Molecular factors in intervertebral disc degeneration

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Abstract

The aim of this paper is to review the literature on molecular protein and messenger ribonucleic acid (mRNA) factors involved in intervertebral disc degeneration (IVDD). These elements were categorized basing on the changes in i) cell viability or number, ii) extracellular matrix (ECM) and iii) inflammation. Factors found to influence cell number and viability in IVDD included Fas/FasL, tumor necrosis factor related apoptosis-inducing ligand, death receptor-4/-5, bcl2-like 11, p53 inducible nuclear protein 1, p53/p21 factors, basic fibroblast growth factor and transforming growth factor-β. Factors found to affect IVD ECM included a range of matrix metalloproteinase, metalloproteinases with thrombospondin motifs, tissue inhibitors of metalloproteinases and disintegrins. Several proinflammatory factors have been identified in IVDD including interleukin-1β and TNF-α. The advent of protein and mRNA detection techniques has increased the understanding of IVDD from a molecular perspective. Further study of molecular protein and mRNA factors has the potential to identify and optimize therapeutic targets in the future.

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Introduction

It has been estimated that up to 84% of the global population suffers from lower back pain at some point in their lifetime, the most common cause being intervertebral disc (IVD) degeneration (IVDD).[1,2] Recent research has identified current treatments to be less effective than previously thought.[3] This highlights the importance of developing standardized, effective and non-invasive treatments for lower back pain, which requires a thorough understanding of the underlying molecular processes that lead to IVDD.

The IVD is composed of three main components, all of which are susceptible to degeneration. In the center lies the non-innervated gelatinous nucleus pulposus (NP), comprising of extracellular matrix (ECM) proteins such as proteoglycans and type II collagen fibers.[4] The NP serves to absorb shock and cushion impact between vertebrae. Surrounding the NP is the annulus fibrosus (AF), which is innervated and contains type I collagen and elastin fibers.[5] The AF facilitates mobility of the spine as well as providing resistance to radial forces from the NP during compressive loading.[6] Finally, cartilaginous end plates (CEP) above and below the AF, as well as the AF itself, modulate diffusion of nutrients between the vertebrae and IVD.[7,8] Vascular supply from the AF to the CEP is present at birth, but by adulthood the IVD undergoes a regression in vascularity.[9] This increases IVD susceptibility to degeneration because the NP ECM degrades without access to sufficient nutrients.

The current literature indicates that IVDD is mediated by changes in three major factors: i) cell viability or numbers, ii) ECM and iii) inflammatory cytokines.[10-14] Although we consider the contributions of these three factors separately, there exists a complex interplay between them, as well as with other local and systemic disease-related conditions. Collectively, these processes contribute to the progression of IVDD. For example, abnormal production of inflammatory cytokines by cells of IVD components such as the NP, the AF, and various cells of the immune sys-

tem has been reported to induce degradation of the ECM.[15] Moreover, these cytokines can lead to changes in cell viability and growth by promoting apoptosis and autophagy.[16,17]

This review aims to provide a current understanding of the molecular processes involved in IVDD, as derived from human studies involving protein expression analysis. Significant findings focus on the differences between degenerated and control IVDs. We define *expression studies* as studies that examine changes in mRNA and/or protein levels in response to altered microenvironments. These molecular studies investigate protein and mRNA expression by quantitative detection techniques as detailed in Tables 1-3.[14,18-42] Particular focus will be given to the microdynamics of cellular death and senescence, ECM and inflammatory cytokines within the IVD.

Cell death, senescence and growth factors

Cell death observed in IVDD either occurs with a specific biological intent (programmed cell death) or as a non-specific consequence of compromising general viability of cells and tissues (necrosis).[43] Programmed cell death in IVDD reflects the activation of intrinsic cell death-provoking physiological mechanisms, which are operative and essential for normal mammalian development to maintain tissue homeostasis and to counter viral or bacterial infections.[43] While many forms of programmed cell death are known, including apoptosis, autophagy, pyroptosis and necroptosis, expression of proteins that trigger apoptosis in IVD tissue indicates the mediation of a deliberate biological response during IVDD. Cellular senescence represents a biological state characterized by a terminal proliferative arrest and reduced metabolic rates. This becomes more prevalent during ageing and likely contributes to IVDD in elderly patients. Senescent cells have high levels of cell cycle inhibitors such as p16/CDKN2A. These are linked to the activities of tumor suppressors such as retinoblastoma protein (RB1) and p53.[44] Multiple factors linked to growth arrest and cell death have been identified that contribute to cell loss observed in IVDD (Table 1).[14,18-22]

Degeneration of the IVD is accompanied by changes in cell density, although this biological parameter is not unequivocal. A decrease in cell density has frequently been associated with IVDD, however increase in cell density has also been reported.[45] Observations of increased cell density may perhaps reflect a stage in the disease where the ECM has become dehydrated or depleted of its constituent structural glycosylated proteins. Alternatively, increased cell density could result from an initial tissue repair response in which cell proliferation is stimulated to restore IVD tissue. Liebscher *et al.*[46] reported a decrease in cell density measured using morphometric analysis that inversely correlated with histologic disc degeneration scores in human specimens. Contributing to this decrease in cell density is programmed cell death occurring during IVDD. This however has not been the only correlation observed. Initial observations by Gruber and Hanley found there increased apoptotic cells in control specimens relative to degenerated specimens.[47] Perhaps not surprisingly, these studies also showed that the presence of apoptotic cells in specimens not only correlates with the degree of disease progression but also increases with the age of the patient.

Park *et al.*[18] used immunohistochemistry (IHC) to observe expression of programmed cell death receptor Fas on degenerated IVD cells. They could not identify a correlation with IVDD using magnetic resonance imaging (MRI), although there was a correlation with age, supporting the initial findings by Gruber and Hanley.[47] In a follow-up study Park *et al.,*[19] observed Fas ligand (FasL) on other IVD cells within the same unit and postulated that Fas mediated programmed cell death in IVD occurs via an autocrine or paracrine mechanism.

Table 1. Expression of cell death, senescence and growth factors in human intervertebral disc degeneration.[14,18-22]

Study	Factor (protein/mRNA)	Expression in degenerated IVD	Outcome in the IVD	Study methodology
Park *et al.*, 2001[18]	Fas (protein)	Up	Increased cell death	IHC
Park *et al.*, 2001[19] Bertram *et al.*, 2009[20]	Fas ligand (protein)	Up	Increased cell death	IHC
Bertram *et al.*, 2009[20]	TNF-related apoptosis-inducing ligand (protein)	Up	Increased cell death	IHC
Bertram *et al.*, 2009[20]	Death receptor 4 (protein)	Up	Increased cell death	Flow cytometry
Bertram *et al.*, 2009[20]	Death receptor 5 (protein)	Up	Increased cell death	Flow cytometry
Gruber *et al.*, 2010[14]	bcl2-like 11 (mRNA)	Up	Increased cell death	Microarray
Gruber *et al.*, 2010[14]	p53 inducible nuclear protein 1 (mRNA)	Up	Increased cell death	Microarray
Zhou *et al.*, 2016[21]	p53/p21 factors (protein)	Up	Increased cell death	IHC
Tolonen *et al.*, 2006;[22] Gruber *et al.*, 2010[14]	Basic fibroblast growth factor (protein, mRNA)	Up	Increased or decreased cells survival	IHC; microarray
Gruber *et al.*, 2010[14]	Transforming growth factor-β (mRNA)	Down	Decreased cell survival	Microarray

IVD, intervertebral disc degeneration; IHC, immunohistochemistry.

Recently, Bertram *et al.*[20] used IHC to analyze TNF-related apoptosis-inducing ligand (TRAIL). Both TRAIL and FasL expressions and death receptors DR4 and DR5 were detected on degenerated IVD. Using IHC to detect levels of poly(ADP-ribose) polymerase (PARP) p85, a downstream factor in the TRAIL apoptotic signaling pathway, they confirmed an increased rate of apoptosis in degenerated IVDs. Subsequent findings supported these observations with microarray analysis of IVD that detected increased levels of apoptotic mediators, such as BCL2L11 (bcl2-like 11), and TP53INP1 (p53 inducible nuclear protein 1) in degenerated IVD.[14]

Beyond the disease-related effects of cell death in IVDD, cell senescence also contributes to the onset and progression of IVDD. Senescent cells reduce the intrinsic repair capacity of IVD tissue. This is due to their diminished metabolic activity and capacity to produce extracellular matrix proteins, as well as simultaneously being incapable of proliferative expansion to restore appropriate cell numbers after induction of apoptosis in other cells within the tissue. Senescence has been observed in IVDD with the classical senescence biomarker beta-galactosidase, which is detected at significantly high levels.[48] Zhou *et al.*[21] used enzyme-linked immunosorbent assay (ELISA) to detect beta-galactosidase and concluded higher levels of cellular senescence in CEP of degenerated IVD cells compared to control. They were further able to use IHC to identify the p53/p21 pathway as the predominant senescence mechanism in IVDD.

Programmed cell death signals in IVDD operate in concert with proliferative or growth factor signals in maintaining tissue homeostasis. Apart from induction of cell death that has been observed in IVDD, a difference in growth signals has been observed in IVDD as well. Tolonen *et al.*[22] detected higher levels of basic fibroblast growth factor (bFGF/FGF2) in degenerated IVD cells relative to control IVD cells using IHC. Although bFGF is a very potent mitogen that provokes proliferative responses, stimulation by bFGF can also promote apoptosis.[49,50] The latter may reflect a state in which bFGF2 signaling is provoked while other conditions required for cell proliferation such as other growth factors, nutrition and metabolic activity are not met. Gruber *et al.*[14] employed microarray analysis to show that annulus cells from degenerated IVDs express different patterns of growth factors, in particular a downregulation of TGF-β1 and upregulation of bFGF relative to control.

In IVDD, there is a loss of IVD cells through programmed cell death. This is exacerbated by the development of IVD cell senescence. Loss of these cells along with aberrant expression of growth factors is believed to affect the integrity of ECM and contribute to IVDD as disc chondrocytes are vital to maintaining a healthy ECM and preventing IVDD.[51]

Extracellular matrix changes

The ECM of skeletal tissues is composed primarily of water and macromolecules such as proteins and polysaccharides.[52] IVD tissues contain specifically collagen type II that is characteristic of other cartilaginous tissues and non-collagenous proteins tissues that are extensively glycosylated such as proteoglycans. The ECM in IVD cells is involved in IVD tissue homeostasis in addition to serving as physical support for the cells it surrounds. It influences AF and NP cell orientation, supports cell viability and cell differentiation and maintains the biophysical properties of the microenvironment such as hydration levels and local pH.[53] Many factors have been identified that contribute to a loss of ECM integrity in IVDD (Table 2).[23-34]

Cell viability in the IVD is dependent on the balance of catabolic and anabolic processes within the ECM.[51] ECM production is dependent on cellular secretion within IVD chondrocytes to maintain integrity in IVDD. In fact, it has be postulated that this process promotes IVDD.[51] The major catabolic factors involved in IVD ECM degradation are matrix metalloproteinases (MMPs), disintegrins and metalloproteinases with thrombospondin motifs (ADAMTSs) and tissue inhibitors of metalloproteinases (TIMPs).[11] MMPs are a family of calcium-dependent, zinc cofactor-containing endopeptidases involved in the breakdown of specific ECM components such as collagen, gelatin, laminin and proteoglycans.[54-56] ADAMTSs are a family of multidomain metalloproteinases that has a distinct set of substrates to MMPs and lecticans.[57,58] TIMPs maintain ECM homeostasis and inhibit both MMPs and ADAMTSs.[59]

Increased MMP expression in IVDD has been reported in the literature.[11] Crean *et al.*[26] were the first to investigate the differences in MMP expression between degenerated and control human IVD cells. Using gelatin-gel zymography, they detected that MMP-2 and -9 were increased in degenerated IVDs. A few years later, Roberts *et al.*[23] did comprehensive IHC analysis of MMP expression in degenerated IVDs. They demonstrated increased MMP-1, -2, -3, -7, -8, -9 and -13 staining in degenerated IVD cells compared to the control IVD cells. Specifically, MMP-8 had the greatest difference in expression between the two population samples. Weiler *et al.*[24] followed with a similar study, using IHC to detect the temporo-spatial distribution of MMP-1, -2, -3 and -9 expression in degenerated discs *versus* non-degenerated discs. Expression of MMP-1, -2 and -3 was found to significantly correlate with the degree of IVDD, where MMP-1 showed the greatest significant correlation. MMP-9 on the other hand was detected at very low levels across all samples, its expression not significantly correlated with IVDD.

In the following years, multiple studies determined specifically the tissue localization of MMPs in IVDD. Le Maitre *et al.*[28] found that MMP-1 increased mostly in the NP and, to a lesser degree, in the AP of degenerated IVDs. In their study, MMP-13 was the only MMP observed to have a statistically significant difference between degenerated and non-degenerated IVD. Furthermore, IHC was utilized to demonstrate the greatest increase in MMP-7 to the NP in degenerated IVD cells. Bachmeier *et al.*[27] used QRT-PCR,

IHC and zymography to show that MMP-3 and MMP-8 were significantly and consistently upregulated in degenerated IVDs relative to healthy IVDs. In the same year, Gruber *et al*.[29] observed MMP-28 in the ECM of degenerated IVDs only by using IHC. Deng *et al.*[25] recently elucidated significantly greater MMP-1 expression in lumbar disc tissue and serum from IVDD patients compared to control patients.

ADAMTSs have also been reported to be upregulated in IVDD recently. Le Maitre *et al.*[28] used IHC to localize a significant increase in ADAMTS-4 expression to the NP in degenerated IVD. Patel *et al.*[30] used Western blot (WB) analysis to confirm increase in ADAMTS-4 correlated to the severity of degeneration in IVDD. They observed no difference in ADAMTS-5 expression, although a few years later, two studies by Pockert *et al.*[31] and Zhao *et al.*[32] found using QRT-PCR, a significant increase in mRNA expression of ADAMTS-5 in degenerated IVD tissue. Pockert *et al.*[31] also observed an increase in ADAMTS-4 and -15.

The deregulation of ECM homeostasis in degenerated IVDs has been attributed to changes in TIMP. Roberts *et al.*[23] observed higher expression of TIMP-1 in degenerated IVDs compared to the control using IHC. TIMP-2 was not observed to be significantly different. Le Maitre *et al.*[28] also used IHC to localize TIMP-1, -2 and -3 expression. They reported significant expression increase of TIMP-1 in the NP and inner AF; TIMP-2 in the NP only; and TIMP-3 in the inner AF only. The increase in TIMP-3 expression was mod-

Table 2. Expression of extracellular matrix modulating factors in human intervertebral disc degeneration.[23-34]

Study	Factor (protein or mRNA)	Degenerated IVD expression	Outcome in the IVD	Study methodology
Roberts *et al.*, 2000;[23] Weiler *et al.*, 2002;[24] Deng *et al.*, 2015[25]	MMP-1 (protein)	Up	Increased ECM breakdown	IHC; ELISA (of serum)
Crean *et al.*, 1997;[26] Roberts *et al.*, 2000;[23] Weiler et al 2002[24]	MMP-2 (protein)	Up	Increased ECM breakdown	Gelatin-gel zymography; IHC
Roberts *et al.*, 2000;[23] Weiler *et al.*, 2002;[24] Bachmeier *et al.*, 2009[27]	MMP-3 (protein)	Up	Increased ECM breakdown	IHC; QRT-PCR, zymography
Roberts *et al.*, 2000[23]	MMP-7 (protein)	Up	Increased ECM breakdown	IHC
Roberts *et al.*, 2000;[23] Le Maitre *et al.*, 2004;[28] Bachmeier *et al.*, 2009[27]	MMP-8 (protein)	Up	Increased ECM breakdown	IHC
Crean *et al.*, 1997;[26] Roberts *et al.*, 2000[23]	MMP-9 (protein)	Up	Increased ECM breakdown	Gelatin-gel zymography; IHC
Roberts *et al.*, 2000;[23] Le Maitre *et al.*, 2004[28]	MMP-13 (protein)	Up	Increased ECM breakdown	IHC
Gruber *et al.*, 2009[29]	MMP-28 (protein)	Up	Increased ECM breakdown	IHC
Le Maitre *et al.*, 2004;[28] Patel *et al.*, 2007;[30] Pockert *et al.*, 2009[31]	ADAMTS-4 (protein, mRNA)	Up	Increased ECM breakdown	IHC; WB; QRT-PCR
Pockert *et al.*, 2009;[31] Zhao *et al.*, 2011[31]	ADAMTS-5 (mRNA)	Up	Increased ECM breakdown	QRT-PCR
Pockert *et al.*, 2009[31]	ADAMTS-15 (mRNA)	Up	Increased ECM breakdown	QRT-PCR
Roberts *et al.*, 2000;[23] Le Maitre *et al.*, 2004[28]	TIMP-1 (protein)	Up	Imbalanced ECM homeostasis	IHC
Le Maitre *et al.*, 2004[28]	TIMP-2 (protein)	Up	Imbalanced ECM homeostasis	IHC
Le Maitre *et al.*, 2004[28]	TIMP-3 (protein)	Up	Imbalanced ECM homeostasis	IHC
Chen *et al.*, 2016[33]	SOX-9 (mRNA, protein)	Down	Dysregulated ECM homeostasis	QRT-PCR, IHC
Chen *et al.*, 2016[33]	CA12 (mRNA, protein)	Down	Dysregulated ECM homeostasis	QRT-PCR, IHC
Chen *et al.*, 2016[33]	HIF-1a (mRNA, protein)	Down	Dysregulated ECM homeostasis	QRT-PCR, IHC
Yee *et al.*, 2016[34]	CILP (protein)	Up	Decreased ECM gene expression	Mass spectrometry, WB, IHC, IF
Yee *et al.*, 2016[34]	HTRA1 (protein)	Up	Decreased ECM gene expression and increased ECM breakdown	Mass spectrometry, WB, IHC, IF

IVD, intervertebral disc; ECM, extracellular matrix; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; TIMP, tissue inhibitors of metalloproteinases; SOX, sry-related HMG box; CA, carbonic anhydrase; HIF, hypoxia-inducible factor; CILP, cartilage intermediate layer protein; HTR, serine protease; IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; QRT-PCR, quantitative reverse transcription-polymerase chain reaction; WB, western blot; IF, immunofluorescence.

est compared to the other two. However, recently Pockert *et al.*[31] and Bachmeier *et al.*[27] detected TIMP-1, TIMP-2 or TIMP-3 mRNA expression using QRT-PCR and observed no correlation with disc pathology and these metalloproteinase inhibitors. Likewise, Deng *et al.*[25] reported no difference in TIMP-1 expression between IVDD patient and control samples. Although these data are conflicting, it is clear there is an association between aberrant TIMP expression and IVDD that likely contributes to a loss of ECM homeostasis in the IVD.

In the past year, ECM modulating factors other than metalloproteinases or their inhibitors have been identified in IVDD. Chen *et al.*[33] used QRT-PCR, confirmed with IHC and histopathology staining, to show that ECM components, type II collagen and aggrecan are downregulated in degenerated disc tissue, along with the transcription factor SOX-9. Oh *et al.*[60] had previously demonstrated the involvement of SOX-9 in the regulation of many chondrocyte ECM genes. Chen *et al.*[33] also had demonstrated using QRT-PCR the downregulation of CA12 and HIF-1α in degenerated IVD cells.

Using mass spectrometry to analyze differential protein expressions in IVDD cells, Yee *et al.*[34] found there was an increase in the expression of cartilage intermediate layered protein (CILP) and high temperature requirement A (HTRA1). These findings were confirmed by WB, IHC and immunofluorescence analysis. The protein CILP has been shown to inhibit TGF-β1-mediated induction of cartilage matrix gene expression.[61] Serine protease HTRA1 has been shown to modulate TGF-β1, bone morphogenic protein and degrade ECM components such as aggrecan and collagen.[62]

There are a multitude of proteins that contribute to the loss of ECM homeostasis and drive the pathophysiology of IVDD. The more thoroughly researched proteins include the MMPs. There is some potential that ADAMTSs and TIMPs are also involved in IVDD etiology. Along with cell death in the IVD, inflammation also affects ECM integrity. Indeed, pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumour necrosis factor (TNF) have also been implicated in ECM modulation in IVDD.[15,63]

Pro-inflammatory factors

Inflammatory processes aim to re-establish homeostasis in response to insult such as infection, injury or tissue malfunction.[64] It is likely that in the context of IVDD inflammation manifests due to physiological changes, rather than as a result of infection or injury. Although the inflammation mechanism of IVDD remains unclear, multiple studies have reported the presence of inflammatory mediators in human degenerated IVDs.[10,36-42,65] Inflammation in IVDD is largely mediated by pro-inflammatory cytokines, such as belonging to the interleukin-1 (IL-1) and tumor necrosis factor (TNF) families.[66] Binding of these cytokines to their respective receptors initiate signaling cascades that ultimately lead to recruitment of immune cells and prostaglandin release. These reactive products then modulate IVDD pain and inflammatory symptoms.[67] Many pro-inflammatory factors in the IVD that contribute to the pathophysiology of IVDD have been identified (Table 3).[35-42]

The most characterized and long established inflammatory cytokines found in IVDD are IL-1β and TNF-α. Igarashi *et al.*[35] first detected IL-1β, TNF-α and IL-6 via ELISA and CLEIA in lumbar facet joint cartilage and synovium of individuals known to have IVDD. They hypothesized that inflammatory cytokines are involved in pain gen-

Table 3. Expression of pro-inflammatory factors in human intervertebral disc degeneration.[35-42]

Study	Factor (protein or mRNA)	Degenerated IVD expression	Outcome in the IVD	Study methodology
Igarashi *et al.*, 2004;[35] Park *et al.*, 2011[36]	IL-1β (protein, mRNA)	Up	Inflammation, pain generation	ELISA, CLEIA; QRT-PCR
Igarashi *et al.*, 2004;[35] Weiler *et al.*, 2005;[37] Lee *et al.*, 2009[38]	TNF-α (protein)	Up	Inflammation, pain generation	ELISA, CLEIA; IHC; WB
Igarashi *et al.*, 2004[35]	IL-6 (protein)	Up	Inflammation, pain generation	ELISA, CLEIA
Lee *et al.*, 2009[38]	IL-8 (protein)	Up	Inflammation, pain generation	WB
Gruber *et al.*, 2013[39]	IL-17 (protein, mRNA)	Up	Inflammation	IHC, microarray
Wang *et al.*, 2013[40]	CCL3 (mRNA, protein)	Up	Promote macrophage infiltration	QRT-PCR, IHC
Gruber *et al.*, 2014[41]	RANTES (protein, mRNA)	Up	Promote macrophage, monocyte and T cell chemoattraction	IHC, microarray
Klawitter *et al.*, 2014[42]	TLR-1 (mRNA)	Up	Inflammation	QRT-PCR
Klawitter *et al.*, 2014[42]	TLR-2 (mRNA, protein)	Up	Inflammation	QRT-PCR, WB
Klawitter *et al.*, 2014[42]	TLR-4 (mRNA)	Up	Inflammation	QRT-PCR

IVD, intervertebral disc; IL, interleukin; TNF, tumor necrosis factor; CCL, chemokine ligand; RANTES, regulated on activation, normal T cell expressed and secreted; TLR, toll-like receptor; ELISA, enzyme-linked immunosorbent assay; CLEIA, chemiluminescent enzyme immunoassay; IHC, immunohistochemistry; WB, western blot; QRT-PCR, quantitative reverse transcription-polymerase chain reaction.

eration. These authors continued in a later study[65] to confirm that the expression of these inflammatory cytokines correlates with pain symptoms in IVDD patients. Weiler *et al.*[37] confirmed a statistical correlation between expression of TNF-α and IVD degeneration. TNF-α was only found in specimens sourced from adults of advanced age, indicating that TNF-α is more likely to aggravate rather than initiate IVDD. Furthermore, inflammatory cytokines TNF-α and IL-8 were found by Lee *et al.*[38] to have greater expression in IVDD patients compared to those with just a NP herniation. This potentially explains why those with IVDD experience more severe back pain than patients with NP herniation alone. Park *et al.*[36] found greater expression of IL-1β and TNF-α expression in degenerated IVD compared to control, suggesting that the expression of inflammatory cytokines is directly related to the severity of IVDD pathophysiology.

In the past few years, other inflammatory proteins have been observed in degenerated IVD, through stimulation by IL-1β and TNF-α. Gruber *et al.*[39] detected significantly higher IL-17 protein, via IHC, and gene, via microarray, expression in degenerated IVD cells upon stimulation with IL-1β and TNF-α compared to non-degenerated IVD cells. In a similar study, Wang *et al.*[40] used QRT-PCR and IHC to detect chemokine CCL3 at higher levels in NP cells after being treated with IL-1β and TNF-α. A year later, Gruber *et al.*[41] went on to assess changes in the expression of another chemokine, that is regulated upon activation, normal T-cell expressed, and secreted (RANTES) in degenerated IVDs after IL-1β and TNF-α challenge. They used IHC and QRT-PCR to detect elevated RANTES in degenerated IVD as a result of IL-1β and TNF-α exposure. Finally, Klawitter *et al.*[42] used QRT-PCR to assess toll-like receptor (TLR) mRNA expression in cells from IVDD patients after stimulation with IL-1β and TNF-α. They found a correlation between TLR-1/-2/-4/-6 expression and the degree of IVD degeneration, however, upon stimulation with IL-1β and TNF-α, TLR-1/-4 had a moderate increase while TLR-2 was strongly increased. The increase in TLR-2 expression was further confirmed at the protein level with a WB.

Inflammatory factors can reflect the intensity and severity of IVDD, and thus are a major component of IVDD etiology. Of these inflammatory factors, the most thoroughly studied are IL-1β and TNF-α, however other factors that are part of the same pathway are emerging as possible therapeutic targets.

Conclusions

Molecular study the pathogenesis of IVDD has provided a new perspective for researchers and clinicians, identifying never before seen mechanisms and molecular pathways. Continued molecular studies of degeneration in human IVD will assist in the identification and clarification of potential molecular targets for future therapeutic approaches.

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