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Review

TGF- β signaling in intervertebral disc health and disease

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SUMMARY

Objective: This paper aims to provide a comprehensive review of the changing role of transforming growth factor- β (TGF- β) signaling in intervertebral disc (IVD) health and disease.

Methods: A comprehensive literature search was performed using PubMed terms 'TGF- β ' and 'IVD'.

Results: TGF- β signaling is necessary for the development and growth of IVD, and can play a protective role in the restoration of IVD tissues by stimulating matrix synthesis, inhibiting matrix catabolism, inflammatory response and cell loss. However, excessive activation of TGF- β signaling is detrimental to the IVD, and inhibition of the aberrant TGF- β signaling can delay IVD degeneration.

Conclusions: Activation of TGF- β signaling has a promising treatment prospect for IVD degeneration, while excessive activation of TGF- β signaling may contribute to the progression of IVD degeneration. Studies aimed at elucidating the changing role of TGF- β signaling in IVD at different pathophysiological stages and its specific molecular mechanisms are needed, and these studies will contribute to safe and effective TGF- β signaling-based treatments for IVD degeneration.

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Introduction

Low back pain (LBP) is a common and frequently recurring health condition, and over 80% of adults will suffer LBP during their lifetime^{1,2}. Moreover, LBP is the leading cause of years lived with disability and can cause huge economic losses each year both in developed and developing countries^{3,4}. Intervertebral disc (IVD) degeneration is frequently associated with LBP and is generally thought to be the major cause of LBP⁵.

The IVD is the largest avascular organ in the human body and can be macroscopically divided into three parts, including nucleus pulposus (NP), annulus fibrosus (AF) and cartilaginous endplate (CEP)^{6,7}. The centrally situated gelatinous NP is constituted by type II collagen (Col2), the glycosaminoglycans (GAGs) and NP cells (NPCs), and the outer fibrocartilaginous AF is mainly composed of type I collagen fibers and AF cells (AFCs). The upper and lower CEPs are similar to hyaline cartilage and enclose the disc⁸. Current evidences demonstrate that the IVD progressively degenerates with IVD cell loss, inflammatory response, extracellular matrix (ECM) degradation increase and synthesis reduction⁹. Growth factors,

such as the multifunctional growth factor transforming growth factor- β (TGF- β), are essential in maintaining IVD homeostasis¹⁰. On the basis of current literature, we highlight the TGF- β signaling pathway and changes in TGF- β expression, multiple functions as well as the treatment prospect of TGF- β signaling in IVD, and aim to help understand the changing role of TGF- β signaling in IVD health and disease.

TGF- β signaling

In mammals, the TGF- β family includes three TGF- β members, TGF- β 1, TGF- β 2 and TGF- β 3¹¹. Although these three members are highly homologous in terms of molecule structure, each member has different biological activity and distinct temporal and spatial expression pattern^{12,13}. All TGF- β s are expressed as inactive precursors, which consist of a latency-associated polypeptide (LAP) and a mature polypeptide (Fig. 1). The latent TGF- β is commonly bound to and deposited in ECM via a latent TGF- β binding protein (LTBP), which is disulfide-linked to the LAP¹⁴. In the IVD, mechanical loading can promote the activation and liberation of TGF- β by integrins¹⁰. It has been reported that matrix metalloproteinases (MMPs), reactive oxygen species (ROS), proteases and other factors can also participate in the latent TGF- β activation^{15,16}.

In the process of latent TGF- β activation, the mature polypeptide will be converted into a 25 kD dimer that acts as a ligand

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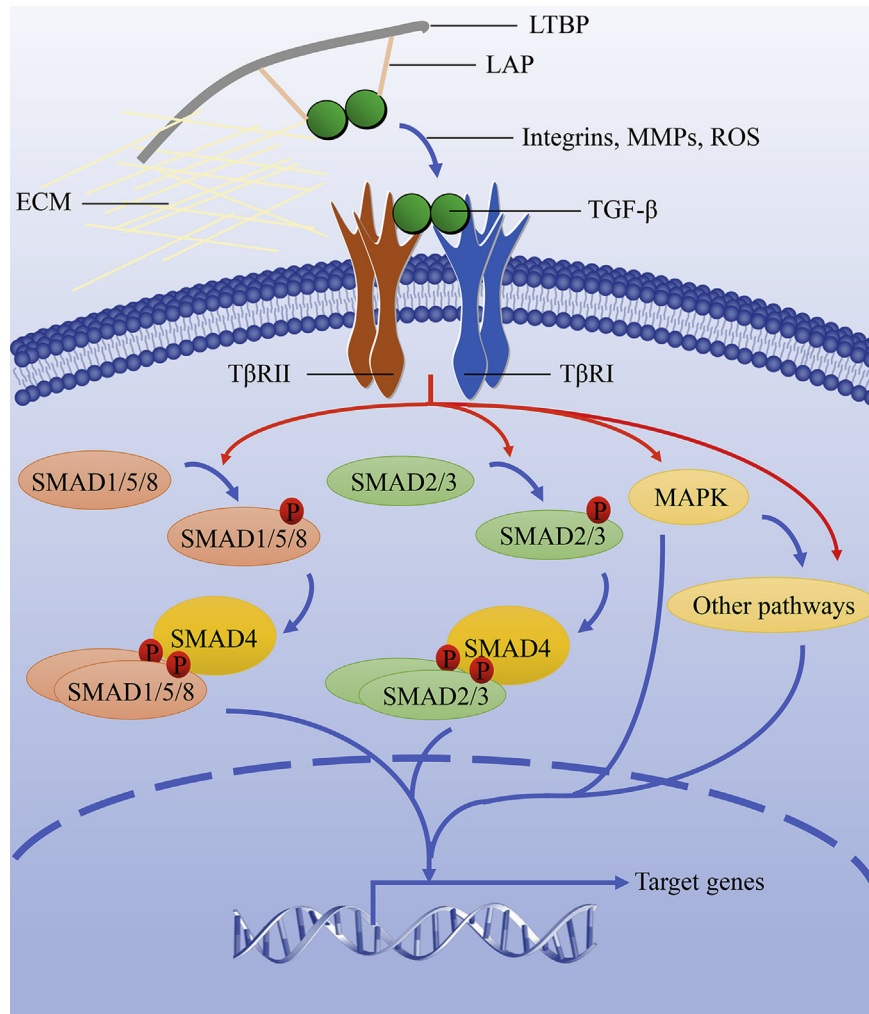


Fig. 1. Simplified scheme of TGF- β signaling in intervertebral disc (IVD). The latent TGF- β , consisting of LAP and a mature polypeptide, is deposited in extracellular matrix (ECM) via LTBP. With the participation of integrins, matrix metalloproteinases (MMPs) or reactive oxygen species (ROS), the mature polypeptide is converted into the activated TGF- β and binds to T β RI and T β RII. T β RII transphosphorylates T β RI and can induce small mother against decapentaplegic (SMAD)2/3 pathway, SMAD1/5/8 pathway and non-SMAD-dependent noncanonical signaling pathways, such as the MAPK pathway.

binding to cell surface TGF- β receptor complex and activates TGF- β signaling¹⁷. The TGF- β receptor complex is a tetramer composed of two transmembrane TGF- β type I receptors (T β RI) and two transmembrane TGF- β type II receptors (T β RII), which are endowed with serine/threonine kinase activity¹⁸. In the initial of tetrameric complex formation, TGF- β ligands bind to T β RII, which trans-phosphorylate the glycine-serine domains of T β RI in juxtamembrane regions at specific serine and threonine residues¹⁹. T β RI is also termed as activin receptor-like kinases (ALKs). In the canonical signaling pathway, the consequently activated T β RI (ALK5) can phosphorylate small mother against decapentaplegic (SMAD)2 and SMAD3 on C-terminal serines, and activated R-SMADs then form trimeric complexes with the common mediator SMAD4²⁰. These trimeric complexes translocate into the nucleus, where they stimulate or suppress target gene expression²¹. When T β RI comprises two distinct types, e.g., ALK5 and ALK1, the activated ALK1 can mediate the phosphorylation of SMAD1/5/8, and phosphorylated SMAD1/5/8 and SMAD2/3 often have opposing functions in many tissues^{22,23}. In addition, TGF- β can also activate non-SMAD-dependent noncanonical signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway^{24,25}.

TGF- β signaling in the development and growth of IVD

The IVD develops from cells contained in both the sclerotome and notochord²⁶. The notochord derived from the axial mesoderm forms the NP, and the sclerotome derived from somite gives rise to the AF and CEP^{27,28}. At embryonic stage during the IVD development, TGF- β directs matrix development within the notochord and promotes the differentiation of the sclerotome into AFCs^{29–31}. It has been reported that the IVDs are incomplete and even missing in T β RII conditional knock-out embryos targeted by Col2a1-Cre transgenic mice^{32,33}. In SMAD3 gene knock-out mice, the spine presents with kyphosis and malformation, and the IVDs degenerate, which is indicated by the pathological changes of the declined height of CEP, decreased collagen and proteoglycan content³⁴. Recently, Peck SH *et al.*³⁵ have found a significant increase in TGF- β 1 expression at P0 relative to E12.5 in mouse, which underscores the central role of TGF- β signaling in the embryonic development of the IVD. Besides, TGF- β signaling also plays an essential role in the growth of IVD at the postnatal stage. Jin H *et al.*³⁶ generated T β RII^{Col2ER} mice using the Col2a1-CreER^{T2} transgenic mice to specifically inactivate the TGF- β signaling in inner AFCs in the IVD and surrounding growth plate chondrocytes. In the

early postnatal transgenic mice (P14), they observed a noticeable reduction in the area and length of CEP tissue and a significant increase in the expression of genes related to matrix degradation, such as MMP13. These results suggest that TGF- β signaling is required for the normal development and growth of the IVD.

The changes in the expression of TGF- β signaling in IVD

Research shows that the expression of TGF- β s and TGF- β receptors changes with the increasing age and the progression of the IVD degeneration. Interestingly, the results from different research groups exhibit different and even opposite changes in the expression of TGF- β signaling in IVD. In a senescence-accelerated mouse model, immunohistochemical staining results showed that the expressions of TGF- β s and TGF- β receptors decreased with age in IVDs^{37,38}. And it was also reported that the expression of pSMAD2/3 in the IVDs was significantly decreased in old mice (18 months) relative to young mice (2 months)³⁹. However, the results from another study suggested that the mRNA levels of TGF- β 1 in both NP and AF tissues from old rabbits (3 years) were higher than that in the young rabbits (6 months)⁴⁰. And some other studies found that NP and AF tissues had a different change in the expression of TGF- β signaling with the increasing age in mice or rats^{41,42} (Table 1).

In human IVD tissues, the change in the expression of TGF- β signaling has not reached consensus either. Nerlich AG *et al.*⁴³ performed a study, which included 30 IVD specimens from cadavers (age range 0–86 years, without consuming illness or known back problem) in the autopsy group and 12 IVD specimens from patients (age range 31–76 years, underwent surgery for IVD degeneration) in the surgical group, and they observed an evident association between increased TGF- β 1 expression and IVD degeneration. Some other research groups also confirmed that TGF- β s and TGF- β receptors were highly expressed in the human degenerative IVD tissues compared with the normal control IVD tissues^{44–46}. And further studies indicated that the expression level of TGF- β 1 was upregulated in the degenerative disc tissues and significantly positively associated with the pathological grades of degenerative IVDs^{47,48}. However, Schroeder M *et al.*⁴⁹ found a significant decrease of the TGF- β s expression in degenerated NP tissues and a significant increase of the TGF- β s expression in degenerated AF tissues. Abbott RD *et al.*⁵⁰ observed that T β RI was downregulated in severely degenerated NPCs compared to

moderately degenerated NPCs. And findings from the study of Tsarouhas A *et al.*⁵¹ identified no significant differences in the mRNA expressions of TGF- β 1 between herniated and control IVD tissues. From these contradictory results, we can conclude that the changes in the expression of TGF- β signaling in IVD may differ highly depending on species, age, tissue, cell type and detection method. Of note, TGF- β s are highly present in the IVD in a latent inactive form, and the expression of TGF- β s might not be the same as the actual TGF- β signaling.

The mechanisms of TGF- β signaling in the degenerated IVD

The pathophysiological characteristics of IVD degeneration mainly include the reduction of ECM content, cell loss and inflammatory response^{52–54}. Encouragingly, TGF- β signaling can repair the degenerated IVD by targeting these three aspects (Fig. 2).

Inhibition of ECM degradation and increase of ECM synthesis

Altered ECM homeostasis is one of the most important hallmarks of the progression of IVD degeneration, which manifests as an imbalance between catabolic and anabolic metabolism of ECM⁵⁵. Previous studies have shown that some proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , can be released from immune cells and disc cells within the IVD and promote the ECM degradation by upregulating catabolic enzymes, including MMPs and disintegrins and metalloprotease with thrombospondin motifs (ADAMTSs)^{56,57}. Expectedly, TGF- β can partially reverse the proinflammatory cytokines-induced matrix-degrading enzymes upregulation by regulating the MAPK pathway and NF- κ B pathway^{25,58–60}. Recent studies also indicate that microRNAs participate in the ECM degradation of the IVD by targeting TGF- β signaling, and the regulation of microRNAs through TGF- β signaling may be a novel target for IVD degeneration^{61,62}.

In addition to the inhibition of ECM degradation of the IVD, TGF- β signaling can also promote the synthesis of ECM^{63–65}. GAG, collagen and aggrecan are important components of ECM in the IVD. Aggrecan can bind to hyaluronan and form large aggregate, and the negatively charged GAG, which is attached to the proteoglycan, can attract cations to create a high osmotic pressure in NP^{6,66}. Accumulating evidences suggest that TGF- β can stimulate the GAG synthesis via SMAD2/3, Ras homolog gene family member

Table 1
The changes in the expression of TGF- β signaling in the intervertebral disc (IVD) of different animals

Animal	Age	IVD area	Measure method	The measured components of TGF- β signaling	Expression changes	Reference
Senescence-accelerated mice	8, 24, 50 weeks	AF, NP	Immunohistochemistry	TGF- β 1, TGF- β 2, TGF- β 3, T β RI, T β RII	The immune-positive cell ratio decreases with age	37,38
C57BL/6J mice	2, 18 months	NP	Immunohistochemistry, Western blot	pSMAD2/3, pSMAD2	The pSMAD2/3-positive cell ratio in NP decreases with age, and the expression of pSMAD2 decreases with age	39
New Zealand white rabbits	6 months, 3 years	AF, NP	Quantitative real-time polymerase chain reaction	TGF- β 1	The mRNA levels of TGF- β 1 increases with age	40
Sprague–Dawley rats	12, 32 weeks	AF, NP	Reverse transcription polymerase chain reaction	TGF- β 1, TGF- β 2, TGF- β 3, SMAD1, 2, 3, 5, 8	The mRNA expression of TGF- β 2, TGF- β 3, SMAD3 and SMAD5 decreases with age in NP, while the mRNA expression of SMAD3 increases with age in AF	41
FVB mice	4 day, 2, 9, 12, 26, 48 weeks	AF, NP, CEP	Immunofluorescence	pSMAD1/5/8, pSMAD2/3	The pSMAD1/5/8-positive cell ratio decreases with age in all parts of IVD. The pSMAD2/3-positive staining persists in the CEP and NP, but is downregulated in the AF	42

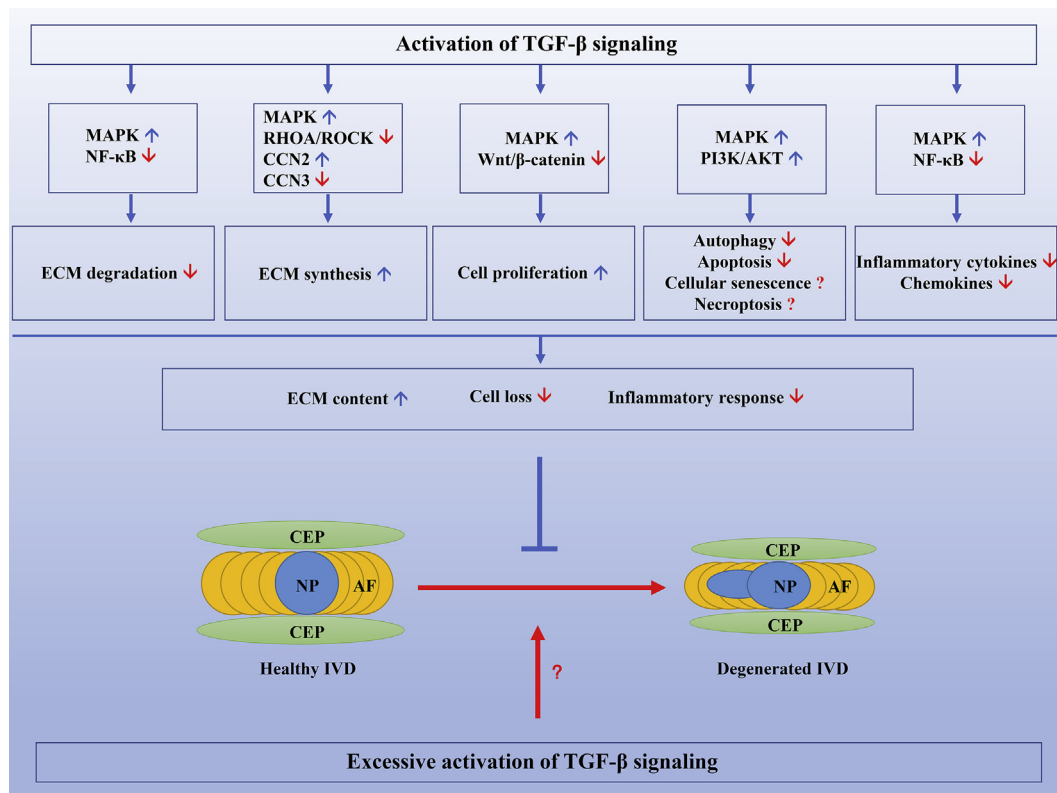


Fig. 2. Illustration of the positive and deleterious effects of TGF- β signaling on degenerated IVD. Activation of TGF- β signaling can delay IVD degeneration by increasing ECM content and inhibiting cell loss as well as inflammatory response via various signaling pathways, including MAPK^{25,58–60,67,86}, NF- κ B^{25,97}, Ras homolog gene family member A (RHOA)/ROCK⁶⁷, CCN2^{70,72}, CCN3⁷¹, Wnt/ β -catenin⁸³ and PI3K/AKT⁸⁶ pathways. But excessive activation of TGF- β signaling can promote the progression of IVD degeneration. Further studies to reveal the effects of TGF- β signaling on cellular senescence and necroptosis of IVD cells and the negative role of TGF- β signaling in IVD degeneration are warranted.

A (RHOA)/Rho-associated protein kinase (ROCK) and MAPK pathways^{67–69}. And in NPCs, researches show that TGF- β can induce the expression of aggrecan by increasing CCN family protein 2 (CCN2) expression and suppressing CCN3 expression^{70–72}. Furthermore, TGF- β signaling has been widely confirmed to increase the expression of aggrecan, proteoglycan and Col2 in IVD degeneration organ culture models and in IVD degeneration animal models^{73–76}. But it is noteworthy that the animals used in these studies are young, and the function of TGF- β as matrix synthesis stimulating growth factor in IVD may be vary with age. Thus, further studies to investigate the function of TGF- β as matrix synthesis stimulating growth factor in aged IVD model systems are needed.

Promotion of cell proliferation and inhibition of cell death

In the degenerated IVD, IVD cells are exposed to a more adverse microenvironment, consisting of hypoxia, mechanical loading, low pH, nutrient deficiency and high osmotic pressure, which is harsh to cell viability and can lead to increased cell death^{77–79}. It has been reported that TGF- β 1 can upregulate the AFCs proliferation, and have synergic effects with some growth factors on cell proliferation, including insulin-like growth factor-I (IGF-I) and fibroblast growth factor-2 (FGF-2)^{80,81}. In rat NPCs, exogenous administration of TGF- β 1 can promote cell cycle progression and cell proliferation by regulating c-Myc signaling and MAPK pathway⁸². The study of Hiyama A *et al.*⁸³ also revealed that SMAD signal could inhibit Wnt/ β -catenin signaling and maintain the cell proliferation of rat NPCs treated with lithium chloride.

Apart from the promotion of cell proliferation, TGF- β signaling can delay IVD degeneration by mitigating cell death in the IVD^{76,84}. Apoptosis and autophagy have been confirmed to play a key role in the progression of IVD degeneration⁸⁵. Ni BB *et al.*⁸⁶ reported that TGF- β could protect against apoptosis of AFCs under starvation condition by inhibiting excessive autophagy, and PI3K/AKT/mTOR and MAPK pathways might be involved in this process. Recently, results from our research group have suggested that receptor-interacting protein kinase (RIPK)1 mediated necroptosis might play an essential role in NP cell death during IVD degeneration^{87,88}. Regrettably, there have been no additional studies to discuss the effect of TGF- β on the necroptosis of IVD cells. Growing evidences demonstrate that cellular senescence is one of the major contributors to IVD degeneration⁸⁹, and TGF- β signaling can regulate cellular senescence in many other cell types^{90,91}. However, the effect of TGF- β signaling on the regulation of cellular senescence in IVD is still unclear, and further studies are needed.

Alleviation of inflammatory response

Inflammatory response is also strongly associated with the progression of IVD degeneration⁹². During IVD degeneration, the degenerated IVD tissue is able to spontaneously produce some chemokines, such as monocyte chemoattractant protein (MCP)-1, C–C motif chemokine ligand 4 (CCL4), which can attract macrophages to the degenerated location⁵⁴. The extensive macrophage infiltration then leads to the production of inflammatory cytokines, particularly IL-1 β and TNF- α , and accelerates the progression of IVD degeneration by upregulation of the matrix catabolic enzymes⁹³. Numerous studies indicate that TGF- β treatment can not only

suppress the release of IL-1 β and TNF- α , but also inhibit the increased expression of inflammatory cytokines-induced MMPs^{94,95}. On the other hand, TGF- β signaling can alleviate inflammatory response in the IVD by inhibiting the expression of chemokines. Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine, which plays a key role in the macrophage recruitment into the herniated disc tissue and the initiation of inflammatory response by stimulating MCP1⁹⁶. Evidences have proved that endogenous TGF- β activity could limit the expression of TSLP in a steady state by inhibiting NF- κ B activation⁹⁷. Moreover, Zhang J *et al.*⁹⁸ reported that TGF- β 1 could downregulate the expression of CCL4 by activating the MAPK pathway. Hence, activated TGF- β signaling can alleviate inflammatory response in the IVD by blocking both the initiation and maintenance of inflammation.

Excessive activation of TGF- β signaling may contribute to IVD degeneration

TGF- β is recognized as a pleiotropic cytokine, which has both deleterious and positive effects due to various tissues and disease states^{99–101}. For example, in lung and liver, activation of TGF- β signaling is validated to be necessary for organogenesis during embryogenesis and tumor suppression at early stage, while excessive activation of TGF- β signaling could promote tissue fibrosis and the later tumor progression^{14,19}. This changing role of TGF- β signaling is particularly evident in joints under different pathophysiologic conditions¹⁰². In young and healthy joints, the articular cartilage has a basic expression level of TGF- β signaling and the activation of TGF- β signaling maintains the differentiated chondrocyte phenotype and tissue homeostasis mainly through SMAD2/3 pathway. However, in old or osteoarthritic joint, due to the altered expression level of TGF- β and TGF- β receptor, activation of TGF- β signaling promote the development of osteoarthritis mainly through SMAD1/5/8 pathway¹². Recently, it has been reported that overactivation of TGF- β signaling may contribute to the progression of IVD degeneration^{103,104}. In spine instability mouse models, Bian Q *et al.*^{10,103} observed that aberrant mechanical loading could lead to the excessive activation of TGF- β signaling and IVD degeneration, and administration of T β RI (ALK5) inhibitor suppressed the R-SMAD signaling and attenuated the IVD degeneration. And in a rabbit annular puncture model, Hu Y *et al.*¹⁰⁵ found that halofuginone could delay the IVD degeneration and the inactivation of TGF- β signaling might be involved. Furthermore, the study of Kwon YJ *et al.*¹⁰⁶ suggested that TGF- β 1 was increased and could activate both SMAD2/3 and SMAD1/5/8 pathways in bovine NPCs under degenerative condition, and the activated SMAD1/5/8 pathway could negatively regulate the SMAD2/3 signaling, which resulted in further IVD degeneration. In human chondrocytes, it has been confirmed that that ALK1-dependent SMAD1/5/8 signaling can inhibit TGF- β /ALK5-dependent SMAD3-driven transcriptional activity, which may provide a mechanistic explanation for the R-SMAD activation differences in IVD¹⁰⁷. Although the results above suggest that aberrant activation of TGF- β signaling may contribute to IVD degeneration, there are a few points to note here. First, these results have not been verified in human IVD tissues, so further studies with human cells are needed. Second, the expression of the TGF- β signaling pathway components and the R-SMAD activation change with the increasing age and the progression of the IVD degeneration, then it can be speculated that the changing role of TGF- β signaling is probably related to age and the different IVD degeneration stages. However, studies on TGF- β signaling in the IVD have always focused on young animal tissues and cells, while for the human tissues and cells there is a bias towards aged and degenerated material. Therefore, the studies of the role of TGF- β signaling in different age and degeneration stage model systems

are needed. Finally, the precise mechanism of the negative role of TGF- β signaling in IVD degeneration is still not clear and needs more investigations (Fig. 2).

The treatment prospect of TGF- β signaling in IVD degeneration

Currently, TGF- β signaling-based treatments for IVD degeneration can be roughly divided into two main categories: (1) activated TGF- β signaling directly repairs the IVD degeneration by increasing the content of ECM, inhibiting the cell loss and inflammatory response. (2) TGF- β signaling repairs the IVD degeneration indirectly by combining with tissue engineering technology. Because the direct treatment effects of TGF- β signaling have been introduced in detail above, the treatments of TGF- β combined with tissue engineering technology for IVD degeneration will be mainly discussed below. Mesenchymal stem cell (MSC)-based tissue engineering treatment has been widely contemplated for the repair of IVD degeneration and has shown promising perspectives^{108,109}. Studies show that TGF- β plays a vital role in it^{110,111}. On one hand, TGF- β can synergize with other growth factors to promote the differentiation of MSCs towards NP-like cells, which supplies the quantity of IVD cells for the repair of IVD degeneration^{112,113}. But in order to better understand the physiology and function of the NP-like cells, the cells should be evaluated by specific NP phenotypic markers, in accordance with the consensus stated by The Spine Research Interest Group at the 2014 Annual ORS Meeting¹¹⁴. On the other hand, TGF- β mediates the communication between NPCs and MSCs, which can improve the quality of IVD cells and promote the regeneration of degenerated IVD tissue¹¹⁵. Furthermore, with the development of various biomaterials, such as injectable hydrogels and self-assembling polypeptide scaffolds, the TGF- β can be released slowly to stimulate the TGF- β -induced MSCs differentiation and drive the MSCs-mediated IVD regeneration process^{116–118}. However, considering that excessive activation of TGF- β signaling is detrimental to the IVD, the activation level of TGF- β signaling for IVD degeneration treatment should be appropriate to ensure the safety and effectiveness. Of course, from another point of view, inhibition of the aberrant TGF- β signaling might have a therapeutic potential for IVD degeneration. Local application of the TGF- β signaling inhibitors might be a relatively good treatment strategy, although there are many challenges needed to be solved, such as the choice of administration dose, time and method.

Conclusion

As outlined in this review, TGF- β signaling plays a key role in the development, growth and tissue homeostasis of the IVD. Activation of TGF- β signaling has a promising treatment prospect for IVD degeneration. However, excessive activation of TGF- β signaling may contribute to the progression of IVD degeneration. Thus, it is urgent to elaborate the changing role of TGF- β signaling in IVD at different pathophysiological stages and its specific molecular mechanisms in further studies. Only when this key point is solved can TGF- β signaling-based treatments for IVD degeneration be safe and effective.

Authors' contributions

Zengwu Shao contributed to the conception and design of this review article. Sheng Chen performed searches, analyses, and interpretations. Lei Zhao and Sheng Liu drafted the paper, Hui Lin and Kaige Ma substantially revised the paper. Zengwu Shao gave final approval of the version to be submitted.

Conflicts of interest

None.

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