Review

Molecular mechanisms involved in intervertebral disc degeneration and potential new treatment strategies

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Lower back pain (LBP) is a major cause of pain and disability. However, current treatment strategies are focused primarily on relieving its symptoms and have varying degrees of success. For future treatments to be proactive, they must target the underlying pathogenic alterations in cellular biology. Intervertebral disc degeneration (IVDD) has been linked to a high percentage of LBP cases, therefore, inhibition of the processes contributing to IVDD and, regeneration of the intervertebral disc (IVD) matrix lost during IVDD are the primary focuses of current research. Therapies aimed at the inhibition of the cytokine interleukin-1 that is increased during IVDD have been investigated as potential treatments aimed at inhibiting the pathogenic processes of IVDD. In addition, the application of growth factors, such as insulin-like growth factor, transforming growth factor and bone morphogenetic protein or alternatively replacement of abnormal IVD cells, either by injection of mesenchymal stem cells or autologous disc cell transplantation, has been investigated as potential therapeutic agents aimed at regeneration of the IVD matrix. However, for research into these therapeutic techniques to progress, a more detailed knowledge of the complex cellular biology of the IVD is required.

Key words: intervertebral disc degeneration, cytokine, growth factor, gene therapy, mesenchymal stem cells, autologous disc cell transplantation.

Lower Back Pain

Lower back pain (LBP), sometimes referred to as lumbago, is a highly prevalent cause of pain and disability in developed countries. Epidemiological studies estimate that approximately 80% of the population will suffer from LBP at some point of time in their lives. An additional factor to consider when assessing the morbidity of LBP is the economic burden as LBP has been estimated to cost the UK approximately £12 billion per annum. Two, three

LBP symptoms range from pain specific to areas of the lower back, and can lead to sciatica, where the pain radiates down into either one or both of the legs. Although the exact pathogenesis of LBP has not yet been elucidated, a variety of causes such as mechanical stress, herniated disc, tissue injury and intervertebral disc degeneration (IVDD) have been implicated as potential causes, however these features can also be observed in asymptomatic individuals. In addition, it has been suggested that between 52 and 74% of LBP cases could arise from genetic origin. However, although some genes have been identified, the genetic influence is still poorly understood.

Existing therapies for LBP are mainly symptomatic and achieve inconsistent results. The current therapies range from simple non-operative treatments such as medication aimed at pain relief to more extreme operative procedures aimed at alleviating the clinical symptoms of LBP. These therapies do not target the pathogenic changes which are seen in the degenerate disc and have been linked to approximately 40% of LBP cases. Thus, future treatment options under investigation are targeted at the underlying pathogenesis of LBP rather than the symptoms which arise as a result of pathogenic processes. However, for the pathogenesis of LBP to be targeted, a detailed knowledge of the anatomy and physiology of the spine is required.
The human spine is composed of 33 individual bones, 24 of which are movable vertebrae, divided into the cervical, thoracic and lumbar regions in addition to seven fused vertebrae consisting of the sacral and coccygeal regions. The vertebrae are separated by intervertebral discs (IVDs), which allow bending and torsion of the spine and protect it during mechanical loading. The individual vertebrae and IVDs are held together by ‘ligaments’ that provide the tension that facilitates the curved posture of the spine. LBP is associated with abnormalities in the IVDs of the lumbar region, which is under continuous pressure from bending, twisting and lifting, and supports the weight of the entire upper body.

**Intervertebral Discs**

Collectively the IVD contribute 25% of the length of the spine. Each IVD is composed of the annulus fibrosus (AF) and nucleus pulposus (NP).

The AF consists of densely arranged, circumferential, lamellae rich in collagen fibres. The AF is mainly composed of collagen type I, but also contains other types of collagen fibres. The peripheral layer of the AF has a high concentration of collagen type I fibres that progressively become more proportionally collagen type II fibres towards the centre of the IVD. The AF is responsible for encasing the gelatious NP and enabling the IVD to sustain considerable compressive loads. The outer AF is composed of fibroblast-like cells, but is populated by chondrocyte-like cells towards the inner AF region. The NP core of the IVD is comprised of chondrocyte-like cells, collagen (mainly type II) fibres and proteoglycans, mainly aggrecan with smaller amounts of versican, decorin, biglycan, fibromodulin and lumican. The proteoglycan-rich matrices, especially the aggrecans, are negatively charged and attract water resulting in a hydrophilic core. This hydrophilic composition of the NP enables the structure to withstand compressive force and acts as the shock absorber in the spine.

Cartilaginous end plates (CEPs) are located superior and inferior to the IVD and are involved in the attachment of each individual IVD to their respective vertebrae. The CEP is a thin structure approximately 1 mm in width, composed of hyaline cartilage and populated by chondrocyte cells. As the IVD is mainly avascular, the CEP is heavily involved in the nutrition of, and removal of waste products from, the IVD. Nutrients such as glucose and oxygen enter the IVD by passive diffusion in a process which is facilitated by the blood vessels which penetrate the CEP and the peripheral AF. Waste products such as lactic acid and carbon are removed in a reciprocal manner. As the CEP ages, calcification occurs and consequently the ability to supply the IVD with nutrients deteriorates.

**Intervertebral Disc Degeneration**

IVDD has been identified in approximately 40% of LBP diagnoses, however current knowledge of the principal pathogenesis resulting in this condition is limited. Degeneration of the disc occurs during the normal ageing process, however accelerated degeneration is seen in diseased discs. While IVD height does undergo some decrease with age, the reduction is not as significant as during IVDD, hence the loss of disc height is characteristic of IVDD and can be used in the diagnosis of the condition. IVDD is a multifactorial process influenced by mechanical pressures, genetic inheritance and alterations in the cellular biology of the IVD.

The chondrocyte-like cells of the NP are responsible for the production of growth factors such as bone morphogenetic protein (BMP), transforming growth factor (TGF) and insulin-like growth factor (IGF) and cytokines such as interleukin (IL)-1 (IL-1), IL-6 and tumour necrosis factor (TNF). Growth factors are involved in the regulation of IVD matrix by stimulating production of matrix proteins. In contrast, the cytokines inhibit matrix synthesis and stimulate the production of degradative enzymes such as matrix metalloproteinases (MMPs) and a disintegrin-like and metalloprotease with thrombospondin motifs (ADAMTS) which break down the extracellular matrix of the IVD. The chondrocyte-like cells of the IVD are involved in both the synthesis of the IVD matrix and also the production of the enzymes involved in its degradation. Chondrocyte-like cell abnormalities result in alteration of the homeostatic processes within NP cells and have therefore been identified as potential pathogenic agents involved in IVDD.

Increased MMP and ADAMTS enzyme activity is a characteristic of IVDD, particularly, MMP 7, 13 and MMP 13, and ADAMTS 4 and ADAMTS 5, which mediate the degradation of the IVD matrix components collagen type II and aggrecan.

MMP enzymes are secreted in their latent form and so require activation to enable their proteolytic properties. In addition, MMP activity is blocked by endogenous tissue inhibitors of metalloproteinases (TIMP) of which there are four types: TIMP 1, 2, 3 and 4. TIMP 1 and 2 are the major inhibitors of MMPs. However, as production of TIMP 1 and 2 is also increased in the IVD during cases of IVDD, the MMPs such as MMP 7, which demonstrate resistance to TIMP 1 and 2, have been implicated as key enzymes involved in the pathogenesis of IVDD. ADAMTS, however, are inhibited specifically by TIMP 3, which, in contrast to TIMP 1 and 2, is not upregulated during cases of IVDD; therefore, the aggrecanase activity of the ADAMTS is increased in cases of IVDD, because the increased production in ADAMTS enzymes are not controlled by a concurrent increase in their inhibitor.

In addition to increased matrix degradation, cells in IVDD become abnormal and display cell senescence, which has been linked to the increase in matrix degradation enzymes. A reduction in the number of active chondrocyte-like cells leads to a decrease in the production of the growth
factors required to stimulate matrix synthesis. Consequently, any matrix degradation cannot be repaired and will also result in a decrease in overall IVD matrix.

The matrix composition and cellular activity of the IVD is integral to its functionality. IVDD results in loss of the IVD matrix, loss of chondrocyte-like cells of the NP, decreased structural integrity, hydration and ability to withstand mechanical loads, angiogenesis and innervation. These biological changes result in IVDD and the associated decreased disc height, which can result in the symptoms associated with LBP.23

**Future Treatment Strategies**

For future LBP therapies to be successful, they must be aimed at the underlying pathogenesis such as the alterations in cellular biology, which have been linked to LBP.23,26 Consideration has been given to the role of the cytokines IL-123 and TNFα28 in the pathogenesis of IVDD. During IVDD, the expression of both cytokines is increased, and both have been shown to inhibit matrix synthesis and to increase gene expression for matrix degradation enzymes in vitro.23–26 However, although the functional receptors of IL-1 are upregulated throughout IVDD, expression of the TNFα receptors is actually decreased.25 Therefore, while both cytokines are expressed in instances of IVDD, IL-1 has been implicated as the major mediator of IVDD.25,26 Indeed, using *in situ* zymography techniques, inhibitors of IL-1 have been shown to inhibit matrix degradation in both normal and degenerate discs, however inhibition of TNFα failed to reduce the matrix degradation, thus supporting the theory that IL-1 is a key cytokine involved in the pathogenesis of IVDD.26

The natural inhibitor of IL-1, IL-1 receptor antagonist (IL-1Ra), is also produced by IVD cells. In non-degenerate IVDs, the actions of IL-1 appear to be controlled by the levels of IL-1Ra present, however during IVDD an increase is seen in the production of IL-1 with no parallel increase in IL-1Ra and thus the balance is lost.24 As such, IL-1Ra has been identified as a possible therapeutic agent to target the inhibition of IL-1-mediated pathogenesis of IVDD.6

IL-1Ra has been administered previously in clinical procedures such as limiting cartilage degradation in rheumatoid arthritis, and consequently the related pharmacology and side effects are well elucidated.27 However, in these circumstances IL-1Ra is injected subcutaneously, a technique which cannot be applied to the IVD due to their avascular composition.16 Any passive diffusion into the IVD would not be sufficient to elicit a clinical effect and additionally there is a risk that inhibition of IL-1 in the systemic circulation would occur.6 Direct administration of cytokines to the IVD by injection is another previously proposed method; however, due to the short biological half-life of IL-1Ra, repeated administration would be necessary to achieve the desired clinical impact.6 Gene therapy has therefore been identified as a potential solution to the administration problems as a single dose of IL-1Ra could be administered via gene therapy to the IVD which would provide long-lasting effects.6

Genetic material can be delivered into host cells by vectors via one of two methods *in vivo* or *ex vivo*. *In vivo* refers to the direct injection or inhalation of the vector. *Ex vivo* is the process where host cells are removed and the vector is applied *in vitro*, and then the modified cells are returned to the host. There are two classifications of vectors which can be used in gene therapy: viral or non-viral vectors. Viral vectors can be further subdivided into two categories: genome incorporating, which include retroviruses and lentiviruses and non-genome incorporating viruses, such as herpes viruses, adenoviruses and adeno-associated viruses. Viral vectors are genetically modified viruses which have been engineered to lack the genetic material which makes them pathogenic while retaining the genetic information which enables insertion of their genes into host cells. Additionally, a copy of the therapeutic gene is inserted into the viral vector so that it may be transferred into the host cell.

Adenoviral vectors have been utilized in various gene therapy investigations on the IVD.6,15,28–30 As adenoviruses do not incorporate into the host cell’s genetic material, the potential risk of oncogenesis associated with genome-incorporating vectors is eliminated.6 One potential drawback of adenoviral vectors is that therapeutic genes are not transferred to daughter cells when the IVD cells replicate; however, due to the low cell turnover in the IVD, this potential disadvantage should not be a significant problem in practice.6

*Ex vivo* administration of IL-1Ra to human IVD explants, using adenovirus-mediated gene therapy, significantly inhibits the activity of the degradative enzymes in degenerated discs.6,15 In addition, other treatment strategies, such as oral administration of glucosamine and chondroitin sulfate31 and gene therapy targeting the anti-catabolic gene TIMP1, which will inhibit the increased production of MMPs seen during disc degeneration, aimed at inhibiting the processes of IVDD have been recently reviewed, but will not address the increased production of ADAMTS which also appear important in the pathogenesis of IVDD.32

Preventing the loss of IVD matrix composition would ideally be used in conjunction with an approach aimed at regenerating the IVD matrix in order to begin reversing the matrix composition. Therefore, regeneration of the degenerated IVD matrix must be considered as an additional and equally important factor in the treatment of IVDD.6

**Regeneration of Disc Matrix**

Both the application of growth factors such as IGF, TGF and BMPs and alternatively replacement of abnormal IVD cells,
either by injection of adult mesenchymal stem cells (MSCs) or autologous IVD cells, have been investigated as potential therapeutic agents aimed at regeneration of the IVD matrix. Growth factors are peptides the function of which is to regulate the stimulation of cellular proliferation, differentiation and migration and to stimulate matrix synthesis. In the IVD specific growth factors such as IGF, TGF and BMP are produced by the chondrocyte-like cells of the NP and act to stimulate matrix synthesis. As loss of IVD matrix composition is a characteristic feature of IVDD, growth factors have been investigated as potential therapeutic agents, aimed at promoting matrix synthesis in the degenerated IVD. 

Preliminary work implementing exogenous application of growth factors, such as TGF-β1, IGF-1 and BMP-7, demonstrated increased IVD matrix synthesis following administration. However, as growth factors are known to have short biological half-lives, this method of administration would not be feasible in clinical scenarios as IVDD is a chronic condition and therefore requires the growth factor application to be prolonged. The requirement for a sustainable method of growth factor application resulted in the development of the gene therapy delivery system for the application of growth factors. Initial gene therapy studies examined the possibility of introducing exogenous genes into IVD cells utilizing retroviral vectors and both in vitro and in vivo studies utilizing adenoviral vectors. The success of gene therapy studies on rabbit IVD cells was the foundation for similar experiments on human IVD cells. Adenoviral vectors were implemented to transduce TGFβ1 genes into human IVD cells, which resulted in increased TGFβ1 protein expression which in turn increased synthesis of proteoglycan and collagen by over 300%, which was significantly more than exogenous application. Studies have also examined gene therapy delivery of other growth factors such as BMP-2 and IGF-1 which have also been implicated in the processes of IVD matrix synthesis. Future gene therapy approaches could potentially combine the various growth factors implicated in the processes of IVD matrix synthesis such as TGFβ1, IGF-1 and BMP-2, in order to treat IVDD, and an additive effect on the amplification of proteoglycan synthesis has been observed. 

However, an important observation that TGF and IGF receptors are expressed in the blood vessels of the IVD suggests that the therapeutic use of these particular growth factors to regenerate the IVD matrix could cause excessive angiogenesis and subsequently nerve ingrowth and therefore would not be a viable treatment strategy. As BMP receptor expression was not observed in the IVD blood vessels, growth factors targeted at these receptors have been identified as preferable therapeutic agents for therapies aimed at regenerating IVD matrix in cases of IVDD. An alternative to the use of growth factors is the utilization of transcription factors. Complex signalling cascades involving various transcription factors are involved in the production of growth factors and the matrix components of the IVD, especially collagens and proteoglycans. Sox 9, c-Jun and LIM mineralization protein (LMP)-1 are transcription factors involved in chondrogenesis. Sox 9 has been shown to upregulate type II collagen synthesis. Therefore, the delivery of a recombinant adenoviral vector which expresses Sox 9 has been investigated as a novel therapeutic treatment for IVDD. Sox 9 production is significantly increased in degenerated human IVD cells transfected using adenovirus-mediated gene therapy and an increase in type II collagen synthesis is also recorded. As type II collagen is an essential constituent of the NP, which in cases of IVDD is notably reduced, gene therapy targeting Sox 9 has enormous potential in the treatment for IVDD. In addition to Sox 9, LMP-1 has been shown to mediate proteoglycan production through its action on BMPs. In vitro experiments using adenoviral vectors to deliver LMP-1 demonstrate increased proteoglycan aggrecan content in NP-like cells. In addition, adenoviral vector delivery of LMP-1 in vivo causes a significant increase in the levels of LMP-1, BMP-2 and BMP-7. A characteristic feature of IVDD is the reduction in metabolically active IVD cells and IVD cell number. A sufficient cell population is essential to maintain its homeostatic mechanisms. A suggested method of rectifying this is autologous IVD cell transplantation (ADCT). ADCT involves the explantation of IVD chondrocyte-like cells from herniated or degenerate discs, which are subsequently cultured in vitro and replaced into the autologous IVD tissue from which they were derived.

Promising results have been attained from clinical trials evidencing the success of ADCT. After 24 months, the control group of IVDD patients, who underwent surgery but did not receive ADCT, suffered a 75% reduction in IVD fluid content. In comparison, the IVDs of the IVDD patients that underwent ADCT only demonstrated a 59% reduction in fluid content. In addition, the pain suffered by the ADCT-treated patients was less, according to the score attained from questionnaires, in comparison to the control group after 24 months. However, there was still a significant decrease in fluid content. A number of studies have demonstrated that the cells of a degenerated disc act abnormally and display senescence and the use of degenerated cells as a therapeutic source is inherently flawed.

In addition, replacement of degenerated IVD cells with autologous IVD cells has a restricted potential for the treatment of IVDD because of the limited population of IVD cells which can be explanted for culture. Despite this, ADCT is currently the only therapeutic technique aimed at IVDD which has been clinically tested and the results demonstrate that effective pain relief and changes in IVD matrix composition are achieved long term. However, the removal of
autologous cells and culture in vitro is limited to a few particular scenarios such as IVD herniation, because of the limited number of cells which could be harvested and the possibility that implantation of the autologous cells could cause detrimental damage to the structure of the IVD and accelerate its degeneration.45 More recently, attention has turned to the potential of MSCs.48

Stem cells are undifferentiated cells with two defining properties; the ability to differentiate into specialized cells and the capability of self renewal long term. After fertilization, a single totipotent cell is formed, which is capable of giving rise to all the cell types of the organism. During embryonic development, this cell differentiates into pluripotent stem cells which have the ability to differentiate into all cell lineages. These cells then further differentiate into multipotent stem cells which can only differentiate into cells of a particular germ layer. Adult MSCs are undifferentiated multipotent stem cells which can be derived from several tissues including skeletal muscle, synovial membranes, adipose, dermis and bone marrow.5 In addition to being easily accessible, MSCs have a high plasticity and able to differentiate into bone, cartilage, fat and fibrous tissues.49 The use of adult stem cells bypasses the controversial ethical issues regarding the acquisition of embryonic stem cells. Adult stem cells are involved in the replacement of cells lost during normal matrix turnover. Various methods have been tried in order to differentiate MSCs into the chondrocyte-like cells found in the NP.48, 50–52

The exogenous application of growth factors to MSCs in vitro can influence the differentiation of these cells51 using current knowledge of the effects of growth factors on NP cells; studies have attempted to culture MSCs to initiate differentiation into chondrocyte-like cells using the growth factors TGFβ and BMP.53 However, inducing MSC differentiation with individual growth factors is problematic, because multiple growth factors are involved in the normal development of the IVD.

Co-cultivation of MSCs with IVD cells in vitro may be sufficient to induce differentiation into the chondrocyte-like cell phenotype.50 However, because current knowledge of the IVD NP cell phenotype is incomplete, a definitive conclusion on whether differentiation has been successful is impossible. For MSC-based therapy to progress, a better knowledge of the complex cellular biology of the IVD is required than is currently available. The discovery that differentiation of MSC cells into IVD-like cells can be achieved by co-culture of MSCs with IVD cells suggests that rapid production of NP cells could be possible without the removal of IVD tissue.48, 50 This evidence supports the theory that harvested MSCs could potentially be administered directly to the IVD, circumventing the requirement for differentiation to be induced in vitro, however, the in vivo experiments required to validate the use of this approach in clinical scenarios have not been conducted to a sufficient standard to date.5

At present, it is unclear if MSC differentiation can be achieved long term or whether differentiated cells will replicate. In addition, the stage of degeneration may also influence the effectiveness of this type of therapy.54 These factors would need to be sufficiently evaluated before MSC therapy for the treatment of IVDD could be evaluated in clinical settings.55 MSC implantation therapy if used individually may counter the progression of IVDD, however, for full regeneration of the IVD, an approach aimed at the cessation of IVDD delivered in tandem would be required.49

In addition to these major therapeutic approaches, other novel therapies have been proposed. Artificial IVD implantation strategies, aimed at replacing degenerate IVDs, show promising clinical potential and have recently been reviewed.31 Alternatively, whole disc transplantation has been proposed. However, the problems associated with identifying suitable donors make this a less viable clinical option.52 Tissue engineering techniques which aim to augment the function of degenerate IVDs have also been developed. Some of these techniques utilize biomaterials that mimic the properties of the NP which are injected into the IVD in order to provide a scaffold for the existing chondrocyte-like cells and thus prevent further damage such as decreased IVD matrix synthesis, which is caused by the additional mechanical stress to the IVD as a result of reduced IVD height.52

It has been postulated that there may be a threshold beyond which the damage caused during IVDD becomes irreversible,13 however, recent research suggests that therapies which are delivered in later stages of IVDD are actually more effective.54 Multifactorial conditions with poorly defined pathogenic mechanisms such as IVDD are a particular challenge for gene therapy36 and for the potential of gene therapy to be maximized it is essential that the pathogenic processes of IVDD are fully elucidated to enable targeting to the correct molecule or molecules. The therapeutic use of growth factors has a vast potential for stimulating the synthesis of IVD matrix components. However, for the full potential to be accomplished the knowledge of the complex cellular biology of the IVD must be improved. For example, although application of TGFβ has a significant effect on proteoglycan and collagen synthesis, receptors are found in the blood vessels in the periphery of the IVD.16 This is not the case with BMP suggesting that it has better therapeutic potential.41 With a greater understanding of the complex cellular biology of the IVD, potential agents for use in gene therapy and regeneration strategies could be identified.37

**Conclusions**

It is clear that future treatments aimed at the underlying pathogenesis of IVDD are required, as current therapeutic strategies are purely symptomatic. The literature cited in this review highlights inhibition of the processes contributing to IVDD and promotion of IVD matrix synthesis as the primary focuses of
current research on future treatments targeting IVDD. However, for the advancement of these therapeutic techniques, a greater knowledge of the complex cellular biology of the IVD is required, which at present is not available.

Combined approach therapies targeted at inhibiting the pathogenic mechanisms of IVDD while promoting IVD matrix synthesis could provide the greatest clinical potential. A gene therapy ‘cocktail’ approach could potentially achieve this. However, the principal pathogenesis of IVDD is inadequately defined to provide a solid foundation for such a future treatment at the current time. A more comprehensive knowledge of the complex cellular biology of the IVD disc and the pathogenic mechanisms of IVDD could potentially enable the development and application of efficient treatments to the common complaint of LBP.

References


