differentiation to chondrogenic lineage[31-33].

In discussing the more contentious member of the fibroblast growth factor family, several studies have shown bFGF to be mitogenic and anabolic much like PDGF and IGF-1[24,32,34]. Recent studies have analyzed the effects of bFGF on bovine NP cell growth and differentiation cultured in monolayer and alginate, and found that bFGF stimulated increased sulfated proteoglycan (PG) synthesis, lower aggrecan turnover, and differentiation of NP cell phenotype by maintaining responsiveness to TGF-β[29,30,32,34,35]. However, other studies suggest that the mitogenic effect of bFGF in human articular chondrocytes or spine disc cells signal pathologic degeneration rather than regeneration[29,36,37]. While the literature leaves the exact anabolic/catabolic role of bFGF open for debate, more recent research shows bFGF exert catabolic effect on human articular chondrocytes and IVD tissue by activation of FGFR1 and up-regulation catabolic enzymes and inhibition of extracellular matrix synthesis"[29]. Hence,

Table 2: Unpublished Clinical trials investigating Growth factor injection therapy for IVD degeneration.

Author	Title	No. of Patients	Growth Factor used	Follow up	Status	Clinical trial Number
DePuy Spine	Phase I/II, Multicenter, Open-label, Single Administration, Dose Finding, Clinical Trial to Evaluate the Safety and Tolerability of Intradiscal rhGDF-5 for the Treatment of Early Stage Lumbar Disc Degeneration	32	rhGDF-5	36	Completed	NCT00813813
Lutz GE et al.	A Multicenter, Randomized, Double-blind, Placebo Controlled, Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Effectiveness of 2 Doses of Intradiscal rhGDF-5 (Single Administration) for the Treatment of Early Stage Lumbar Disc Degeneration	45	rhGDF-5	36	Ongoing	10156

the exact mechanisms and pathophysiology necessitate further research, but it stands to argue that there is likely some utility in the modification of FGF regulation in the therapeutic arena.

Growth and Differentiation Factor-5 (GDF-5)

While not technically a member of the BMP family, GDF-5 is closely related to this family of proteins and serves a similar role in cell growth and differentiation in both adult and embryonic tissues; specific to our interest, GDF-5 has been shown to influence joint and skeletal development via matrix production and disc cell proliferation[38-42]. In a bovine model of NP and AF cells, Chujo et al. (2006)[43] showed that recombinant rhGDF-5 increased matrix synthesis and cell proliferation in both sets of cell groups, with a higher response being documented in the NP cells. Recently, Luo et al. (2016)[42] demonstrated similar results in human NP cells when cells were infected with adenovirus-mediated GDF-5. Moving toward in vivo responses, Walsh et al. (2006)[44] were able to produce a notable increase in disc height after a single GDF-5 injection in a mouse disc compression model. Interestingly, in the same study, the team was unable to elicit a significant disc height increase from IGF-1, bFGF, or TGF-β injection. More recently, Liang et al. (2010)[45] developed a murine degenerative disc model via needle puncture, and investigated the effects of adenoviral (Ad) GDF-5 gene therapy. Utilizing bioluminescent imaging, radiographic, and MRI scanning, they showed the percent disc height index at two weeks in the mice injected with Ad-GDF5 increased significantly compared with that of the control mice; the increase was sustained for the rest of the experiment period. The T2-weighted signals were detected in the Ad-GDF-5 at 6 and 8 weeks after injection while none were detected in the control group. On histological evaluation, the GDF-5 group also significantly confirmed the hypothesized disc improvement. They demonstrated notable decreases in glycosaminoglycan levels at two weeks post-insult and decreased DNA levels at four weeks; contrastingly, the discs treated with GDF-5 revealed no decrease in glycosaminoglycan or DNA levels throughout the 8-week trial period[45]. There are two ongoing multicentric clinical trials investigating the safety, tolerability, effectiveness of intradiscal recombinant human GDF-5 injection for treatment of early stage lumbar disc degeneration [Table 2].

Transforming Growth Factor Beta-1 (TGF-β1)

TGF-β1 is secreted polypeptide acting in multiple cellular functions including proliferation, growth, and cell differentiation. In a rabbit model, researchers have shown after one week of in vivo injection of hTGF-b1/adenovirus construct in IVD, there was a 100% increase in PG expression when compared with controls[46]. Similar to results found with GDF-5, studies have shown increased deposition of PGs compared with basal media or chondrogenic media alone, and further showed abundant aggrecan and type II collagen deposition[47]. Similar IVD restorative results via increased PG synthesis have been further proven in canine studies[48,49]. In a study examining the role of TGF-β1 in platelet-rich plasma on IVD regeneration, Chen et al. (2006)[50] concluded that growth factors in platelet-rich plasma can effectively react as a growth factor cocktail to induce NP proliferation and differentiation, promote tissue-engineered NP formation, and serve as a possible therapeutic deterrent to IVD disease.

In recent approaches aimed at potential therapeutic treatment, researchers have qualitatively and quantitatively shown the stimulatory effects of a combination treatment with TGF-β1 and IGF-1 on the synthesis of sulphated glycosaminoglycan and type I and II collagen by AF cells; they suggest the role for TGF-β1 in pushing cells towards a

fibrocartilaginous phenotype[51]. From a signaling cascade perspective, a study recently demonstrated that BMP-2 and TGF-β regulate β1,3-glucuronosyl transferase-1 expression and chondroitin sulfate synthesis in NP cells through a signaling network comprising MAPK, AP1, Sp1, and TonEBP[52]. These researchers draw the conclusion that by controlling glycosaminoglycan and aggrecan synthesis, these growth factors positively influence disk cell function. In another recent rabbit model, Yang et al. (2010) [53] looked at the effects of the combination therapy of mesenchymal stem cells (MSC) and pure fibrinous gelatin (PFG)-TGF-β1 on disc height. They reported that the MSC-PFG-TGF-β1 group had less degeneration and a slower decrease in disc height compared with both the degenerative model and pure PFG-TGF-β1 groups Furthermore, they showed up-regulation of type II collagen content in NP cells while demonstrating a decrease in the rate of cell apoptosis in the MSC-PFG-TGF-β1 group. Recently, similar results were reported in in vivo rabbit IVD model using lentivirus and adenoviral mediated transfer of TGFβ1[54,55].

Transforming Growth Factor Beta-3 (TGF-β3)

Transforming growth factor (TGF-β3) plays a pivotal role in maintain and induce transformation of IVD structure[56] . TGF-β3 has a synergy with other growth factors to induce discogenic differentiation and reduce the damage caused by degeneration[56] . Risbud et al.[57] showed that TGF-β3 could enhance NP and AF structure and function by elevating the levels of activated ERK1/2, which in turn would regulate the TGF-β-RI and TGF-β-RII. Hegewald et al.[58] confirmed the previous finding and demonstrated that TGF-β3 administration is a possible candidate as a biological treatment of AF degeneration. They presented that stimulation of AF with TGF-β3 was associated with more production of collagen type X.

Bone Morphogenetic Protein-7 (BMP-7)

BMP-7 is also known as osteogenic protein-1 (OP-1), BMP-7 is a known player in bone homeostasis and the transformation of mesenchymal cells into bone and cartilage[59]. Specifically, BMP-7 treatment has been shown to induce all the genetic markers of osteoblast differentiation and extracellular matrix synthesis[59,60]. Similar to many of the previously addressed growth factors, BMP-7 has been implicated in proteoglycan metabolism and extracellular matrix synthesis in IVD cells exposed to known inflammatory factors such as interleuken-1[61,62]. Pertinent to possible future clinical trials, this enhanced extracellular matrix synthesis has been demonstrated in both rabbit and human IVD cells in vitro[19,63,64]. Regarding in vivo studies, BMP-7 has shown to have a disc height restoration effect similar to GDF-5. Rabbit studies have shown that a single intradiscal administration of BMP-7 yielded both an increase in NP proteoglycan content as well as an overall heightened disc measurement, neither of which was seen in the control groups[63]. In another rabbit model, BMP-7 injection showed a sustained disc height re-establishment at 8-, 12- and 24-week time points, suggesting a sustainable increased water content of the NP of treatment vs. control subjects[19,64].

Shifting to in vivo techniques and response, Wang et al.[65] (2011) argued that the recombinant adeno-associated viral vector rAAV2 vector has empirical advantages over adenovirus vector Ad-hBMP-7 to transfer exogenous genes into cells, especially in the clinical use. They found that NP cells transfected by the rAAV-humanhBMP-7 vector expressed hBMP-7 for at least two weeks while promoting a remarkable and significant accumulation of both proteoglycans (42% and 77% higher than non-transfected cell, $p<0.05$) and collagen type II (63% and 94% higher than non-transfected cells, $p<0.05$). Based on the results of Masuda, Wang, and others, it seems feasible that there is an opportunity to restore degenerative discs by a single NP injection of BMP-7. Recently, Liao et al (2016)[66] performed in vitro and in vivo studies using bone marrow derived mesenchymal stem cells with overexpression of BMP-7. They reported that BMP-7 transfected stem cells significantly slowed progression of disc degeneration in a rat tail disc model.

Bone Morphogenetic Protein-2 (BMP-2)

Like BMP-7, BMP-2 is a known osteoinductive protein that stimulates osteoblast differentiation and bone formation. Moreover, in vitro and in vivo studies in different animal and human IVDs have shown BMP-2 acting similarly to BMP-7 in upregulation with both aging and induced disc injury[67]. Demonstrating its anabolic nature in DDD, studies have shown that direct administration of BMP-2 to IVD cells results in increased production of components of the extracellular matrix; again, this is a shared commonality between BMP-2 and BMP-7[67-69]. Furthermore, research has shown upregulation of the BMP pathway via other agents, namely simvastatin and LIM mineralization protein-1, which has resulted in similar outcomes as the direct administration of bone morphogenic proteins[70]. To this extent, BMP-2 is currently available in the United

States and has shown some clinical efficacy as an adjunct to anterior lumbar interbody fusion procedures for degenerative disc disease. Burkus et al. (2009)[71] combined BMP-2 on an absorbable collagen sponge with the use of dual tapered threaded fusion cages to demonstrate obtained and maintained intervertebral spinal fusion, improved clinical outcomes, and reduced pain after anterior lumbar interbody arthrodesis. Based on the current literature, it stands to argue that by way of either direct or indirect stimulation of BMPs, there is a possible role for these proteins in the treatment of discogenic pain. Use of recombinant growth factors has shown promising results, but contain some risks such as uncontrolled bone formation, immunogenicity, and malignancies. Malignancies has specifically been a concern with the use of recombinant BMP-2[72-74]. Vavken et al.[74] conducted meta-analysis to assess the risk profile of the use of rhBMP-2 in spine fusion. They reported an increase in general risk of complication such as heterotopic ossification, retrograde ejaculation, and neck swelling in cervical fusion. Moreover, they reported that there is slight increased risk of new tumor, but findings were not statistically significant[74]. Recently, Dettori et al. (2006)[75] performed retrospective cohort study reporting that there was no increase in overall cancer incidence among patients those receiving rhBMP. Use of BMP-2 in spine fusion is controversial, and has been well described in the review article published by Hustedt et al. (2014)[73]. It is clear that more basic and clinical research is needed to evaluate the harmful and beneficial effects of BMP-2.

Summary

Clearly the key to understanding the pathophysiology of DDD is a stronger understanding of the molecular mechanism underlying IVD disease. Specifically, the prospect of being able to harness the power of the growth factors and cytokines we discussed, and applying these to structural modification in human models is encouraging. The aim is that the injection of growth factors and mitogens could potentially surmount the complex degenerative changes of the IVD disease phenotype. Growth factor prolotherapy could be considered as a reasonable line of management for intervertebral disc degeneration and associated low back pain. It would provide a true not palliative and long-term cure for the patient. In addition, it might effectively reduce the narcotic consumption and surgical intervention for early stage cases. However, it may not be effective for late stage IVD, and there are still many questionable aspects in regards to alternative sources, numbers of injections, frequency, and safety (malignancies). We believe that numerous growth factors may be administrated. However, administration of the right one based on the level of degeneration is crucial. Unfortunately, the bulk of existing research remains confined to animal and in vitro studies. Certainly, the goal is to apply these principles to growth factor modulation and tissue engineering in human models to attain matrix restoration. A follow up concern to be addressed in the future would be determining the ideal timing of these interventions. Beyond that, little has been discussed regarding the precarious in vivo environment around which the IVD exists, thus opening new questions as to the drawbacks and side effects along with the therapeutic and practical challenges of administration. Currently in the United States, the Food and Drug Administration has only recently allowed the initiation of investigational new drug clinical trials on OP-1/BMP-7 and GDF-5 based on the aforementioned disc height restoration potential seen in multiple animal models[76]. We hope future collective efforts by researchers and clinicians will yield safe and effective therapies that address both functional disc restoration and back pain alleviation.

Conflict of interests: The authors declare no conflict of interests.

Funding: This work is supported by the Department of Orthopaedic Surgery, Augusta, GA.

Acknowledgements: We like to thank the Department of Orthopaedic Surgery for their support.

References

1. Andersson GB. Epidemiological features of chronic low-back pain. Lancet (London, England). 1999;354(9178):581-585.
2. Crow WT, Willis DR. Estimating cost of care for patients with acute low back pain: a retrospective review of patient records. The Journal of the American Osteopathic Association. 2009;109(4):229-233.
3. Hoy D, March L, Brooks P, et al. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. Annals of the Rheumatic Diseases. 2014;73(6):968-974.
4. Freemont AJ. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology (Oxford, England). 2009;48(1):5-10.
5. Hart LG, Deyo RA, Cherkin DC. Physician office visits for low back pain. Frequency, clinical evaluation, and treatment patterns from a U.S. national survey. Spine. 1995;20(1):11-19.

6. Praemer A. FS, Rice DP. Musculoskeletal conditions in the United States. Rosemont AAUS; 1992.
7. Taylor VM, Deyo RA, Cherkin DC, Kreuter W. Low back pain hospitalization. Recent United States trends and regional variations. Spine. 1994;19(11):1207-1212; discussion 1213.
8. Goswami A. Prolotherapy. Journal of pain & palliative care pharmacotherapy. 2012;26(4):376-378.
9. policy UCM. Prolotherapy For Musculoskeletal Indications. 2018.
10. Masuda K, Oegema TR, Jr., An HS. Growth factors and treatment of intervertebral disc degeneration. Spine. 2004;29(23):2757-2769.
11. Mwale F, Roughley P, Antoniou J. Distinction between the extracellular matrix of the nucleus pulposus and hyaline cartilage: a requisite for tissue engineering of intervertebral disc. European cells & materials. 2004;8:58-63; discussion 63-54.
12. Kepler CK, Anderson DG, Tannoury C, Ponnappan RK. Intervertebral disk degeneration and emerging biologic treatments. The Journal of the American Academy of Orthopaedic Surgeons. 2011;19(9):543-553.
13. Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine. 2006;31(18):2151-2161.
14. Buckwalter JA. Aging and degeneration of the human intervertebral disc. Spine. 1995;20(11):1307-1314.
15. Choi YS. Pathophysiology of Degenerative Disc Disease. Asian Spine Journal. 2009;3(1):39-44.
16. Hilton R.C. BJ. 1984. Ann Rheum Dis Vertebral rim lesions in the dorsolumbar spine;43:302–307.
17. David G. Angiogenesis in the degeneration of the lumbar intervertebral disc. 2010;3(2):154-161.
18. Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing research reviews. 2007;6(3):247-261.
19. Masuda K, Takegami K, An H, et al. Recombinant osteogenic protein-1 upregulates extracellular matrix metabolism by rabbit annulus fibrosus and nucleus pulposus cells cultured in alginate beads. Journal of Orthopaedic Research. 2003;21(5):922-930.
20. Paglia DN, Singh H, Karukonda T, Drissi H, Moss IL. PDGF-BB Delays Degeneration of the Intervertebral Discs in a Rabbit Preclinical Model. Spine. 2016;41(8):E449-458.
21. Gruber HE, Norton HJ, Hanley EN, Jr. Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro. Spine. 2000;25(17):2153-2157.
22. Pratsinis H, Constantinou V, Pavlakis K, Sapkas G, Kletsas D. Exogenous and autocrine growth factors stimulate human intervertebral disc cell proliferation via the ERK and Akt pathways. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2012;30(6):958-964.
23. Presciutti SM, Paglia DN, Karukonda T, et al. PDGF-BB inhibits intervertebral disc cell apoptosis in vitro. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2014;32(9):1181-1188.
24. Murakami H. YST, Attallah-Wasif E.S., Tsai K.J., Fei Q., Hutton W.C., . The expression of anabolic cytokines in intervertebral discs in age-related degeneration. Spine. 2006;31(16):1770-1774.
25. Osada R, Ohshima H, Ishihara H, et al. Autocrine/paracrine mechanism of insulin-like growth factor-1 secretion, and the effect of insulin-like growth factor-1 on proteoglycan synthesis in bovine intervertebral discs. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 1996;14(5):690-699.
26. Takayama B, Sekiguchi M, Yabuki S, Kikuchi S, Konno S. Localization and function of insulin-like growth factor 1 in dorsal root ganglia in a rat disc herniation model. Spine. 2011;36(2):E75-79.
27. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN. IGF-1 rescues human intervertebral annulus cells from in vitro stress-induced premature senescence. Growth Factors. 2008;26(4):220-225.
28. An JL, Zhang W, Zhang J, Lian LC, Shen Y, Ding WY. Vitamin D improves the content of TGF-beta and IGF-1 in intervertebral disc of diabetic rats. Experimental biology and medicine (Maywood, NJ). 2017;242(12):1254-1261.
29. Ellman MB, An HS, Muddasani P, Im HJ. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene. 2008;420(1):82-89.
30. Friedl A, Chang Z, Tierney A, Rapraeger AC. Differential binding of fibroblast growth factor-2 and -7 to basement membrane heparan sulfate: comparison of normal and abnormal human tissues. The American journal of pathology. 1997;150(4):1443-1455.
31. Ellman MB, Yan D, Ahmadinia K, Chen D, An HS, Im HJ. Fibroblast growth factor control of cartilage homeostasis. Journal of cellular biochemistry. 2013;114(4):735-742.
32. Shu C, Smith SM, Little CB, Melrose J. Use of FGF-2 and FGF-18 to direct bone marrow stromal stem cells to chondrogenic and osteogenic lineages. Future science OA. 2016;2(4):Fso142.
33. Zhou X, Tao Y, Wang J, et al. Roles of FGF-2 and TGF-beta/FGF-2 on differentiation of human mesenchymal stem cells towards nucleus pulposus-like phenotype. Growth Factors. 2015;33(1):23-30.
34. Tolonen J, Gronblad M, Virri J, Seitsalo S, Rytomaa T, Karaharju E. Basic fibroblast growth factor immunoreactivity in blood vessels and cells of disc herniations. Spine. 1995;20(3):271-276.
35. Tsai TT, Guttapalli A, Oguz E, et al. Fibroblast growth factor-2 maintains the differentiation potential of nucleus pulposus cells in vitro: implications for cell-based transplantation therapy. Spine. 2007;32(5):495-502.
36. Li X, An HS, Ellman M, et al. Action of fibroblast growth factor-2 on the intervertebral disc. Arthritis Research & Therapy. 2008;10(2):R48.
37. Qu Z, Huang XN, Ahmadi P, et al. Expression of basic fibroblast growth factor in synovial tissue from patients with rheumatoid arthritis and degenerative joint disease. Laboratory investigation; a journal of technical methods and pathology. 1995;73(3):339-346.
38. Buxton P, Edwards C, Archer CW, Francis-West P. Growth/differentiation factor-5 (GDF-5) and skeletal development. The Journal of bone and joint surgery American volume. 2001;83-A Suppl 1(Pt 1):S23-30.
39. Feng C, Liu H, Yang Y, Huang B, Zhou Y. Growth and differentiation factor-5 contributes to the structural and functional maintenance of the intervertebral disc. Cellular physiology and biochemistry : international journal of

experimental cellular physiology, biochemistry, and pharmacology. 2015;35(1):1-16.
40. Francis-West PH, Abdelfattah A, Chen P, et al. Mechanisms of GDF-5 action during skeletal development. Development (Cambridge, England). 1999;126(6):1305-1315.
41. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN, Jr. Growth and differentiation factor-5 (GDF-5) in the human intervertebral annulus cells and its modulation by IL-1ss and TNF-alpha in vitro. Experimental and molecular pathology. 2014;96(2):225-229.
42. Luo XW, Liu K, Chen Z, et al. Adenovirus-mediated GDF-5 promotes the extracellular matrix expression in degenerative nucleus pulposus cells. Journal of Zhejiang University Science B. 2016;17(1):30-42.
43. Chujo T, An HS, Akeda K, et al. Effects of growth differentiation factor-5 on the intervertebral disc--in vitro bovine study and in vivo rabbit disc degeneration model study. Spine. 2006;31(25):2909-2917.
44. Walsh AJ, Bradford DS, Lotz JC. In vivo growth factor treatment of degenerated intervertebral discs. Spine. 2004;29(2):156-163.
45. Liang H, Ma SY, Feng G, Shen FH, Joshua Li X. Therapeutic effects of adenovirus-mediated growth and differentiation factor-5 in a mice disc degeneration model induced by annulus needle puncture. The spine journal : official journal of the North American Spine Society. 2010;10(1):32-41.
46. Nishida K, Kang JD, Gilbertson LG, et al. Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenovirus-mediated transfer of the human transforming growth factor beta 1 encoding gene. Spine. 1999;24(23):2419-2425.
47. Feng G, Wan Y, Balian G, Laurencin CT, Li X. Adenovirus-mediated expression of growth and differentiation factor-5 promotes chondrogenesis of adipose stem cells. Growth Factors. 2008;26(3):132-142.
48. Guo T, Zeng X, Hong H, et al. Gene-activated matrices for cartilage defect reparation. The International journal of artificial organs. 2006;29(6):612-621.
49. Thompson JP, Oegema TR, Jr., Bradford DS. Stimulation of mature canine intervertebral disc by growth factors. Spine. 1991;16(3):253-260.
50. Chen WH, Lo WC, Lee JJ, et al. Tissue-engineered intervertebral disc and chondrogenesis using human nucleus pulposus regulated through TGF-beta1 in platelet-rich plasma. Journal of cellular physiology. 2006;209(3):744-754.
51. Hayes AJ, Ralphs JR. The response of foetal annulus fibrosus cells to growth factors: modulation of matrix synthesis by TGF-beta1 and IGF-1. Histochemistry and cell biology. 2011;136(2):163-175.
52. Hiyama A, Gogate SS, Gajghate S, Mochida J, Shapiro IM, Risbud MV. BMP-2 and TGF-beta stimulate expression of beta1,3-glucuronosyl transferase 1 (GlcAT-1) in nucleus pulposus cells through AP1, TonEBP, and Sp1: role of MAPKs. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(5):1179-1190.
53. Yang H, Wu J, Liu J, et al. Transplanted mesenchymal stem cells with pure fibrinous gelatin-transforming growth factor-beta1 decrease rabbit intervertebral disc degeneration. The spine journal : official journal of the North American Spine Society. 2010;10(9):802-810.
54. Liu HF, Ning B, Zhang H, et al. Effect of rAAV2-hTGFbeta1 Gene Transfer on Matrix Synthesis in an In Vivo Rabbit Disk Degeneration Model. Clinical spine surgery. 2016;29(3):E127-134.
55. Liu Y, Yu T, Ma XX, Xiang HF, Hu YG, Chen BH. Lentivirus-mediated TGF-beta3, CTGF and TIMP1 gene transduction as a gene therapy for intervertebral disc degeneration in an in vivo rabbit model. Experimental and therapeutic medicine. 2016;11(4):1399-1404.
56. Frauchiger DA, Heeb SR, May RD, Woltje M, Benneker LM, Gantenbein B. Differentiation of MSC and annulus fibrosus cells on genetically engineered silk fleece-membrane-composites enriched for GDF-6 or TGF-beta3. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2017.
57. Risbud MV, Di Martino A, Guttapalli A, et al. Toward an optimum system for intervertebral disc organ culture: TGF-beta 3 enhances nucleus pulposus and anulus fibrosus survival and function through modulation of TGF-beta-R expression and ERK signaling. Spine. 2006;31(8):884-890.
58. Hegewald AA, Zouhair S, Endres M, et al. Towards biological anulus repair: TGF-beta3, FGF-2 and human serum support matrix formation by human anulus fibrosus cells. Tissue & cell. 2013;45(1):68-76.
59. Xu J, E XQ, Wang NX, et al. BMP7 enhances the effect of BMSCs on extracellular matrix remodeling in a rabbit model of intervertebral disc degeneration. The FEBS journal. 2016;283(9):1689-1700.
60. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth Factors. 2004;22(4):233-241.
61. Takegami K, An HS, Kumano F, et al. Osteogenic protein-1 is most effective in stimulating nucleus pulposus and annulus fibrosus cells to repair their matrix after chondroitinase ABC-induced in vitro chemonucleolysis. The spine journal : official journal of the North American Spine Society. 2005;5(3):231-238.
62. Takegami K, Thonar EJ, An HS, Kamada H, Masuda K. Osteogenic protein-1 enhances matrix replenishment by intervertebral disc cells previously exposed to interleukin-1. Spine. 2002;27(12):1318-1325.
63. Imai Y, Miyamoto K, An HS, Thonar EJ, Andersson GB, Masuda K. Recombinant human osteogenic protein-1 upregulates proteoglycan metabolism of human anulus fibrosus and nucleus pulposus cells. Spine. 2007;32(12):1303-1309; discussion 1310.
64. Masuda K, Imai Y, Okuma M, et al. Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. Spine. 2006;31(7):742-754.
65. Wang C, Ruan DK, Zhang C, Wang DL, Xin H, Zhang Y. Effects of adeno-associated virus-2-mediated human BMP-7 gene transfection on the phenotype of nucleus pulposus cells. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2011;29(6):838-845.
66. Liao JC. Cell Therapy Using Bone Marrow-Derived Stem Cell Overexpressing BMP-7 for Degenerative Discs in a Rat Tail Disc Model. International journal of molecular sciences. 2016;17(2).
67. Than KD, Rahman SU, Vanaman MJ, et al. Bone morphogenetic proteins and degenerative disk disease. Neurosurgery. 2012;70(4):996-1002; discussion 1002.

68. Belykh E, Giers M, Bardonova L, Theodore N, Preul M, Byvaltsev V. The Role of Bone Morphogenetic Proteins 2, 7, and 14 in Approaches for Intervertebral Disk Restoration. World neurosurgery. 2015;84(4):871-873.
69. Li Z, Lang G, Karfeld-Sulzer LS, et al. Heterodimeric BMP-2/7 for nucleus pulposus regeneration-In vitro and ex vivo studies. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2017;35(1):51-60.
70. Zhang H, Wang L, Park JB, et al. Intradiscal injection of simvastatin retards progression of intervertebral disc degeneration induced by stab injury. Arthritis Research & Therapy. 2009;11(6):R172.
71. Burkus JK, Gornet MF, Schuler TC, Kleeman TJ, Zdeblick TA. Six-year outcomes of anterior lumbar interbody arthrodesis with use of interbody fusion cages and recombinant human bone morphogenetic protein-2. The Journal of bone and joint surgery American volume. 2009;91(5):1181-1189.
72. Epstein NE. Complications due to the use of BMP/INFUSE in spine surgery: The evidence continues to mount. Surgical neurology international. 2013;4(Suppl 5):S343-352.
73. Hustedt JW, Blizzard DJ. The controversy surrounding bone morphogenetic proteins in the spine: a review of current research. The Yale journal of biology and medicine. 2014;87(4):549-561.
74. Vavken J, Mameghani A, Vavken P, Schaeren S. Complications and cancer rates in spine fusion with recombinant human bone morphogenetic protein-2 (rhBMP-2). European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society. 2016;25(12):3979-3989.
75. Dettori JR, Chapman JR, DeVine JG, McGuire RA, Norvell DC, Weiss NS. The Risk of Cancer With the Use of Recombinant Human Bone Morphogenetic Protein in Spine Fusion. Spine. 2016;41(16):1317-1324.
76. Zhang Y, Chee A, Thonar EJ, An HS. Intervertebral disk repair by protein, gene, or cell injection: a framework for rehabilitation-focused biologics in the spine. PM & R : the journal of injury, function, and rehabilitation. 2011;3(6 Suppl 1):S88-94.
77. An HS, Takegami K, Kamada H, et al. Intradiscal administration of osteogenic protein-1 increases intervertebral disc height and proteoglycan content in the nucleus pulposus in normal adolescent rabbits. Spine. 2005;30(1):25-31; discussion 31-22.
78. Huang KY, Yan JJ, Hsieh CC, Chang MS, Lin RM. The in vivo biological effects of intradiscal recombinant human bone morphogenetic protein-2 on the injured intervertebral disc: an animal experiment. Spine. 2007;32(11):1174-1180.