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Review

The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain

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In 2007, three times as many peer reviewed publications covering the biology and biotherapeutics of intervertebral disc (IVD) disease appeared in the literature than in 1997. This is testimony to the upsurge in interest in the IVD, mainly driven by the openings that modern molecular pathology has generated to investigate mechanisms of human disease and the potential offered by novel therapeutic technologies to use data coming from these studies to positively influence chronic discogenic back pain and sciatica. Molecular pathology has shown IVD degeneration, a major cause of low back pain, to be a complex, active disorder in which disturbed cytokine biology, cellular dysfunction and altered load responses play key roles. This has translated into a search for target molecules and disease processes that might be the focus of future, evidence-based therapies for back pain. It is not possible to describe the totality of advances that have been made in understanding the biology of the IVD in recent years, but in this review those areas of biology that are currently influencing, or could conceivably soon impinge on, clinical thinking or practice around IVD degeneration and discogenic back pain are described and discussed.

Key words: Intervertebral disc, Back pain, Pathobiology, Degeneration.

Introduction

It is estimated that more than half the population will experience significant low back pain (LBP) during their lives [1]. LBP is a major cause of morbidity and impacts considerably on the economy, both through loss of work (~15% of all sickness leave in the United Kingdom) and the cost of health care and societal support for the affected individual and their family [2, 3].

Although an important public health issue, the pathogenesis of LBP is poorly understood. Most is thought to arise from disturbances in the lumbar spine and associated structures. Studies examining the problem from different directions (e.g. examination of volunteers [4] and patients [5], imaging investigations [6], trials of intervention [7]) have produced evidence implicating the intervertebral disc (IVD) in a significant proportion (at least 40%) of cases of chronic back pain, leading to the use of the term 'discogenic back pain'.

From the work that has been carried out to date two processes stand out as being important in the origins of discogenic back pain, disc degeneration and nociceptive nerve ingrowth into the normally aneural IVD.

Only in the last 10–15 yrs have the mechanisms underlying human IVD degeneration been studied in any detail, but the arrival of molecular pathology and similar techniques for examining disease mechanisms in human tissue (e.g. immunohistochemistry [8], *in situ* zymography [9], *in situ* hybridization [10] and quantitative image analysis [11]) and the advent of biotherapeutics [12], stem cell therapy [13] and tissue engineering [14] have brought both methods for and reasons to investigate IVD degeneration.

During these studies it became evident that there was vascular ingrowth into the degenerate IVD and that in painful degenerate IVD the vessels were accompanied by nociceptive nerves. Further investigation is required, but if it transpires that nociceptive nerve ingrowth is a major cause of discogenic back pain, the processes driving this ingrowth could become key therapeutic targets for its management.

To understand the pathology and pathogenesis of IVD degeneration and discogenic back pain, it is first necessary to have an overview of the normal IVD and IVD cell function.

The normal IVD

The IVD, adjacent two vertebrae and their posterior elements are described as the 'motion segment'. The nature of the specialized matrix of the IVD allows movement (e.g. twisting and bending), offers resilience under compression, and is key to the 'spacer' function of the IVD necessary for generating the optimal biomechanical environment within the motion segment.

The central component of the IVD [the nucleus pulposus (NP)], has a matrix that consists of type II collagen and the proteoglycan aggrecan in a ratio of 1 : 20 (cf. articular cartilage 1 : 2) [15]. Aggrecan is highly hydrophilic, imbibing water with such avidity that it generates a swelling pressure sufficient to force apart the vertebral bodies.

The matrix of the NP is maintained by the cells within it. They have a chondroid phenotype which is characterized by the expression of the matrix molecules aggrecan and type II collagen and the regulatory molecule, Sox9 [16]. There is now developing evidence that the cytokine IL-1 is important in normal IVD cell function [17].

The NP is confined above and below by the end plates of the vertebral bodies and circumferentially by the fibres of the annulus fibrosus (AF).

The end plates consist of a layer of articular cartilage (cartilage end plate) in contact with the NP and separating it from the cortical bone of the vertebral body (bony end plate). The cartilage and bony end plates are together known as 'the end plate' (EP). The EP gives the resilience that prevents the load transmitted through the IVD fracturing the bone of the vertebral body.

The swelling pressure of the NP is resisted by tension in the type I collagen fibres of the AF. The AF consists of a number of lamellae. In each, the collagen fibres are parallel to one another and run diagonally between the vertebrae. The fibres in each lamella run at an angle (120°) to those in the two immediately adjacent lamellae such that in rotational movements some fibres

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are put into tension and others become slack. At rest the balance between the swelling pressure in the NP and the tension in the AF maintains the distance between adjacent vertebral bodies.

The matrices of all three structures (NP, AF and EP) are highly regulated by the cells they contain through continuous matrix breakdown and synthesis.

With the exception of the outermost AF, the normal IVD is both avascular and aneural.

'Degeneration' of the IVD

The tissue changes of degeneration are increased breakdown of matrix, altered matrix synthesis (consisting largely of a change from type II to type I collagen synthesis and decreased synthesis of aggrecan), cell loss through apoptosis and *in situ* replication of surviving cells to form clusters [18–20]. The process extends to the AF largely as a result of altered loading consequent upon reduced separation between vertebrae ('loss of disc height') as the amount of aggrecan and the swelling pressure of the NP fall. In this setting, the normal balance between forces generated in the NP and AF is lost, resulting in decreased tension in the collagen fibres in the AF, which promotes shock loading at the enthesis during normal movement, leading to microtrauma and pain. The microtrauma damages both the AF and the bone into which the fibres of the AF insert, allowing blood vessels and nerves a route into the IVD [21]. Similar changes occur in the EP as a result of fracture. When the spacing effect of the normal NP is lost and the vertebral bodies approximate to one another, abnormal movement and loading occurs throughout the entire motion segment causing traumatic damage to the facet joints and other structures [22, 23].

Although dysfunction of the motion segment is caused by adverse changes in the matrix of the IVD, these changes are mediated by disturbances in the biology of the cells of the NP, AF and EP [24]. These have been the focus of much of the recent study of degeneration.

New advances in understanding the altered cell biology of IVD degeneration

Five major factors influence IVD cell function in health and degeneration:

(i) Diffusion of nutrients and oxygen across the IVD matrix.
(ii) Soluble regulators of cell function.
(iii) Genetic influences.
(iv) Ageing and senescence.
(v) Mechanical load.

Diffusion of nutrients and oxygen across the IVD matrix

Cells of the IVD receive oxygen and nutrients by diffusion across the discal matrix. The outer AF probably gains its nutrients from the local vasculature but the remainder of the IVD is nourished from the bone marrow. As the lower lumbar discs are nearly 1 cm thick, the diffusion pathway to cells in the centre of the disc is long. Thus, the cells are believed to be adapted to function in an environment that is relatively oxygen and nutrient poor [25].

There is strong evidence that reduced blood flow to the margins of the IVD is associated with early and established degeneration. This may occur because of changes in the local vasculature (e.g. those initiated by smoking) or by disturbance of the physical structure of the EP [26, 27]. Whilst this might explain the cell changes that initiate degeneration, the evidence for this still needs to be fully tested and any hypothesis linking hypoxia to disc degeneration will need to explain the vascularization of the IVD that occurs in progressive degeneration [28].

This remains a very interesting field of research, particularly as this is one area of research endeavour that might shed some light on the still elusive events that initiate degeneration.

Soluble regulators of cell function

IL-1. There is accumulating evidence that both isoforms of the pleiotropic cytokine IL-1 (IL-1α ad IL-1β) are the normal regulators of IVD cell function and that IL-1 effects are controlled in this tissue as in others by synthesis of IL-1 through IL-1 converting enzyme (ICE), and balanced production of the activating receptor (IL-1RI), the exported decoy receptor (IL-1RII) and the inhibitor of IL-1: IL-1 receptor antagonist (IL-1Ra) [17].

In degeneration, there is a breakdown in IL-1 regulation with increased production of IL-1 isoforms by native disc cells associated with a failure to up-regulate IL-1Ra. This imbalance in the IL-1 system has been shown to be able to induce all the tissue changes associated with degeneration. These include:

- Up-regulation of zinc-based matrix degrading enzymes, notably MMPs and ADAMTSs [20, 29–32].
- Abnormal synthesis of aggrecan and collagen II and their replacement by collagen I [20, 33].
- Angiogenesis [34, 35].
- Neuronogenesis [36].
- Apoptosis of native IVD cells [37].

Furthermore, exogenous IL-1Ra applied to IVD cells and human tissue explants will reverse the molecular pathology of degeneration [38–40].

The factors initiating the imbalance in the IL-1 system are unknown. Load has been implicated [41], but a role has not been proved. Interestingly genetic epidemiology has shown an association between back pain, IVD degeneration and the inheritance of specific genes of the IL-1 family [42–44], raising the possibility that suboptimal function of the protein products of these genes might pre-dispose to the development of IVD cell dysfunction. This is clearly not the whole story as non-back pain patients express these haplotypes and not all the discs in those expressing these genes become degenerate.

TNF-α. This has been discovered within the degenerate IVD and to a lesser extent the normal disc [45]. It is particularly expressed by the cells in prolapsed disc tissue.

In animal models, NP tissue has been applied directly onto spinal nerve roots in the epidural space [46]. This resulted in functional, vascular and morphological abnormalities of the nerve root, which were often followed by intraradicular fibrosis and nerve fibre atrophy. Extrapolating from the finding that TNF was expressed by cells in disc protrusions and that tissue found in prolapsed discs induced nerve damage, it was hypothesized that TNF might be the chemical mediator of discogenic radiculopathy. It was subsequently demonstrated that TNF-α applied to nerve roots caused vascular and radicular abnormalities similar to those seen following application of NP tissue [47], implicating TNF-α in nerve root damage and sciatic pain. Furthermore, application of TNF-α blockers [48] prevented the processes and symptoms. It was therefore hypothesized that TNF-α blockade might have a therapeutic role in sciatic pain [49]; however, such studies as have been performed using anti-TNF in patients with back pain have been less encouraging than might have been hoped [50]. An alternative explanation for the role of TNF-α in back pain comes from a recent study in the TNF-α-deficient mouse which has provided evidence that TNF-α can induce sensory nerve growth into the IVD [51], which is of considerable interest as it has been previously noted that nerve ingrowth is a feature of the painful degenerate IVD [52].

More recently, TNF has been implicated in the catabolic processes leading to matrix degradation in the degenerate IVD [53, 54]. The data around this are inconsistent. For instance, whilst there is no question that with increasing degrees of degeneration IVD cells exhibit increased TNF-α expression [55], the IVD cells that would be the putative target do not express its

receptor [56], and anti-TNF does not inhibit *in situ* matrix degrading activity [40].

Other cytokines implicated in IVD catabolism. Other cytokines have been described in the degenerate IVD that could influence matrix breakdown [57, 58] but a precise role for them has yet to be discovered.

TGF-β superfamily. Inarticular cartilage members of the TGF-β superfamily are anabolic. Does this also apply to the IVD? In what surely will turn out to be a seminal paper on several fronts, TGF-β delivered by gene therapy was shown to increase aggrecan production by rabbit NP cells [59]. Others have shown that TGF-β can cause NP cell proliferation [60] and the formation of NP-like cells from mesenchymal stem cells [61].

However, current interest is focused not on TGF-β itself, but on other members of the TGF-β superfamily, and in particular the bone morphogenetic proteins (BMPs) [62, 63]. Of these, BMP-7 [osteogenic protein-1 (OP-1)] has received particular attention [64]. Preliminary data indicate that it may be a potent anabolic agent in regenerating the degenerate IVD.

Therapeutic implications. Importantly, with the advent of molecular medicine, cytokines and cytokine regulation pathways have the potential to be key therapeutic targets, as has happened in rheumatoid disease and OA. Although still relatively nascent, there is no doubt that the next few years will see increasing research focused on translating our understanding of molecular pathways underlying degeneration into novel therapies for managing discogenic pain [65] through prevention of progression or reversal of the pathology of degeneration. The greatest challenge, as in all areas of regenerative medicine that try to restore normal tissue within a disease system, is normalizing the biology of the diseased tissue 'niche' in which regeneration is being attempted. In this respect, normalizing the cytokine environment alone is clearly insufficient, and other factors such as abnormal load, and altered nutrient and metabolite transport, will need to be addressed in concert.

Genetic influences

Twin and other studies have shown that a significant proportion of IVD degeneration cases can be explained on the basis of genetic factors [66, 67]. Quite what those factors are has yet to be properly determined. However, a number of genetic associations have been reported over the last 20 yrs but only a few have been replicated convincingly. Of those molecules investigated, only VDR [68] and collagen IX [69] polymorphisms have been consistently associated with degeneration in reasonably sized populations. Other candidate genes linked to degeneration of the IVD include: collagen I α1 [70], interleukin-6 [71], aggrecan [72], MMP 3 [73], thrombospondin, cyclo-oxygenase, TIMP1 [74], cartilage intermediate layer protein [75] and IL-1 family members, as described earlier.

A better understanding of the significance of these findings can only come from a more thorough functional analysis of these polymorphisms within the context of the molecular pathology of the degenerate IVD.

Ageing and senescence

The nature of collagenous tissues is such that their physical properties change with time and age consequent upon progressive internal cross-linking of matrix molecules and the nutritional status of these poorly vascularized tissues. With age, these changes lead to modifications in collagen and proteoglycan composition of the IVD [76]. As the incidence of discal degeneration also increases with age, distinguishing 'normal ageing' from 'disease' becomes paramount [77]. This is complicated by the high frequency of disc degeneration at some spinal levels (e.g. L3-4, L4-5 and L5-S1), making the definition of 'normality' problematic.

Disc cell numbers and viability decrease in degenerate IVD. This has been attributed to apoptosis and, more recently, cellular senescence. Senescent cells lose their ability to divide but are viable and synthetically active, although gene expression is different from that in normal cells. The accumulation of senescent cells *in vivo* with age, together with their changed pattern of gene expression implicates cellular senescence in ageing and age-related pathologies [78] of other chondroid tissues such as articular cartilage in OA [79], where chondrocyte senescence correlates with disturbed matrix homoeostasis. This has raised the possibility that the changes seen within the diseased IVD are also senescence related.

There are two types of senescence: replicative senescence (RS) and stress-induced premature senescence (SIPS) [80]. RS is generally regarded as the result of telomere shortening accumulated as cells undergo repeated cell divisions, whereas SIPS occurs in response to stress-inducing factors such as exposure to cytokines or oxidative stress [81]. Certain cellular changes indicative of senescence are shared by RS and SIPS including: growth arrest, a large, flat cell morphology with increased staining for senescence-associated β-galactosidase (SA-βgal) and increased expression of cell cycle inhibitors.

The investigation of cellular senescence within human IVD is a relatively new area of research. In 2006 [82] and 2007 [83], two groups showed increased staining for SA-βgal in cells from prolapsed and degenerate IVD when compared to non-degenerate discs. A more comprehensive study of senescence biomarkers has recently been described [84]. This showed that: mean telomere length decreased with age in cells from non-degenerate tissue and also decreased with progressive stages of degeneration; and expression of the cell cycle inhibitor p16INK4a protein (which is up-regulated during cellular senescence) increased with both subject age and degeneration, indicating that degeneration is a form of accelerated, tissue-specific cellular senescence. Furthermore, the study showed a direct relationship between expression of p16INK4a and the genes for two matrix degrading enzymes, MMP-13 and ADAMTS5, important in IVD degeneration [8, 20]. Whilst this might be an epiphenomenon, it might also link senescence and a catabolic phenotype.

Mechanical load

There is increasing evidence that load has a profound and fundamental influence on the biology of IVD cells [41] and, indeed that 'normal' mechanical loading is essential for maintaining a normal phenotype [85, 86]. Excessive spinal loading (e.g. as caused by lifestyle and increased body weight [87]) can lead to the development of the radiological and biochemical features of degeneration. Not only does excessive load lead to changes in the IVD but so too do other factors such as significant traumatic injury (e.g. EP fracture) [18] and scoliosis [88], which reduce or alter the load in other ways.

The precise mechanisms linking load and cell function in the IVD are poorly understood. However, there is increasing interest in mechanotransduction (the science that investigates the relationships between load, load recognition, intracellular signalling pathways, gene transcription and cell function, including regulation of extracellular matrices), which is gradually aiding an understanding of how the excellent work on the altered mechanical environment in the IVD that causes [89] and is caused by [90] degeneration, translates into altered cell and matrix biology [91] and can be employed in therapeutic regeneration [92]. This is likely to become a key area of IVD research in the next 5 yrs.

Nerve ingrowth

A factor that has been a constant finding in the analysis of excised painful IVD has been the presence of nerves and blood vessels within the usually aneural and avascular tissues of the IVD.

Generally, nerve and vessel ingrowth into usually aneural and avascular tissues can come about as a consequence of either loss of anti-angiogenic/-neuronogenic factors naturally present in avascular and aneural tissues or local production of angiogenic and neuronogenic factors in disease. It transpires that both processes might be at work in disc degeneration.

In 1997, two groups [52, 93] described ingrowth of nociceptive nerves into the degenerate IVD. This was based on identifying nerve fibres using a combination of nerve stains in histological sections of IVD. These nerves have the shape of nociceptive nerves and express GAP43, a marker of nerve growth, and substance P, a nociceptor (and vasoregulatory) neurotransmitter.

There have been a number of studies evaluating the mechanisms leading to nerve ingrowth. They can be summarized as falling into three groups. Nerve ingrowth: associated with angiogenesis; induced by an alteration in IVD matrix biology; and initiated by altered IVD cell function.

Angiogenesis-associated nerve ingrowth

Nerves growing into degenerate IVD do so in physical association with ingrowing blood vessels [94]. In the current state of knowledge, it would appear that the nerves growing into the disc initially have a vasoregulatory role, but at some stage and for unknown reasons they send off nociceptive shoots into the disc tissue. During angiogenesis, endothelial cells of vessels growing into the IVD synthesize the neurogenic stimulator, nerve growth factor (NGF), one of a family of neurotrophins [95]. Furthermore, the accompanying nerves expressed the high-affinity receptor for NGF, TrkA, a phenomenon entirely commensurate with a vasoregulatory role for nerves accompanying blood vessels.

An important aspect of these studies is that nerves with the structure and biology of nociceptive nerves are only seen in IVD that had been classified clinically as 'pain level discs'. By this it is usually meant that insulting these discs (e.g. by discography or direct probing) specifically reproduces the patient's symptoms of back pain and/or sciatica. IVD showing similar degrees of degeneration but that did not come from 'pain levels' do not show nerve ingrowth.

Altered matrix biology and nerve ingrowth

In some very elegant experiments, Johnson and co-workers [96] examined the *in vitro* effects of aggrecan removed from normal human AF and NP had on neurite outgrowth. They showed that aggrecan derived from normal IVD inhibited the growth of neurites, but that aggrecan that had been deglycosylated to make it more akin to that found in the degenerate IVD had a reduced inhibitory effect. This implies that normal aggrecan is an inhibitor of nerve ingrowth into the IVD, and that in degeneration nerve ingrowth may occur as a consequence of changed aggrecan biology. Aggrecan from both the AF and NP were inhibitory but perhaps a little unexpectedly that from the AF was more inhibitory.

Altered IVD cell function

In a similar series of experiments, Johnson *et al.* [97] have also examined the effects of cells derived from normal and degenerate IVD on neurite outgrowth. They found that the normal inhibition of neurite outgrowth by aggrecan could be reversed by cells derived from degenerate IVD. The extent of the effect was related to the number of IVD cells. Conditioned media had no such effect.

Overview

Overall, current data indicate that normal IVD matrix prevents nerve ingrowth into the IVD, but that in degeneration changes in the structure of aggrecan, coupled with altered IVD cell biology lead to nerve ingrowth into pain level IVD and that this is enhanced by the production of neurogenic cytokines during neovascularization of the degenerate IVD.

Clinical implications/applications

At the present time, therapy for discogenic back pain is largely empirical and aimed at relieving symptoms rather than addressing the underlying disease mechanisms. The continually increasing burden of disease and the patient experience suggest that this approach has limited success. It could be argued that therapeutic advances might be facilitated were more known about the causes of back pain and the underlying tissue processes.

There is a body of evidence suggesting that degeneration of the IVD underlies a significant proportion of cases of debilitating back pain. This has triggered a new interest in the biology of IVD degeneration. It is too soon to see the clinical translation of much of this new knowledge into clinical practice, but the advances in understanding that have been made in the last few years are already driving a body of research directed towards preventing, halting or reversing the processes of disc degeneration.

Arguably, the two main foci of this work are in restoring the normal environment of the IVD and in regenerating functional IVD tissue. In the former, the major targets are the altered load consequent upon disturbed matrix composition and the abnormal cytokine environment of the degenerate IVD. These have given rise to research on delivery of cytokine modulators to the degenerate IVD [98, 39], novel biomaterials to replace the function of the NP [99, 100] and the use of stem cells to replace deficient IVD cells [101–103]. Reliable new treatments for discogenic back pain based on this new knowledge are a long way off, but the tide of translational research is running in that direction. There are also new research areas developing particularly around mechanotransduction and prevention of degeneration based on recognizing genetically programmed 'at risk' groups.

One area that has been neglected to some extent is in the clinical subtyping of patients to identify those who might benefit from the new therapies. Advance in this area will be essential if the new therapeutics are to be of any value, and will go wider than the history and clinical examination but will also encompass clinical technologies such as novel imaging and the wealth of different 'omics'.

This is an exciting time to be working on back pain and there is little doubt that the clinical management of the patient with discogenic back pain and/or IVD degeneration will be distinctly different in 10 yrs time.

Rheumatology key messages

- IVD degeneration is a significant cause of back pain.
- Degeneration is an 'active' process mediated by cytokines, altered load and premature senescence.
- Understanding the molecular pathology of degeneration will lead to novel back pain treatments.

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References

1 McBeth J, Jones K. Epidemiology of chronic musculoskeletal pain. Best Pract Res Clin Rheumatol 2007;21:403–25.
2 Dagenais S, Caro J, Haldeman S. A systematic review of low back pain cost of illness studies in the United States and internationally. Spine J 2008;8:8–20.
3 Asche CV, Kirkness CS, McAdam-Marx C, Fritz JM. The societal costs of low back pain: data published between 2001 and 2007. J Pain Palliat Care Pharmacother 2007;21:25–33.

4 Kelgren JH. The anatomical source of back pain. Rheumatol Rehab 1977;16:3–12.
5 Kuslich SD, Ulstrom CL, Michael CJ. The tissue origin of low back pain and sciatica: a report of pain response to tissue stimulation during operations on the lumbar spine using local anesthesia. Orthop Clin North Am 1991;22:181–7.
6 Luoma K, Riihimäki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low back pain in relation to lumbar disc degeneration. Spine 2000;25:487–92.
7 Barrick WT, Schofferman JA, Reynolds JB *et al.* Anterior lumbar fusion improves discogenic pain at levels of prior posterolateral fusion. Spine 2000;25:853–7.
8 Le Maitre CL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. J Pathol 2004; 204:47–54.
9 Freemont AJ, Byers RJ, Taiwo YO, Hoyland JA. In situ zymographic localisation of type II collagen degrading activity in osteoarthritic human articular cartilage. Ann Rheum Dis 1999;58:357–65.
10 Mee AP, Hoyland JA, Braidman IP, Freemont AJ, Davies M, Mawer EB. Demonstration of vitamin D receptor transcripts in actively resorbing osteoclasts in bone sections. Bone 1996;18:295–9.
11 Braidman I, Baris C, Wood L *et al.* Preliminary evidence for impaired estrogen receptor-alpha protein expression in osteoblasts and osteocytes from men with idiopathic osteoporosis. Bone 2000;26:423–7.
12 Sasaki N, Kikuchi S, Konno S, Sekiguchi M, Watanabe K. Anti-TNF-alpha antibody reduces pain-behavioral changes induced by epidural application of nucleus pulposus in a rat model depending on the timing of administration. Spine 2007;32:413–6.
13 Jandial R, Aryan HE, Park J, Taylor WT, Snyder EY. Stem cell-mediated regeneration of the intervertebral disc: cellular and molecular challenge. Neurosurg Focus 2008;24:E21.
14 O'Halloran DM, Pandit AS. Tissue-engineering approach to regenerating the intervertebral disc. Tissue Eng 2007;13:1927–54.
15 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. Eur Cell Mater 2004;8:58–63.
16 Gruber HE, Norton HJ, Ingram JA, Hanley EN Jr. The SOX9 transcription factor in the human disc: decreased immunolocalization with age and disc degeneration. Spine 2005;30:625–30.
17 Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 2005;7:R732–45.
18 Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine 2006;31:2151–61.
19 Johnson WE, Roberts S. 'Rumours of my death may have been greatly exaggerated': a brief review of cell death in human intervertebral disc disease and implications for cell transplantation therapy. Biochem Soc Trans 2007;35:680–2.
20 Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA. Matrix synthesis and degradation in human intervertebral disc degeneration. Biochem Soc Trans 2007;35:652–5.
21 Hilton RC, Ball J. Vertebral rim lesions in the dorsolumbar spine. Ann Rheum Dis 1984;43:302–7.
22 Brown KR, Pollintine P, Adams MA. Biomechanical implications of degenerative joint disease in the apophyseal joints of human thoracic and lumbar vertebrae. Am J Phys Anthropol 2008;136:318–26.
23 Adams MA, Pollintine P, Tobias JH, Wakley GK, Dolan P. Intervertebral disc degeneration can predispose to anterior vertebral fractures in the thoracolumbar spine. J Bone Miner Res 2006;21:1409–16.
24 Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev 2007;6:247–61.
25 Soukane DM, Shirazi-Adl A, Urban JP. Computation of coupled diffusion of oxygen, glucose and lactic acid in an intervertebral disc. J Biomech 2007;40:2645–54.
26 Urban JP, Smith S, Fairbank JC. Nutrition of the intervertebral disc. Spine 2004;29:2700–9.
27 Benneker LM, Heini PF, Alini M, Anderson SE, Ito K. 2004 Young Investigator Award Winner: vertebral endplate marrow contact channel occlusions and intervertebral disc degeneration. Spine 2005;30:167–73.
28 Kauppila LI. Ingrowth of blood vessels in disc degeneration. Angiographic and histological studies of cadaveric spines. J Bone Joint Surg Am 1995;77:26–31.
29 Studer RK, Gilbertson LG, Georgescu H, Sowa G, Vo N, Kang JD. p38 MAPK inhibition modulates rabbit nucleus pulposus cell response to IL-1. J Orthop Res 2008;26:991–8.
30 Jimbo K, Park JS, Yokosuka K, Sato K, Nagata K. Positive feedback loop of interleukin-1beta upregulating production of inflammatory mediators in human intervertebral disc cells in vitro. J Neurosurg Spine 2005;2:589–95.
31 Shen B, Melrose J, Ghosh P, Taylor F. Induction of matrix metalloproteinase-2 and -3 activity in ovine nucleus pulposus cells grown in three-dimensional agarose gel culture by interleukin-1beta: a potential pathway of disc degeneration. Eur Spine J 2003;12:66–75.
32 Anderson DG, Izzo MW, Hall DJ *et al.* Comparative gene expression profiling of normal and degenerative discs: analysis of a rabbit annular laceration model. Spine 2002;27:1291–6.
33 Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res 2004;(427 Suppl.):S27–36.
34 Voronov E, Carmi Y, Apte RN. Role of IL-1-mediated inflammation in tumor angiogenesis. Adv Exp Med Biol 2007;601:265–70.
35 Maruotti N, Cantatore FP, Crivellato E, Vacca A, Ribatti D. Angiogenesis in rheumatoid arthritis. Histol Histopathol 2006;21:557–66.
36 Brisby H. Pathology and possible mechanisms of nervous system response to disc degeneration. J Bone Joint Surg Am 2006;88(Suppl. 2):68–71.
37 Zhao CQ, Liu D, Li H, Jiang LS, Dai LY. Interleukin-1beta enhances the effect of serum deprivation on rat annular cell apoptosis. Apoptosis 2007;12:2155–61.
38 Maeda S, Kokubun S. Changes with age in proteoglycan synthesis in cells cultured in vitro from the inner and outer rabbit annulus fibrosus. Responses to interleukin-1 and interleukin-1 receptor antagonist protein. Spine 2000;25:166–9.
39 Le Maitre CL, Hoyland JA, Freemont AJ. Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an in situ zymographic and gene therapy study. Arthritis Res Ther 2007;9:R83.
40 Hoyland JA, Le Maitre C, Freemont AJ. Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. Rheumatology 2008;47:809–14.
41 Wang DL, Jiang SD, Dai LY. Biologic response of the intervertebral disc to static and dynamic compression in vitro. Spine 2007;32:2521–8.
42 Solovieva S, Leino-Arjas P, Saarela J, Luoma K, Raininko R, Riihimäki H. Possible association of interleukin 1 gene locus polymorphisms with low back pain. Pain 2004;109:8–19.
43 Solovieva S, Lohiniva J, Leino-Arjas P *et al.* Intervertebral disc degeneration in relation to the COL9A3 and the IL-1ss gene polymorphisms. Eur Spine J 2006; 15:613–9.
44 Solovieva S, Kouhia S, Leino-Arjas P *et al.* Interleukin 1 polymorphisms and intervertebral disc degeneration. Epidemiology 2004;15:626–33.
45 Olmarker K, Larsson K. Tumour necrosis factor alpha and nucleus pulposus-induced nerve root injury. Spine 1998;23:2538–44.
46 Olmarker K, Rydevik B, Nordberg C. Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine cauda equina nerve roots. Spine 1993;18:1425–32.
47 Igarashi T, Kikuchi S, Shubayev V, Myers RR. 2000 Volvo Award winner in basic science studies: exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Molecular, histologic, and behavioral comparisons in rats. Spine 2000;25:2975–80.
48 Olmarker K, Rydevik B. Selective inhibition of tumor necrosis factor-α prevents nucleus pulposus-induced thrombus formation, intraneural oedema, and reduction of nerve conduction velocity: possible implications for future pharmacological treatment strategies of sciatica. Spine 2001;26:863–9.
49 Cooper RG, Freemont AJ. TNF-alpha blockade for herniated intervertebral disc-induced sciatica: a way forward at last? Rheumatology 2004;43:119–21.
50 Korhonen T, Karppinen J, Paimela L *et al.* The treatment of disc-herniation-induced sciatica with infliximab: one-year follow-up results of FIRST II, a randomized controlled trial. Spine 2006;31:2759–66.
51 Hayashi S, Taira A, Inoue G *et al.* TNF-alpha in nucleus pulposus induces sensory nerve growth: a study of the mechanism of discogenic low back pain using TNF-alpha-deficient mice. Spine 2008;33:1542–6.
52 Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O'Brien J, Jayson MI. Nerve ingrowth into diseased intervertebral disc in chronic back pain. Lancet 1997; 350:178–81.
53 Séguin CA, Pilliar RM, Roughley PJ, Kandel RA. Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue. Spine 2005;30:1940–8.
54 Séguin CA, Pilliar RM, Madri JA, Kandel RA. TNF-alpha induces MMP2 gelatinase activity and MT1-MMP expression in an in vitro model of nucleus pulposus tissue degeneration. Spine 2008;33:356–65.
55 Weiler C, Nerlich AG, Bachmeier BE, Boos N. Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. Spine 2005;30:44–53.
56 Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile. Arthritis Res Ther 2007;9:R77.
57 Kang JD, Georgescu HI, McIntyre-Larkin L, Stefanovic-Racic M, Donaldson WF 3rd, Evans CH. Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. Spine 1996; 21:271–7.
58 Tolonen J, Gronblad M, Virri J, Seitsalo S, Rytomaa T, Karaharju EO. Platelet-derived growth factor and vascular endothelial growth factor expression in disc herniation tissue: an immunohistochemical study. Eur Spine J 1997;6:63–69.
59 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;24:2419–25.
60 Zhang R, Ruan D, Zhang C. Effects of TGF-beta1 and IGF-1 on proliferation of human nucleus pulposus cells in medium with different serum concentrations. J Orthop Surg 2006;1:9.
61 Steck E, Bertram H, Abel R, Chen B, Winter A, Richter W. Induction of intervertebral disc-like cells from adult mesenchymal stem cells. Stem Cells 2005;23:403–11.
62 Gilbertson L, Ahn SH, Teng PN, Studer RK, Niyibizi C, Kang JD. The effects of recombinant human bone morphogenetic protein-2, recombinant human bone morphogenetic protein-12, and adenoviral bone morphogenetic protein-12 on matrix synthesis in human annulus fibrosis and nucleus pulposus cells. Spine J 2008;8:449–56.
63 Zhang Y, An HS, Thonar EJ, Chubinskaya S, He TC, Phillips FM. Comparative effects of bone morphogenetic proteins and sox9 overexpression on extracellular matrix metabolism of bovine nucleus pulposus cells. Spine 2006;31:2173–9.
64 Wei A, Brisby H, Chung SA, Diwan AD. Bone morphogenetic protein-7 protects human intervertebral disc cells in vitro from apoptosis. Spine J 2008;8:466–74.
65 Yoon ST. Molecular therapy of the intervertebral disc. Spine J 2005;5(Suppl. 6): 280S–6S.

66 Battié MC, Videman T, Levalahti E, Gill K, Kaprio J. Heritability of low back pain and the role of disc degeneration. Pain 2007;131:272–80.
67 MacGregor AJ, Andrew T, Sambrook PN, Spector TD. Structural, psychological, and genetic influences on low back and neck pain: a study of adult female twins. Arthritis Rheum 2004;51:160–7.
68 Videman T, Leppävuori J, Kaprio J *et al.* Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. Spine 1998;23: 2477–85.
69 Annunen S, Paassilta P, Lohiniva J *et al.* An allele of COL9A2 associated with intervertebral disc disease. Science 1999;285:409–12.
70 Pluijm SM, van Essen HW, Bravenboer N *et al.* Collagen type I alpha1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. Ann Rheum Dis 2004;63:71–7.
71 Noponen-Hietala N, Virtanen I, Karttunen R *et al.* Genetic variations in IL6 associate with intervertebral disc disease characterized by sciatica. Pain 2005; 114:186–94.
72 Kawaguchi Y, Osada R, Kanamori M *et al.* Association between an aggrecan gene polymorphism and lumbar disc degeneration. Spine 1999;24:2456–60.
73 Takahashi M, Haro H, Wakabayashi Y, Kawa-uchi T, Komori H, Shinomiya K. The association of degeneration of the intervertebral disc with 5a/6a polymorphism in the promoter of the human matrix metalloproteinase-3 gene. J Bone Joint Surg Br 2001;83:491–5.
74 Valdes AM, Hassett G, Hart DJ, Spector TD. Radiographic progression of lumbar spine disc degeneration is influenced by variation at inflammatory genes: a candidate SNP association study in the Chingford cohort. Spine 2005; 30:2445–51.
75 Virtanen IM, Song YQ, Cheung KM *et al.* Phenotypic and population differences in the association between CILP and lumbar disc disease. J Med Genet 2007;44: 285–8.
76 Freemont AJ, Hoyland JA. Morphology, mechanisms and pathology of musculoskeletal ageing. J Pathol 2007;211:252–9.
77 Gruber HE, Hanley EN Jr. Analysis of aging and degeneration of the human intervertebral disc. Comparison of surgical specimens with normal controls. Spine 1998;23:751–7.
78 Repanti M, Korovessis PG, Stamatakis MV, Spastris P, Kosti P. Evolution of disc degeneration in lumbar spine: a comparative histological study between herniated and postmortem retrieved disc specimens. J Spinal Disord 1998;11:41–5.
79 Martin JA, Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology 2002;3:257–64.
80 Toussaint O, Medrano EE, von Zglinicki T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp Gerontol 2000;35:927–45.
81 Aigner T, Rose J, Martin J, Buckwalter J. Aging theories of primary osteoarthritis: from epidemiology to molecular biology. Rejuvenation Res 2004;7:134–45.
82 Roberts S, Evans EH, Kletsas D, Jaffray DC, Eisenstein SM. Senescence in human intervertebral discs. Eur Spine J 2006;15:312–6.
83 Gruber HE, Ingram JA, Norton HJ, Hanley EN Jr. Senescence in cells of the aging and degenerating intervertebral disc: immunolocalization of senescence-associated beta-galactosidase in human and sand rat discs. Spine 2007;32:321–7.
84 Le Maitre CL, Freemont AJ, Hoyland JA. Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther 2007;9:R45.
85 Setton LA, Chen J. Mechanobiology of the intervertebral disc and relevance to disc degeneration. J Bone Joint Surg Am 2006;88(Suppl. 2):52–7.
86 Johannessen W, Vresilovic EJ, Wright AC, Elliott DM. Intervertebral disc mechanics are restored following cyclic loading and unloaded recovery. Ann Biomed Eng 2004;32:70–6.
87 Pye SR, Reid DM, Adams JE, Silman AJ, O'Neill TW. Influence of weight, body mass index and lifestyle factors on radiographic features of lumbar disc degeneration. Ann Rheum Dis 2007;66:426–7.
88 Meir A, McNally DS, Fairbank JC, Jones D, Urban JP. The internal pressure and stress environment of the scoliotic intervertebral disc – a review. Proc Inst Mech Eng [H]. 2008;222:209–19.
89 Adams MA, Freeman BJ, Morrison HP, Nelson IW, Dolan P. Mechanical initiation of intervertebral disc degeneration. Spine 2000;25:1625–36.
90 Adams MA, Dolan P. Spine biomechanics. J Biomech 2005;38:1972–83.
91 Iatridis JC, MacLean JJ, Roughley PJ, Alini M. Effects of mechanical loading on intervertebral disc metabolism in vivo. J Bone Joint Surg Am 2006;88(Suppl. 2): 41–6.
92 Schnake KJ, Putzier M, Haas NP, Kandziora F. Mechanical concepts for disc regeneration. Eur Spine J 2006;15(Suppl. 3):S354–60.
93 Coppes MH, Marani E, Thomeer RT, Groen GJ. Innervation of 'painful' lumbar discs. Spine 1997;22:2342–9.
94 Peng B, Wu W, Hou S, Li P, Zhang C, Yang Y. The pathogenesis of discogenic low back pain. J Bone Joint Surg Br 2005;87:62–7.
95 Freemont AJ, Watkins A, Le Maitre C *et al.* Nerve growth factor expression and innervation of the painful intervertebral disc. J Pathol 2002;197:286–92.
96 Johnson WE, Caterson B, Eisenstein SM, Hynds DL, Snow DM, Roberts S. Human intervertebral disc aggrecan inhibits nerve growth in vitro. Arthritis Rheum 2002;46:2658–64.
97 Johnson WE, Sivan S, Wright KT, Eisenstein SM, Maroudas A, Roberts S. Human intervertebral disc cells promote nerve growth over substrata of human intervertebral disc aggrecan. Spine 2006;31:1187–93.
98 Levicoff EA, Kim JS, Sobajima S *et al.* Safety assessment of intradiscal gene therapy II: effect of dosing and vector choice. Spine 2008;33:1509–16.
99 Vernengo J, Fussell GW, Smith NG, Lowman AM. Evaluation of novel injectable hydrogels for nucleus pulposus replacement. J Biomed Mater Res B Appl Biomater 2008;84:64–9.
100 Lally S, Mackenzie P, LeMaitre CL, Freemont TJ, Saunders BR. Microgel particles containing methacrylic acid: pH-triggered swelling behaviour and potential for biomaterial application. J Colloid Interface Sci 2007;316:367–75.
101 Crevensten G, Walsh AJ, Ananthakrishnan D *et al.* Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng 2004;32:430–4.
102 Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA. Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels. Biomaterials 2008;29:85–93.
103 Richardson SM, Hoyland JA. Stem cell regeneration of degenerated intervertebral discs: current status. Curr Pain Headache Rep 2008;12:83–8.