Pathogenesis of Intervertebral Disc Degeneration

11

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11.1 Introduction

It is estimated that as much as 84 % of the population will suffer from low back pain (LBP) at some point in their lifetime (Walker 2000), with around 10 % of sufferers being chronically disabled. As such LBP is one of the most prevalent musculoskeletal conditions affecting Western society (Stewart et al. 2003), and its prevalence has increased over recent decades (Harkness et al. 2005). The socio-economic cost of LBP is also huge, with associated costs, in terms of lost productivity, disability benefits and direct and indirect health-care costs, estimated in the UK to be around £12 billion annually (Maniadakis and Gray 2000) and in the USA to be over $85 billion per annum (Martin et al. 2008). Importantly, increases in both the size and average age of the population both suggest that the prevalence and costs associated with LBP will continue to rise over future decades, unless novel therapies can be developed to alleviate pain and restore long-term function and mobility to the spine. However, in order to develop such therapies, a more thorough understanding of the underlying aetiology is required.

While it is acknowledged that LBP is a multifactorial condition, a strong correlation with degeneration of the intervertebral disc has been shown in 40 % of cases (Cheung et al. 2009). However, until recently, the pathogenesis of disc degeneration and its role in LBP were poorly understood.

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This chapter will describe the normal intervertebral disc and the changes which occur during degeneration at a molecular, cellular and tissue level and review the clinical implications of these pathological changes in the context of LBP.

11.2 The Cell Biology of the Normal Human Intervertebral Disc

The intervertebral disc is located between the vertebrae of the spine and is comprised of three morphologically distinct regions. The central core of the disc, the nucleus pulposus, is a highly hydrated, gelatinous tissue containing small rounded nucleus pulposus cells embedded within a dense extracellular matrix. These cells are routinely described as being chondrocyte-like (Sive et al. 2002), due in part to similarities in both their morphology and composition of the matrix that they synthesise and secrete. Noteworthy, recent microarray studies have shed new light on the exact phenotype of nucleus pulposus and annulus fibrosus cells, illustrating distinct differences in gene expression profiles between these cells and articular chondrocytes (Lee et al. 2007; Sakai et al. 2009; Minogue et al. 2010a, b). The extracellular matrix of the nucleus pulposus is rich in proteoglycans, particularly the large aggregating proteoglycans aggrecan and versican which possess a large number of negatively charged glycosaminoglycan (GAG) side chains. The GAGs attract positively charged ions, which gives the nucleus pulposus a high osmotic potential, which in turn acts to draw in water resulting in a tissue with a water content between 70 and 90 % (Antoniou et al. 1996). A range of other smaller PGs are also present, including biglycan, decorin and fibromodulin, which are thought to play a number of structural and physiological roles, including growth factor binding and mediation of signalling between cells and the extracellular matrix (Roughley 2004; Feng et al. 2006). Further details of the proteoglycans of the disc are presented in Chap. 4. The nucleus pulposus also contains a range of collagens, predominantly collagen II, although III, V, VI, IX and XI have also been described (Nerlich et al. 1998; Roughley 2004). The collagen II fibrils appear randomly distributed within the matrix, meaning the extracellular matrix lacks the structural architecture noted in similar tissues such as articular cartilage (see Chap. 5). While the extracellular matrix possesses many of the same component molecules as articular cartilage, the ratio of PGs/collagens in the nucleus pulposus is substantially higher, at around 27:1, compared to only around 2:1 in cartilage (Mwale et al. 2004). The elevated proteoglycans content of the nucleus pulposus, in conjunction with the associated high water content, results in a high swelling pressure giving it the ability to effectively act as a 'shock absorber' and withstand the high compressive forces experienced within the spine (see Chap. 2).

The nucleus pulposus is constrained circumferentially by the outer region of the disc, the annulus fibrosus, a fibrous ring of tissue with highly ordered collagen I fibrils orientated in 60° oblique lamellae (Marchand and Ahmed 1990). Collagens II and III are also present in the annulus fibrosus and the total collagen content is around 80 %, compared to only around 20 % in the nucleus pulposus (Roughley 2004; Le Maitre et al. 2007d). While the extracellular matrix of the annulus fibrosus does contain proteoglycans, predominantly versican, they are mainly located between the lamellae, along with elastin fibres which are thought to allow flexion or extension during movement (Yu et al. 2002; Melrose et al. 2008; Smith et al. 2009). Morphologically and phenotypically, fibroblastic annulus fibrosus cells appear to orientate with the collagen fibres in each lamella ring. Although the cell and matrix biology differs between the nucleus pulposus and annulus fibrosus, there is no distinct demarcation between the tissues. Instead, the inner annulus fibrosus, sometimes referred to as a 'transition zone', demonstrates a mix of cell types with both rounded nucleus pulposus and flattened annulus fibrosus cells present. The matrix changes are also gradual, with collagens I and II contents being inversely correlated (Eyre and Muir 1976). This integration of tissues allows the disc to bulge in a constrained manner under loading, allowing distribution and dissipation of the mechanical forces, including flexion, tension, compression and torsion, experienced during everyday motion. These concepts are further developed in Chap. 7.

The inferior and superior faces on the disc, where they meet adjacent vertebral bodies, are covered by a thin layer of hyaline cartilage, the cartilaginous end plates. This is a thin layer of hyaline cartilage containing a population of chondrocytic cells. Collagen fibres from the annulus fibrosus embed directly into the vertebral bodies and into the cartilaginous end plates, which prevent the nucleus pulposus bulging into the vertebral bodies (Humzah and Soames 1988). The cartilaginous end plates are also thought to play a crucial role in regulating disc nutrition (Nachemson et al. 1970; Roberts et al. 1996).

The adult human intervertebral disc is both avascular and aneural, with blood vessels and associated nerves found only in the very outer regions of the annulus fibrosus and in the vertebral bodies adjacent to the cartilaginous end plates (Yasuma et al. 1993; Repanti et al. 1998; Roughley 2004). It is these capillaries that provide nutrients to cells within the disc, through a process of diffusion facilitated by fluid transport that occurs during normal movement. Under compressive loading, water is extruded from the nucleus pulposus, taking metabolic waste products such as lactic acid away from cells towards the blood vessels. As load is briefly alleviated, the osmotic potential of the nucleus draws water back in, supplying the cells with nutrients such as glucose and oxygen. However, as cells in the core of the nucleus pulposus

can be as much as 8 mm from the nearest blood vessel, this results in a tissue which is both nutrient and oxygen poor and with a relatively low pH, primarily due to an accumulation of lactic acid (Urban et al. 1982; Katz et al. 1986). This hostile environment is reflected in the cell densities of the tissue which are considerably less than in other cartilaginous tissues. In early life, cellularity decreases to approximately 4,000 cells/mm^3 in the normal adult nucleus pulposus and 9,000 cells/mm^3 in the annulus fibrosus by the time skeletal maturity is achieved (Maroudas et al. 1975). There is also a reduction in the proportion of large, vacuolated, morphologically distinct notochordal cells within the nucleus pulposus and an increase in or transition to smaller mature nucleus cells, which are thought to have a lower metabolic activity than notochordal cells (Guehring et al. 2008). Cell metabolism within the disc is also thought to be relatively low, due in part to the low pH and oxygen concentration, with cells generating ATP predominantly through glycolysis (Urban et al. 2004).

However, while cell number and metabolic activity are both low, the resident cells are responsible for homeostatic turnover of the extracellular matrix, producing catabolic factors and degradative enzymes, as well as anabolic growth factors and new matrix proteins. This process is tightly controlled and any imbalance in degradative and synthetic processes can lead to a matrix breakdown and loss of tissue integrity. While the exact reasons for this imbalance are not fully understood, research over recent years has shed light on the processes which contribute to, or are potentially responsible for, the tissue breakdown observed during intervertebral disc degeneration.

11.3 Intervertebral Disc Degeneration

Disc degeneration is characterised by an overall breakdown of extracellular matrix, combined with altered matrix synthesis and changes in resident cell number, cell phenotype and behaviour. While many of these features are evident during normal ageing, changes are accelerated in degeneration and are associated with discogenic pain or pain caused by spinal instability and impingement of nerve roots in the spine.

11.3.1 Morphological Features of Degeneration

Degeneration is routinely characterised radiographically (Antoniou et al. 1998; Pfirrmann et al. 2001), with the Thompson grading system used to classify gross morphological changes (Thompson et al. 1990). This system describes the decreasing water content within the nucleus pulposus, combined with disc narrowing and bulging and eventually osteophyte formation and end-plate sclerosis. Histologically, degeneration can be characterised using a range of features (Sive et al. 2002). There is progressive loss of demarcation between the nucleus pulposus and annulus fibrosus with loss of the transition zone. This is due, in part, to a change in collagen synthesis by the nucleus cells from collagen II to collagen I; a loss of proteoglycan, which results in dehydration of the nucleus pulposus; presence and extent of fissuring within the nucleus pulposus, which radiate eventually into the annulus fibrosus; and formation of cell clusters due to abnormal cell turnover. Changes also occur in the annulus fibrosus, with disruption of the collagen lamellae as fissures extend, and there is a change in collagen fibre organisation with fibres bifurcating and interdigitating (Lyons et al. 1981). The poor repair capacity of the disc means that in the late stages of degeneration, the nucleus pulposus is replaced by disorganised scar and granulation tissue, and repair of the annulus fibrosus results in scaring and neovascularisation (Peng et al. 2006). Vascular ingrowth extends eventually into the nucleus pulposus and is associated with innervation into the disc causing discogenic pain (Freemont et al. 1997, 2002). The decrease in water content and increasing fibrous nature of the nucleus pulposus cause the disc narrowing observed radiographically. There is also an increase in collagen cross-linking with tissue sugars, making the disc stiffer, more difficult to repair and more easily injured (Hormel and Eyre 1991; Duance et al. 1998; Pokharna and Phillips 1998; Wagner et al. 2006). Importantly, this reduced disc height significantly alters the biomechanics of the spinal motion segment, with decompression of the nucleus pulposus and removal of stress within fibres of the annulus fibrosus that ultimately causes spinal instability and leaves the disc less able to resist forces experienced during motions such as bending (Zhao et al. 2005; Adams and Roughley 2006). Increased pressure is also placed on the neural arch and this can result in non-discogenic nerve pain during movement (Pollintine et al. 2004).

Although the anatomical and morphological features of degeneration have been well documented, the underlying cellular and pathophysiological changes occurring during degeneration have not been thoroughly described. However, in an attempt to identify novel therapies, recent widespread interest in the elucidating mechanisms underlying degeneration has resulted in a more thorough understanding of the pathogenesis of degenerative disc disease.

11.3.2 Genetic Influences

Age and environmental factors, such as smoking, vibration, excessive heavy loading and localised injury, have all been proposed as risk factors (Holm and Nachemson 1988; Hirano et al. 1988; Deyo and Bass 1989; Wilder et al. 1996; Adams et al. 1999, 2000). Genetic and hereditary factors are

considered to play a dominant role in predisposing individuals to disc degeneration and back pain. A familial survey by Postacchini et al. found that in the group of individuals with discogenic LBP, 35 % had at least 1 family member with a history of discogenic LBP and 5 % had one or two members who had undergone disc surgery (Postacchini et al. 1988). This compared to 12 and 1 % respectively in the asymptomatic cohort. Further studies, including identical and nonidentical twin studies, have also shown strong familial links in discogenic LBP predisposition (Richardson et al. 1997; MacGregor et al. 2004; Frino et al. 2006).

In addition to familial studies, a growing body of research has investigated genetic associations with degeneration. The genes for collagen I (COL1A1), IX (COL9A2 and COL9A3), XI (COL11A2), aggrecan, MMP-3, IL-1, IL-6, vitamin D receptor (VDR), cartilage intermediate layer protein (CILP) and hyaluronan and proteoglycan link protein 1 (HAPLN1) have all been associated with disc degeneration (Videman et al. 1998; Annunen et al. 1999; Takahashi et al. 2001; Kawaguchi et al. 2002; Pluijm et al. 2004; Solovieva et al. 2004, 2006; Seki et al. 2005; Kawakami et al. 2005; Roughley et al. 2006). However, to date, in different ethnic groups, only COL1A1, COL9A2, MMP-3 and VDR polymorphisms have been shown reproducibly to be disease associated. This topic is developed further in Chap. 10. In order to prove links between gene polymorphisms and disc degeneration, then more detailed and large-scale linkage studies on families with members who are predisposed to early onset degeneration are required. If genetic predisposition and association with single gene polymorphisms can be established, this may lead to the development of diagnostic tools to screen disc degeneration predisposition. However, these studies may also reveal that disc degeneration is a complex, multifactorial, oligogenic disorder for which a clear predisposition is difficult to detect.

11.3.3 Alterations in Extracellular Matrix Composition

While many of the matrix changes evident during degeneration are due to increased matrix catabolism by degradative enzymes (as will be discussed in the next section), there are also alterations in the synthesis and distribution of matrix components. In the early stages of degeneration, there is an increase in expression of collagen II, thought to be an attempted repair mechanism (Takaishi et al. 1997). However, with advancing degeneration, there is a general decrease in collagen II synthesis and a shift to collagen I production by nucleus pulposus and inner annulus fibrosus cells (Buckwalter 1995; Schollmeier et al. 2000; Le Maitre et al. 2007d). Collagen X has been identified in the disc during advanced degeneration, particularly around clefts and cell clusters (Boos et al. 1997). The induction of collagen X during late-stage degeneration is suggested to be a cellular response to enhance oxidative stress and is thought to signify nucleus pulposus cell hypertrophy as it is often accompanied by increased expression of Runt-related transcription factor 2 (Runx2), osteoprotegerin and alkaline phosphatase in areas of calcification (Boos et al. 1997; Nerlich et al. 1997; Rutges et al. 2010).

Collagen cross-linking also changes during degeneration, with a decrease in pyridinoline cross-links which give stability to collagen fibrils, especially in the nucleus pulposus where the collagen fibres are less densely packed than the annulus fibrosus. There is also an increase in nonenzymatic glycosylation, which causes cross-linking of matrix proteins, with an increase in pentosidine during degeneration being one marker of this process. This increase in advanced glycation end products (AGEs) within the disc during both natural ageing and degeneration has been demonstrated to cause tissue stiffness, particularly in the annulus fibrosus, and may make the disc more susceptible to mechanical damage during degeneration (Hormel and Eyre 1991; Duance et al. 1998; Pokharna and Phillips 1998; Wagner et al. 2006; Adams et al. 2010).

Combined with the changes in collagen expression, there is a decrease in the proteoglycans content of the disc (Pearce et al. 1987; Inkinen et al. 1998; Cs-Szabo et al. 2002; Sztrolovics et al. 2002). While degenerate nucleus pulposus cells are capable of synthesising aggrecan, versican synthesis increases, as does production of biglycan and decorin (Lyons et al. 1981; Buckwalter 1995; Inkinen et al. 1998; Le Maitre et al. 2007d). In contrast, there is increased degradation of aggrecan and versican fragmentation thereby reducing disc overall proteoglycans content. Several versican isoforms have been identified in the disc with varying molecular weights (Sztrolovics et al. 2002). However, one of the key features of all isoforms is that they contain fewer chondroitin sulphate side chains than aggrecan and hence have a lower negative charge which reduces the osmotic potential of versican-containing aggregates. Therefore, the overall loss of aggrecan, combined with the shift to versican production, reduces the water content in the disc, and the shift in collagen production to collagen I results in a more fibrous tissue, less capable of withstanding load.

There are other changes evident in the disc, caused either as a result of degeneration or as an attempt at repair. One example is the increase in fibronectin and importantly fibronectin fragments during degeneration (Oegema et al. 2000). Fibronectin is a large extracellular glycoprotein that contains binding sites for several cell membrane and matrix proteins, including integrins and collagens, respectively, and is thought to play a role in extracellular matrix organisation. Its expression increases in degeneration, although there is also an increase in fibronectin fragments which have been demonstrated in vitro to stimulate matrix metalloprotein

(MMP) production and suppress aggrecan synthesis (Anderson et al. 2005; Aota et al. 2005) and in vivo to stimulate disc degeneration (Greg et al. 2003). One mechanism for this change may be the stimulation of catabolic cytokine expression, which has been demonstrated to occur following the addition of fibronectin fragments to cartilage explants in vitro (Homandberg et al. 1997).

11.3.4 Matrix Degradation

A range of proteolytic enzymes are responsible for breakdown of the extracellular matrix, including members of the MMP and 'a disintegrin and metalloproteinase with thrombospondin motifs' (ADAMTS) families (for details of these enzymes, see Chap. 8). MMPs are capable of cleaving the majority of constituents of the disc extracellular matrix. Most notably, MMPs 1, 8 and 13 degrade intact triple-helical collagens including collagens I and II, while 2 and 9 are gelatinases cleaving partially degraded triple-helical domains (Nagase and Woessner 1999).

MMPs 1, 2, 3, 7, 8, 9, 10, 13, 19 and 28 have all been identified within the disc, with levels of many increasing during degeneration (Roberts et al. 2000; Weiler et al. 2002; Le Maitre et al. 2004, 2006b; Gruber et al. 2005; Richardson et al. 2009; Bachmeier et al. 2009; Klawitter et al. 2011). In particular, the number of cells immunopositive for MMPs 1, 3, 7 and 13 was shown to be increased in degeneration (Le Maitre et al. 2004, 2006b). We have also demonstrated a significant increase in the expression of MMP-10 in symptomatic (painful) degenerate discs and shown a correlation between expression of MMP-10 and IL-1 and NGF, but not TNF-α, suggesting a possible role for MMP-10 in the initiation of nociception during degeneration (Richardson et al. 2009). MMP-10 is also capable of activating proMMPs, including MMPs 1, 7, 8, 9 and 13, and has been shown to be capable of 'superactivating' proMMPs 1, 8 and 13, giving them a higher-than-normal specific activity and potentially shifting the homeostatic balance of activity towards catabolism (Barksby et al. 2006).

In addition to their activity against collagens, members of the MMP family also have the ability to degrade aggrecan at discrete sites within the G1-G2 and G2-G3 interglobular domains, although their activity against this substrate is significantly lower than that of the aggrecanase members of the ADAMTS family. The ADAMTS family includes the aggrecanases ADAMTSs 1, 4, 5, 8, 9 and 15, which are all capable of degrading aggrecan at sites distinct from those of the MMPs and at an activity substantially higher than that of the MMPs (Tortorella et al. 1999; Abbaszade et al. 1999; Cal et al. 2002; Nagase and Kashiwagi 2003; Somerville et al. 2003; Collins-Racie et al. 2004). An increase in aggrecanase-generated aggrecan fragments has been identified in both aged and diseased discs, with a correlation being shown between increasing grade of degeneration and an increase in the presence of aggrecanase-generated fragments (Sztrolovics et al. 1997; Roberts et al. 2000). Studies initially identified ADAMTS 4 (aggrecanase 1) in the disc (Le Maitre et al. 2004; Hatano et al. 2006), with a correlation noted between ADAMTS 4, but not ADAMTS 5 (aggrecanase 2), expression with degeneration (Patel et al. 2007). Subsequently, we have shown that the expression of ADAMTSs 1, 4, 5, 9 and 15 are all increased with intervertebral disc degeneration (Pockert et al. 2009), with their expression potentially being regulated by IL-1β (Demircan et al. 2005). Building on this finding, in vitro stimulation experiments demonstrated increased expression of both ADAMTSs 4 and 5 following IL-1β stimulation, with nitric oxide being the mediating factor (Le Maitre et al. 2005a; Zhao et al. 2011). More recently, ADAMTSs 7 and 12, enzymes which are capable of degrading cartilage oligomeric matrix protein (COMP), were both shown to be upregulated in a rat model of degeneration; however, their presence in human discs or role in degeneration is yet to be elucidated (Yu and Zhu 2012).

Those molecules discussed above, along with a broad spectrum of other proteolytic enzymes including cathepsins D, G, K and L (Konttinen et al. 1999; Ariga et al. 2001), are responsible for the homeostatic turnover of disc extracellular matrix. Their expression and activity are closely controlled by soluble mediators, such as catabolic (pro-inflammatory) cytokines and anabolic growth factors, and through blocking cognate inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). The TIMP family consists of four members (TIMPs 1, 2, 3 and 4) which play various roles, including MMP activation and inhibition and induction of angiogenesis (Brew et al. 2000). However, their main role is the inhibition of MMPs and ADAMTSs, which is achieved primarily through irreversible non-covalent coupling to active MMPs in a 1:1 stoichiometric fashion (Cooper et al. 1985; Stetler-Stevenson et al. 1989). TIMPs 1, 2 and 3 have been identified in the disc, with TIMPs 1 and 2 exhibiting broad specificity of inhibition of members of the MMP family, while TIMP 3 appears to selectively inhibit aggrecanases (Kashiwagi et al. 2001). Both TIMP 1 and 2 are upregulated in degeneration (Le Maitre et al. 2004), although they have higher specificity for certain MMPs than others, suggesting those MMPs (e.g. MMP 7) that are resistant to TIMP inhibition may play a greater role in degeneration (Le Maitre et al. 2006b). Conversely, while TIMP 3 expression levels in nondegenerate human nucleus pulposus cells have been shown to be higher than that of any of the ADAMTSs (Pockert et al. 2009), its expression does not change in degeneration (Le Maitre et al. 2004; Pockert et al. 2009), suggesting a potential imbalance between active ADAMTSs and TIMP 3 that could lead to the matrix, particularly aggrecan, degradation, a characteristic of the degenerate disc.

11.4 Vascular and Nerve Ingrowth

At birth, both the end plates and annulus fibrosus possess blood vessels, although these soon recede, meaning that, with the exception of the external lamellae of the annulus, the normal adult intervertebral disc is both avascular and aneural (Yasuma et al. 1993; Repanti et al. 1998; Roughley 2004; Roberts et al. 2006b). However, during degeneration, both neovascularisation and innervation occur, with blood vessels and nerve fibres infiltrating the annulus fibrosus and eventually the nucleus pulposus (Freemont et al. 1997, 2002; Coppes et al. 1997; Nerlich et al. 2007). Matrix alterations, particularly vascular ingrowth, and increasing angiogenesis have been correlated with decreasing proteoglycan content in an ovine annular lesion model (Melrose et al. 2002a). Indeed, aggrecan has been shown to be inhibitory to endothelial cell adhesion and migration in a concentration-dependant manner (Johnson et al. 2005), a finding that provides a possible mechanism for accelerating the degenerative process. Extensive capillary networks have also been found to be associated with annular clefts and tears (Nerlich et al. 2007), suggesting the breakdown of the normal disc extracellular matrix is permissive for neovascularisation. However, the mechanisms or the factors underlying initiation of angiogenesis have not yet been clearly elucidated. Pleiotrophin, a growth factor reported to be involved in cell migration and differentiation in various cellular processes, has been implicated as an angiogenic factor in the disc as it has been shown that the frequency of pleiotrophin-positive disc cells was significantly correlated with the amount of vascularisation (Johnson et al. 2007). Additionally, other angiogenic factors have also been implicated including vascular endothelial growth factor (VEGF) (Ohba et al. 2009), basic fibroblast growth factor (FGF-2), TGF-β and osteonectin (Melrose et al. 2002b). More recently, it has also been suggested that IL-1β is capable of inducing angiogenesis through stimulation of the growth factors VEGF, NGF and BDNF, although these results were based on immunohistochemical correlation studies rather than direct stimulation with IL-1β (Lee et al. 2011). While the mechanisms underlying vascular ingrowth are still unclear, neovascularisation is thought to provide a route for various cytokines and growth factors to reach the inner disc regions at an accelerated rate than through the usual route of diffusion (Nerlich et al. 2007), which may be one of the driving forces behind the increased nucleus pulposus cell-derived degradative enzyme production and accelerated proteoglycan loss in the inner annulus fibrosus and nucleus pulposus.

During degeneration, there is also an increase in nerve fibres both physically associated with, and distant from, infiltrating blood vessels. In the majority of these cases, nerve fibres are found alongside blood vessels, and during angiogenesis, endothelial cells from infiltrating vessels secrete NGF. Since nerves possess the high-affinity NGF receptor TrkA, it is likely that there is a vasoregulatory role for the nerve fibres (Freemont et al. 2002). These nerve fibres have been shown to be positive for protein gene product 9.5 (PGP9.5), acetylcholinesterase, neurofilament protein (NFP), substance P (SP) and calcitonin gene-related peptide (CGRP), amongst other proteins. On this basis, it has been suggested that these nerve fibres originate from the dorsal root ganglion (Ashton et al. 1994; Brown et al. 1997; Ohtori et al. 2002; Takahashi et al. 2009; Garcia-Cosamalon et al. 2010) and are nociceptive. Studies on degenerate intervertebral disc tissues have demonstrated a similar protein expression profile for nerves infiltrating the inner annulus fibrosus and nucleus pulposus, suggesting a quantitative increase in nociceptive neurite number, rather than a change in type of neurite (Ashton et al. 1994; Freemont et al. 1997; Brown et al. 1997; Johnson et al. 2002; Melrose et al. 2002a; Takahashi et al. 2009). On the other hand, there is evidence that suggests there is an increase in sympathetic afferents in degenerate tissue, which are hypothesised to play a significant role in low back pain (Takebayashi et al. 2006). In vitro studies demonstrated a concentration-dependant inhibition in nerve fibre outgrowth by human aggrecan, suggesting the decreases in proteoglycans content in the disc during degeneration may permit neural ingrowth (Johnson et al. 2002). The same study also indicates that deglycosylation of aggrecan may also be important as enzymatic removal of keratan and chondroitin sulphate from aggrecan abrogated the inhibitory effect of intact aggrecan. As the effect was greater following chondroitinase ABC than keratanase treatment, it was inferred that there was a greater role for chondroitin sulphate than keratan sulphate in inhibiting nerve outgrowth. Since there is an increase in the ratio of keratin sulphate/chondroitin sulphate, the authors also hypothesised that this change may also be important in terms of allowing nerve infiltration into the disc.

Importantly, however, a complex interplay between catabolic cytokines, neurotrophins, neurotrophin receptors and chemorepellant molecules may be responsible for guiding nerve ingrowth during degeneration, in particular semaphorins, a large family of secreted and membrane-bound axonal guidance molecules (Kolodkin et al. 1993). Gene and protein expression studies of the class 3 semaphorin family member Sema3A, which can cause axonal collapse when found in high concentrations, identified high levels of cell immunopositivity in the outer annulus fibrosus of normal tissue (Tolofari et al. 2010). The percentage of semaphorin-positive cells was found to decrease with increasing grades of degeneration, particularly in individuals with symptomatic (painful) degeneration, suggesting an important role for Sema3A in inhibiting nerve ingrowth into normal disc tissue.

Both nucleus pulposus and annulus fibrosus cells from normal discs have been shown to express low levels of the

neurotrophin nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and expression levels increase in degeneration, particularly in individuals with symptomatic (painful) disc degeneration (Abe et al. 2007; Purmessur et al. 2008; Gruber et al. 2008). Interestingly, these disc cells also express the high-affinity NGF receptor TrkA, the high-affinity BDNF receptor TrkB and the low-affinity NGF/BDNF receptor $p75^{NTR}$, as well as SP, suggesting possible autocrine signalling by the disc cells themselves (Purmessur et al. 2008). However, the predominant role for NGF and BDNF may be to act in a paracrine manner on dorsal root ganglion neurons to stimulate nerve ingrowth. We have recently demonstrated that coculture of human nucleus pulposus cells, derived from the degenerate intervertebral disc, with the neural cell line SH-SY5Y cells caused an increase in both percentage of neurite-expressing cells and mean neurite length (Richardson et al. 2011). This finding supports earlier work by Johnson and colleagues who showed that normal inhibition of neurite outgrowth by aggrecan could be prevented by cells derived from degenerate disc, suggesting that such cells release neurotrophins (Johnson et al. 2006). Indeed, our own studies show that these increases in neurite-expressing cells and neurite length could be inhibited by the addition of anti-BDNF antibodies. In contrast, when inhibition was activated by anti-NGF antibodies, there was only a decrease in the percentage number of neurite-expressing cells (Richardson et al. 2011).

Interestingly, both NGF and BDNF expression can be stimulated by addition of recombinant IL-1β and TNF-α (cytokines shown to be increased in intervertebral disc degeneration) to cultured human nucleus pulposus cells in vitro, while TNF-α stimulation also induces expression of substance P (Purmessur et al. 2008). Such in vitro results suggest that these pro-inflammatory cytokines stimulate the production of neurotrophins which promotes the growth of sensory nerve fibres into the intervertebral disc and induce substance P related with pain transmission. Noteworthy, signalling of NGF and BDNF through their receptors initiates activation of a number of pathways, including the NF-κB pathway; activation induces a range of pro-inflammatory cytokines which may then perpetuate the cycle leading to innervation (Wallach et al. 2002). The expression of NGF also correlates with expression of specific MMPs (Richardson et al. 2009), suggesting a potential role for neurotrophins in driving matrix catabolism, possibly to ease nerve ingrowth through the disc. Importantly, this interaction between cytokines and neurotrophins is complex and requires further extensive study before a clear pathway can be elucidated. Unfortunately, however, this is hindered by the inability to study nerve ingrowth in humans and the potential differences between humans and the model animal systems routinely used in disc degeneration research.

11.5 Alterations in Disc Cell Biology in Degeneration

A wide range of factors are thought to be involved in the initiation and progression of degeneration (Fig. 11.1). While individuals may experience discogenic back pain for different reasons, correlation studies and detailed molecular and cellular biology studies suggest that there are four main categories of factors influencing cell function and hence drive intervertebral disc degeneration. These include soluble regulators of disc cell function (mainly cytokines and growth factors), nutritional status, cell ageing and death and response to mechanical load.

11.5.1 Soluble Regulators of Cellular Function

During degeneration, a range of pro-inflammatory cytokines and inflammatory mediators are increased. These include members of the interleukin family, including IL-1, IL-2, IL-6, IL-12 and IL-17, as well as interferon gamma (IFN-γ), TNF-α and the inflammatory mediators prostaglandin E2 (PGE2) and nitric oxide (NOx) (Kang et al. 1996; Olmarker and Larsson 1998; Le Maitre et al. 2005a, 2007b; Bachmeier et al. 2007; Akyol et al. 2010; Gabr et al. 2011; Studer et al. 2011). The pro-inflammatory cytokines are all thought to play independent roles in matrix catabolism, although interplays between the molecules have been identified. For example, IL-6 is thought to potentiate the response of nucleus pulposus cells to both IL-1 and TNF-α (Studer et al. 2011), while IL-17 synergises with both TNF-α and IFN-γ, increasing the catabolic activities of human nucleus pulposus and annulus fibrosus cells and possibly serving as a key regulator of inflammation in the degenerating disc (Gabr et al. 2011).

However, although there is evidence for the involvement of multiple pro-inflammatory cytokines in the pathogenesis of disc degeneration, the predominant catabolic cytokines appear to be interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α). While there is no clear consensus on which molecule mediates degeneration, research suggests both are fundamentally important in controlling the observed cellular and matrix changes.

11.5.1.1 Interleukin-1

Both isoforms of IL-1 (IL-1α and IL-1β) have been identified within the disc, along with their receptor (IL-1R1), the exported decoy receptor (IL-1RII) and their natural inhibitor (IL-1 receptor antagonist or IL-1Ra) (Le Maitre et al. 2005a). During degeneration, expression of IL-1α and β and IL1RI increases significantly in both the nucleus pulposus and inner annulus fibrosus. However, IL-1Ra expression does not increase and this imbalance leads to an excess of IL-1 isoforms in degenerate tissues. Importantly, in vitro studies have

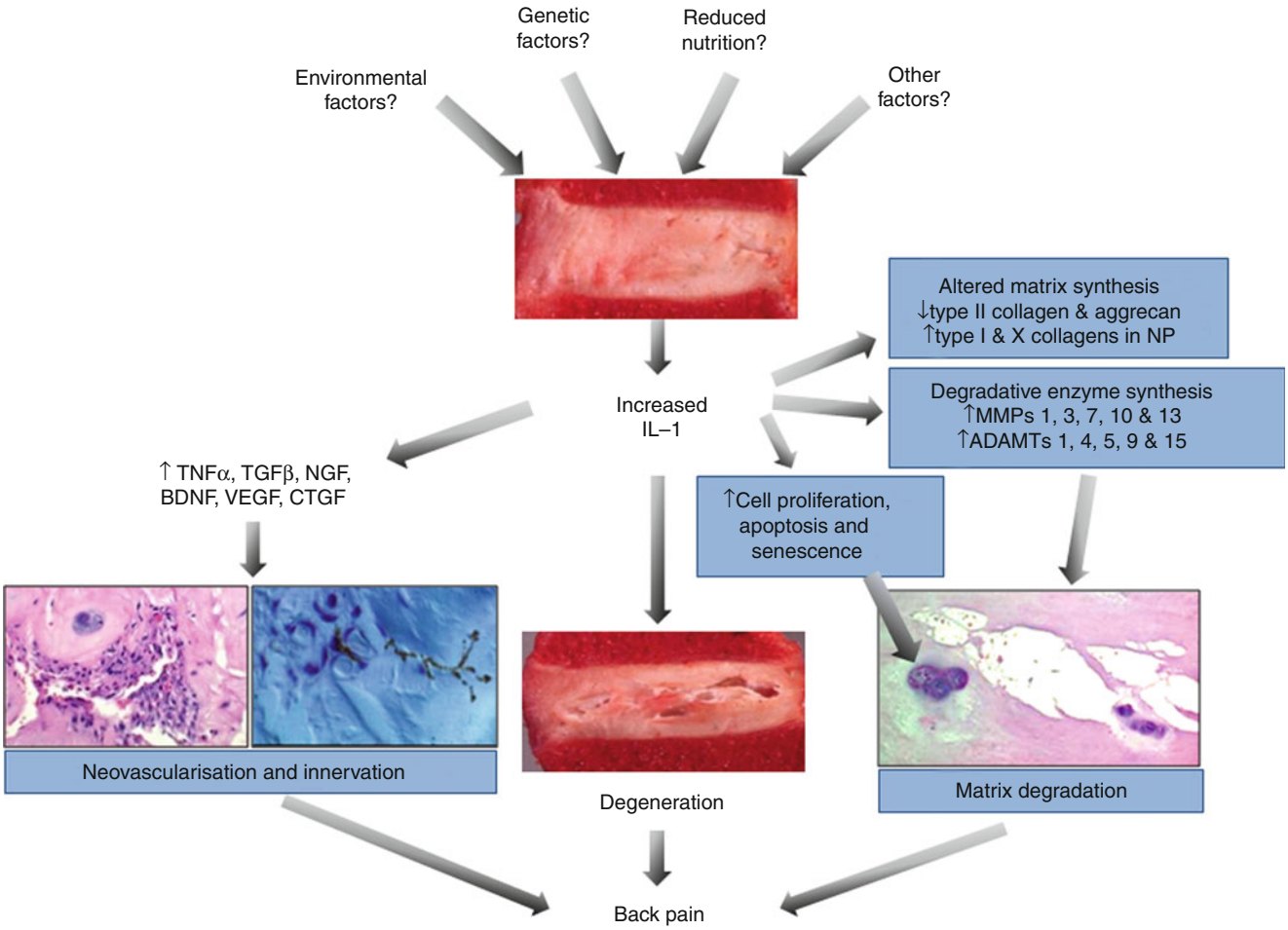


Fig. 11.1 A schematic overview of the pathogenesis of intervertebral disc degeneration demonstrating the involvement of IL-1 in driving the aberrant cell biology and processes involved in matrix catabolism and generation of back pain

shown that IL-1 induces a number of cellular and molecular changes associated with disc degeneration. Stimulation of human nucleus pulposus cells with recombinant IL-1 has been shown to induce an upregulation of both MMPs, including MMPs 3 and 13, and ADAMTSs, including ADAMTS 4, a shift in collagen expression from II to I and reduction in aggrecan expression (Le Maitre et al. 2005a). There appear to be differences in the responses of normal and degenerate disc cells to IL-1 stimulation, with a more catabolic response in degenerate nucleus pulposus cells compared to normal. IL-1 stimulation also resulted in significant increases in both IL-1 isoforms by degenerate nucleus pulposus cells and a decrease in expression by normal nucleus pulposus cells, suggesting a homeostatic response in nondegenerate cells and an aberrant catabolic response once degeneration has been activated. IL-1 has also been shown to induce both angiogenesis (by inducing expression of VEGF) and neuronogenesis (via the stimulation of neurotrophic factors) into disc tissue (Lee et al. 2011) and stimulation of apoptosis (Cui et al. 2007; Zhao et al. 2007a). Conversely an inhibition or reversal of these processes has been demonstrated through the addition of exogenous IL-1Ra, and application of IL-1Ra has been proposed as a potential therapeutic intervention to inhibit intervertebral disc degeneration (Le Maitre et al. 2006a, 2007c; Box 11.1).

Box 11.1: Interleukin-1 as the Driving Force Behind the Pathogenesis of Disc Degeneration

While for some TNF-α has been the focus of investigation as the molecular regulator of disc degeneration, our research has focussed on the involvement of IL-1. These studies have demonstrated an increase in the expression of both isoforms (α and β) of IL-1, along with its receptor (IL-1RI) during degeneration. However, no such increase was demonstrated for its natural inhibitor, IL-1Ra, suggesting an imbalance that may be responsible for driving the cellular and matrix changes evident during degeneration. These roles include inducing expression of both MMPs and ADAMTSs, which are known to catabolise the

extracellular matrix; reducing matrix component molecule expression, most notably aggrecan; inducing apoptosis and senescence of disc cells; and inducing both angiogenesis and innervation into the disc. However, the key question at present is: what initiates the upregulation of IL-1 that then induces the degenerative cascade? Elucidation of this mechanism may lead to the development of novel therapies or allow disc degeneration to be prevented.

11.5.1.2 TNF-α

TNF-α, like IL-1, has been shown to be capable of inducing neural ingrowths into the degenerate intervertebral disc. TNF-α has also been implicated in causing nerve root damage and sciatic pain, with blocking studies supporting this theory (Igarashi et al. 2000; Olmarker and Rydevik 2001). While evidence for a role for TNF-α in nerve ingrowth is compelling, evidence supporting its role in driving matrix catabolism during degeneration is less clear. Although expression of TNF-α is increased in degenerate tissues (Weiler et al. 2005; Bachmeier et al. 2007), other studies have either failed to identify TNF receptor I in degenerate samples or shown that there is no increase in the receptor gene expression in degenerate samples. The result suggests that native disc cells in vivo may not be able to respond to TNF-α (Le Maitre et al. 2007b). However, in studies where recombinant TNF-α was used to stimulate cultured nucleus pulposus cells, there were increases in expression of MMPs 1, 3, 9 and 13, as well as ADAMTSs 4 and 5, although induction of expression of a number of these enzymes was greater following stimulation with IL-1 than with TNF-α (Hoyland et al. 2008). Conversely, in situ zymography studies of normal and degenerate human nucleus pulposus tissue treated with either IL-1 or TNF-α indicated there was only an increase in enzyme activity in the IL-1 group and not the TNF-α group. In this study, addition of IL-1Ra caused a decrease in enzyme activity that was not evident following addition of anti-TNF. While demonstrating an increase in TNF-α in degeneration, the documented low expression of its receptor on disc cells together with differences in findings from the other studies begs the question: what is the target of nucleus pulposus cell secreted TNF-α? Does it play a more fundamental role in innervation and development of discogenic pain, than in matrix catabolism and tissue breakdown?

11.5.1.3 Anabolic Growth Factors

Growth factors have a number of effects on cells, most notably the shift in metabolic balance towards anabolism, promotion of cell proliferation and prevention of cell death. A range of growth factors, including many members of the TGF-β superfamily, are known to be present in the normal intervertebral disc, while evidence for their involvement in disease comes from both in vitro cell stimulation studies and studies of in vivo models of disc degeneration. Many workers have focussed on the ability of growth factors to stimulate proteoglycans synthesis by nucleus pulposus and annulus fibrosus cells and shown positive results following stimulation with transforming growth factor-β (TGF-β), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), connective tissue growth factor (CTGF) and the bone morphogenetic proteins (BMPs) 2, 7 (also known as osteogenic protein-1 or OP-1), 12, 13 (also known as growth and differentiation factor 6 (GDF-6) or cartilage-derived morphogenetic protein-2 (CDMP-2)) and 14 (also known as growth and differentiation factor 5 (GDF-5) or cartilage-derived morphogenetic protein-1 (CDMP-1)) either alone or in combination (Thompson et al. 1991; Osada et al. 1996; Gruber et al. 1997; Masuda et al. 2003; Tim et al. 2003; Imai et al. 2007a; Gilbertson et al. 2008; Le Maitre et al. 2009). Cell proliferation has also been demonstrated in vitro following stimulation with TGF-β and IGF-1, while the same growth factors and platelet-derived growth factor (PDGF) are all capable of reducing disc cell apoptosis and secretion of catabolic cytokines (Gruber et al. 1997, 2000).

In vivo studies also show positive effects of TGF-β, IGF-1, BMPs 2 and 5, fibroblast growth factor-2 (FGF-2) and OP-1 on cell proliferation, matrix synthesis and restoration of disc height in models of disc degeneration (Walsh et al. 2004; An et al. 2005; Masuda et al. 2006; Miyamoto et al. 2006). Of these, the most widely studied is OP-1, with positive effects noted both in vitro and in vivo. Indeed, OP-1 was capable of stimulating proteoglycans and collagen synthesis by human nucleus pulposus cells following IL-1 and chondroitinase ABC treatment in vitro (Takegami et al. 2005; Imai et al. 2007a) and restoring disc height and matrix degradation caused by annular needle injury or chemonucleolysis with chondroitinase ABC (Miyamoto et al. 2006; Imai et al. 2007b). OP-1 also reduced the production of aggrecanase, MMP-13, substance P, TNF-α and IL-1β, suggesting that it may have both anabolic and anti-catabolic effects (Chubinskaya et al. 2007). The effect of OP-1 on substance P expression, combined with the interplay between catabolic cytokines and pain markers or pain modulators, has led to the hypothesis that application of recombinant growth factors may have beneficial effects in terms of pain reduction, as well as matrix restoration during degeneration (Kawakami et al. 2005). However, the clinical translation of growth factor therapies is complicated by their diverse and sometimes biphasic roles. While growth factors such as TGF-β and CTGF are known to promote extracellular matrix synthesis, studies on clinical human samples have linked their overexpression to fibrosis and angiogenesis (Ali et al. 2008; Peng et al. 2009). The application of growth factor therapies is

further complicated by possible changes in receptor distribution. While data in this area is limited, studies suggest that there is no change in the expression levels of major growth factor receptors, including TGFβRII, BMPRII, FGFR3 and IGFRI (Le Maitre et al. 2005b; Peng et al. 2006). However, expression of growth factor receptors, including TGFRII, FGFR3, IGFRI and VEGF receptors I and II, on ingrowing blood vessels (Haro et al. 2002; Le Maitre et al. 2005b) and in granulation tissue in painful degenerate discs (Peng et al. 2006) suggests that the use of growth factors should carefully be controlled to avoid stimulation of unwanted events such as angiogenesis. Therefore, a more detailed understanding of their expression profiles and roles in both disc degeneration and repair are currently required.

Another area where growth factors show potential is in the stimulation of adult mesenchymal stem cell (MSC) differentiation. Previous studies have shown that TGF-β, along with members of the BMP family, most notably BMPs 2 and 14, can stimulate MSC differentiation towards nucleus pulposus-like cells in vitro (Stoyanov et al. 2011; McCanless et al. 2011). Given the increasing information on the nucleus pulposus phenotype, further studies will no doubt shed light on the role that growth factors play in MSC differentiation and may lead to combined regenerative cell/growth factor therapies for treatment of disc degeneration.

11.6 Changes in Disc Nutrition and Oxygen Tension

As mentioned previously, nutrient supply to the disc is predominantly from blood vessels in the vertebral bodies and occurs via diffusion through the cartilaginous end plates. This theory has been well studied and confirmed using a range of tracer diffusion experiments, including MRI contrast media, fluorescent and radioactive tracers and gaseous tracers such as nitrous oxide (Brodin 1955; Holm and Nachemson 1982, 1983; Adams and Hutton 1986; Urban et al. 2001). Exposure to cigarette smoke has also been shown to inhibit transport of oxygen into, and lactic acid out of, the disc in animal experiments due to constriction of the microvasculature in the vertebrae (Holm and Nachemson 1988); this finding supports epidemiological evidence linking smoking to disc degeneration in humans. The size and charge of solutes also affect their penetration into the disc, with anions showing lower rates of diffusion than cations due to the polyanionic nature of the disc (Urban et al. 2004), while larger molecules such as albumin are effectively prevented from diffusing through the cartilaginous end plate (Urban et al. 2004). Compared to uni- or bivalent electrolytes, and the fact that glucose is a relatively large molecule, Urban et al. opined that diffusion into and through the disc may be slow (Urban et al. 2004). This lack of nutrient supply to the core of the nucleus pulposus is reflected in the non-uniform cell distribution throughout the disc: cell number in the outer annulus (which is closer to capillaries surrounding the tissue) is substantially higher than that of the nucleus which can be as far as 8 mm from the nearest capillary.

With age, the nutrient supply to the disc is reduced in part due to reductions in both the density and integrity of capillaries in the vertebral bodies and in part due to calcification of the cartilaginous end plates (Bernick and Cailliet 1982; Roberts et al. 1996). While it is currently unclear whether cartilaginous end-plate calcification is causative of, or the result of, disc degeneration, it is thought to play an important role in disease progression by posing a significant barrier to diffusion of solutes into and out of the disc. However, recent μCT studies on graded normal and degenerate human samples have suggested that, contrary to popular belief, porosity in vertebral end plates increases in degeneration by as much as 130 %, while trabecular thickness decreases by as much as 50 % (Rodriguez et al. 2011, 2012). One result of these changes appeared to be an increase in cell proliferation and decrease in proteoglycan content in the nucleus pulposus. While solute transport was not assessed in this study, the authors proposed that ischemic cell changes in degeneration may reflect capillary transport activity rather than a decrease in end-plate permeability.

Whatever its cause, a reduction in essential nutrients is thought to drive the progression of degeneration. Given that the main energy-generating pathway in disc cells, even in the presence of oxygen, is glycolysis (Holm et al. 1981; Ishihara and Urban 1999), which requires principally glucose and produces lactic acid, free diffusion of solutes to and from the cells is essential. Evidence suggests that reduction in glucose concentration below 0.5 mmol/L, even for a relatively short period, can cause cell death. Likewise, reduction in the pH to below 6.4 can also promote death (Horner and Urban 2001; Bibby and Urban 2004), while less severe reductions in pH can impact cell metabolism (Ohshima and Urban 1992). Evidence from a range of assays, including biochemical assays and microelectrode measurements, shows that this drop in glucose concentration and pH is similar to that observed in degenerate discs. Therefore, while cell activity and even viability may be impaired by a decrease in nutrient supply, enzyme activity is not reduced. In this case, there would be an imbalance between matrix anabolism and catabolism which may contribute to an elevation in matrix degradation in degenerate discs.

The other key metabolite in the disc is oxygen, although its role is less clear than that of glucose. Oxygen levels vary widely in human discs, with no clear correlation between a change in oxygen tension and disc degeneration. However, there exists a steep oxygen gradient within discs – studies in dogs demonstrating a decrease from 8 to 10 % O_2 at the disc-vertebral body interface to 0.3–0.5 % in the centre of the nucleus pulposus (Holm et al. 1981). Human discs show similar trends, with readings as low as 0.7 % O_2 in the core

of the degenerate human nucleus (Bartels et al. 1998). While oxygen is consumed by disc cells, relatively little CO_2 is produced and disc cells can survive for at least 2 weeks without oxygen (Horner and Urban 2001), suggesting oxidative phosphorylation is not the primary mechanism for energy metabolism. Further studies on both canine and bovine disc cells demonstrated that as O_2 concentration decreases from 21 to 1 %, there was a decrease in oxygen consumption of around 75 % and a sharp increase in lactate production (Ishihara and Urban 1999). This suggests a positive Pasteur effect since glycolysis is stimulated under hypoxic conditions. However, studies on the effect of hypoxia on disc cells have also demonstrated cellular inactivity (Horner and Urban 2001) and a loss of matrix synthesis (Ishihara and Urban 1999) below an oxygen concentration of around 5 %. As the increase in lactate production under hypoxic conditions is likely to reduce the pH, this in turn would reduce matrix synthesis (Ohshima and Urban 1992) and eventually cause cell death (Horner and Urban 2001; Bibby and Urban 2004). Hence, the effects of low oxygen and low pH are likely to be cumulative and a destructive influence of disc cell survival. The mechanism by which cells sense oxygen and accommodate to the oxemic state is discussed in exhaustive detail in Chap. 6.

Confounding the problem of evaluating the consequences of oxygen and nutrient limitation is the fact that human tissues are different from those of animal models. Therefore, investigators have increasingly turned to mathematical finite element modelling to elucidate the effect of nutrient limitation on the disc microenvironment and cellular metabolism. However, since all of the variables involved in disc nutrition are poorly understood, the studies published in this area are relatively simple, dealing with only one or two aspects of nutrient supply. Nevertheless, they all suggest that limited nutrient supply affects disc cell viability and metabolic activity (Selard et al. 2003; Yao and Gu 2006; Mokhbi et al. 2009; Malandrino et al. 2011; Jackson et al. 2011).

As with findings from finite element modelling, in vitro studies appear to confirm that during degeneration, glucose limitation and decrease in pH are the predominant factors affecting cell metabolism. While nucleus pulposus cells have mechanisms to regulate intracellular pH, such as the expression of the carbonic anhydrases 9 and 12 (Minogue et al. 2010a), our preliminary data indicates that the expression of these molecules decreases with degeneration, suggesting that cells may not be capable of withstanding the low pH over extended periods (Fig. 11.2). Likewise, changes in the nucleus pulposus membrane glucose transporters (GLUTs), present in degenerative tissues, suggest that there are molecular adaptations to compensate for the reduced glucose concentrations (Richardson et al. 2008b). However, the harsh environment of the degenerate disc is likely to be detrimental not just to resident cells but also to cells that are introduced into the disc. Accordingly, for future cell-based therapies, the introduction of too many cells, or cells which are unable to withstand the conditions of the microenvironmental niche, may exacerbate the degenerative problem rather than provide a cure.

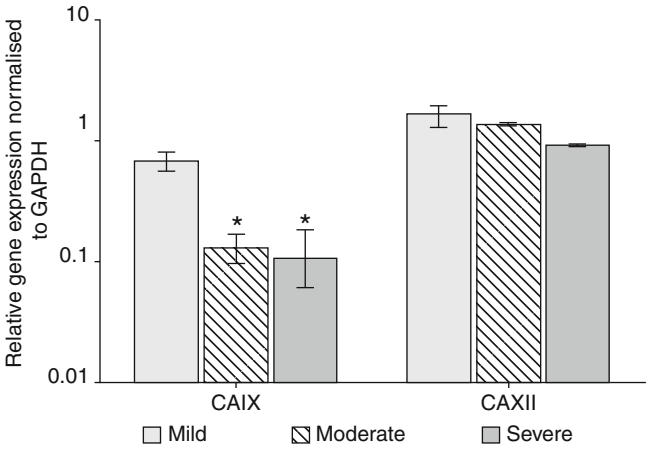


Fig. 11.2 Histogram illustrating real-time quantitative PCR data demonstrating a decrease in expression of carbonic anhydrase 9 (CAIX) and 12 (CAXII) in nucleus pulposus cells during disc degeneration. The expression of CAIX is significantly decreased with disease progression, while a decrease is also seen for CAXII. The carbonic anhydrases play a role in regulating intracellular pH, and a downregulation in their expression may result in cells being incapable of withstanding the harsh physicochemical environment of the degenerate intervertebral disc

11.7 Cell Ageing and Death

During development, the nucleus pulposus is populated by large, vacuolated, metabolically active and morphologically distinct notochordal cells which produce high levels of proteoglycans (Boos et al. 2002; Cappello et al. 2006). However, by the age of 10, this population has been replaced by smaller, chondrocyte-like nucleus pulposus cells which are less metabolically active (Wolfe et al. 1965; Pazzaglia et al. 1989; Boos et al. 2002; Guehring et al. 2008). The period between 3 and 10 years of age, during which identifiable notochordal cells disappear, is also the time during which there is a high level of cell death (Boos et al. 2002). These changes appear to signal the initiation of a transition from a highly hydrated, gelatinous extracellular matrix to a more fibrous, cartilaginous nucleus pulposus seen in adults. It also coincides with the earliest identifiable signs of degeneration seen in MRI studies. In addition, histological studies have identified matrix changes in this age group, which progress throughout adult life (Boos et al. 2002). Although these changes may be considered to be part of the normal ageing process, as discussed previously, the accelerated matrix degradation suggests that in disc degeneration, there is premature ageing.

Several studies have identified necrotic cells within the disc which increase both with age and degeneration; however, more recently, apoptosis has been identified as the

principle mechanism of cell death. Evidence for this has come from a number of studies, using a diverse range of markers including transferase-mediated dUTP nick-end labelling (TUNEL) staining (Gruber and Hanley 1998; Lotz and Chin 2000; Rannou et al. 2004; Kim et al. 2005; Risbud et al. 2005; Heyde et al. 2006; Park et al. 2006; Loreto et al. 2011), annexinV-propidium iodide flow cytometry (Rannou et al. 2004; Risbud et al. 2005; Park et al. 2006), caspase activity analysis (Rannou et al. 2004; Heyde et al. 2006; Park et al. 2006; Tschoeke et al. 2008) and gene expression studies of apoptosis-related markers such as Bax and Bcl-2 (Heyde et al. 2006; Tschoeke et al. 2008; Loreto et al. 2011). The method employed to establish the incidence of apoptosis appears to influence findings both in vivo and in vitro. Serum deprivation resulted in only 1 % cell apoptosis when studied using TUNEL staining (Gruber et al. 2000); in contrast, similar conditions increased in the incidence of apoptosis in rat annulus fibrosus cells to 56 % when annexinV-propidium iodide flow cytometry was used (Risbud et al. 2005). Other studies have also suggested that incidence of apoptosis in disc cells can be as high as 73–74 % (Gruber and Hanley 1998; Ha et al. 2006), although this would be an overestimate since it would result in the de-cellularisation of the disc within days (Alvarez and Ortiz 1999). Conversely, studies using fluorescent cell viability assays on fresh disc tissue have repeatedly demonstrated 60 % to over 90 % cell viability even in degenerate and scoliotic discs, further suggesting that the apoptosis measurements are an overestimation (Bibby et al. 2002; Johnson and Roberts 2007). However, while no causative link has been found between apoptosis and increased extracellular matrix degradation, it is probable that programmed cell death plays an essential role in the pathogenesis of disc disease.

Autophagy, a pathway that may lead to programmed cell death, has recently been identified in the rat nucleus pulposus (Ye et al. 2011) and annulus fibrosus (Shen et al. 2011). We have also demonstrated evidence of autophagy in tissues of the degenerate human intervertebral disc, through immunostaining for the key markers LC-3 and beclin-1 (Fig. 11.3). While autophagy can lead to cell death, it can also maintain cell viability during periods of environmental or nutritional stress by catabolism of intracellular

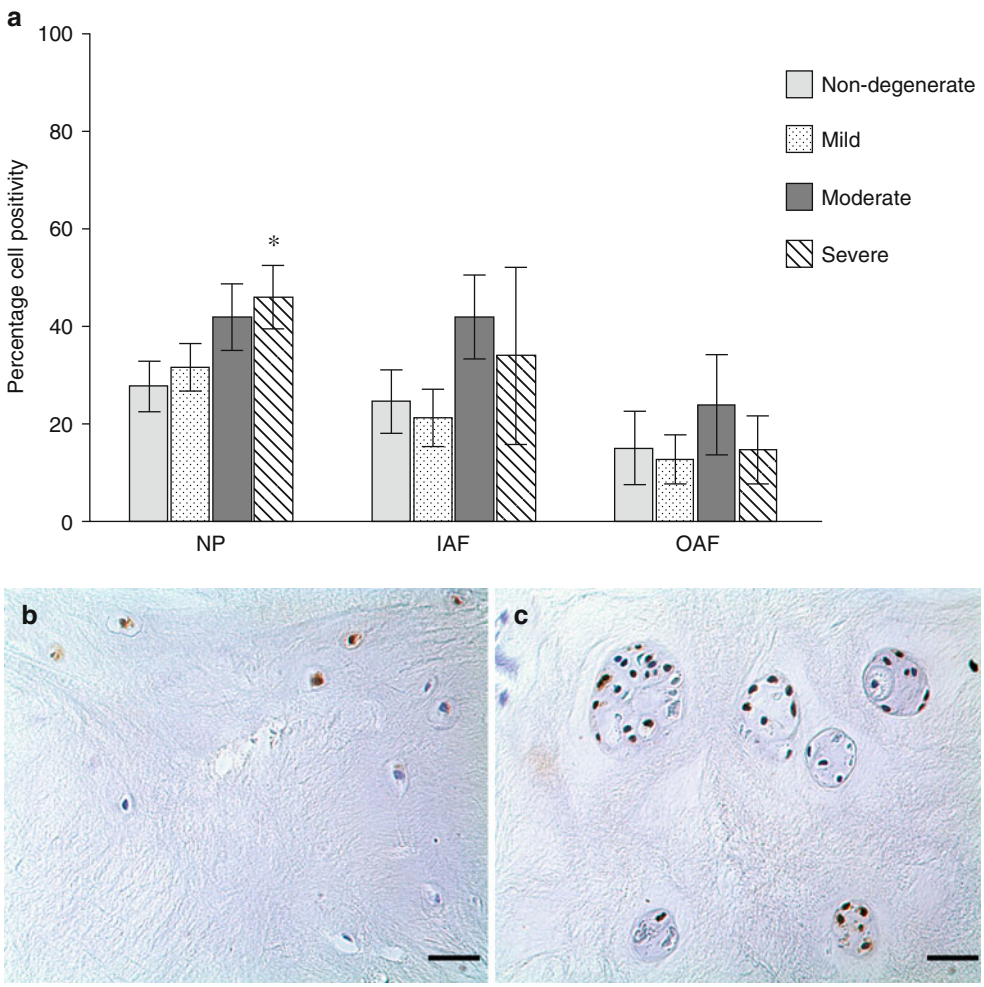
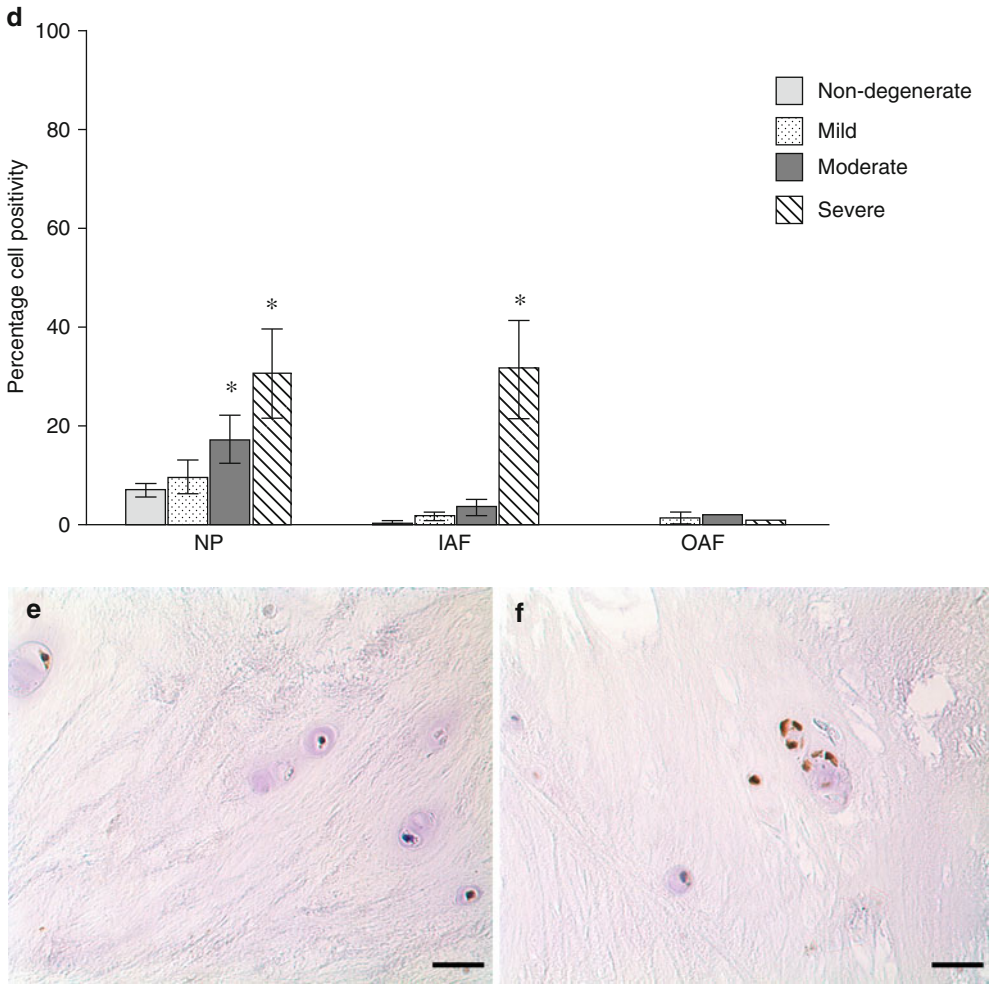


Fig. 11.3 Evidence for the presence of autophagy markers in normal and degenerate human IVD cells. Immunohistochemistry for LC-3 (**a**–**c**) and beclin-1 (**d**–**f**) demonstrated staining for both markers in normal (**b**, **e**) and degenerate (**c**, **f**) nucleus pulposus cells, with strong immunopositivity in degenerate cell clusters. Semi-quantitative analysis of cell positivity demonstrated significant increases in both LC-3 and beclin-1 in the nucleus pulposus with progression of degeneration (**a**, **d**) and in the inner annulus fibrosus (*IAF*), but not outer annulus fibrosus (*OAF*), for beclin-1. Although preliminary data, these findings suggest a role for autophagy in disc cells and that there may be an increase in the number of autophagic cells in intervertebral disc degeneration

Fig.11.3 (continued)



organelles and unfolded or damaged proteins. Therefore, more work is required to establish the role of autophagy in disc degeneration.

Studies on intervertebral disc cell number suggest that rather than a steady decline in cell numbers over time, there is a cyclical pattern of cell death followed by a period of proliferation. Indeed between 11 and 16 years, following the extensive cell death noted between 3 and 10 years, there is notable cell proliferation. The authors hypothesise that this is a compensatory mechanism in response to the early matrix changes seen during this time period (Boos et al. 2002). Cell clustering and proliferation, a characteristic phenomenon of degenerating tissue, is seen in the nucleus pulposus; both parameters increase both with age and with stage of degeneration (Boos et al. 2002). Both histological parameters are routinely identified adjacent to clefts and tears within the tissue, possibly due to a localised increase in the local nutrient supply in these regions (compared to an overall decrease in nutrients within the disc) rather than as a reparative mechanism (Beard et al. 1981; Boos et al. 2002; Zhao et al. 2007b). This hypothesis is further supported by evidence obtained from scoliotic discs which exhibit an increase in cell population in the inner annulus fibrosus in association with regional neovascularisation (Beard et al. 1981). Although signs of cell death are seldom seen, markers of cell proliferation, most notably proliferating cell nuclear antigen (PCNA) and the proliferation-associated Ki-67, have been identified within these cell clusters (Johnson et al. 2001; Zhao et al. 2007b).

A number of potential theories have been proposed to explain this finding. The increased local nutrition may enable cells within clusters to resist apoptosis, or possibly dead cells may be cleared more quickly from clusters than from the rest of the disc. In this respect, other workers have identified morphologically nucleus pulposus-like but CD68-positive cells within cells cluster in the nucleus pulposus of degenerate discs (Nerlich et al. 2002). These cells have the ability to phagocytose apoptotic bodies and are thought to be transformed nucleus pulposus cells, rather than infiltrating cells. In vitro studies have also demonstrated the ability of bovine nucleus pulposus cells to phagocytose apoptotic cells, suggesting that cell clearance from the disc may be undertaken by resident disc cells rather than infiltrating macrophages or monocytes (Jones et al. 2008).

Within clusters, increased proliferation predisposes the resident cells to replicative senescence. Several studies have identified markers of cellular senescence in degenerate discs (Roberts et al. 2006a; Gruber et al. 2007), and in 2007, a link between accelerated cell senescence and degeneration was identified. Through both gene and protein expression studies, Le Maitre et al. (2007a) showed that there was an increase in expression of $p16^{INK4A}$, a cell cycle inhibitor which is upregulated in senescence, a decrease in both mean telomere length and replicative potential and an increase in senescence-associated β-gal staining. While similar trends were identified with ageing, these senescence changes were positively related to stage of degeneration, irrespective of age, clearly demonstrating a role for senescence in degeneration. Such findings are supported by those of Kim and colleagues who also demonstrated an accumulation of senescent cells in degenerate discs. These workers reported that the telomere-based p53-p21-pRB pathway plays an important role in inducing senescence in nucleus pulposus cells (Kim et al. 2009). In addition to replicative senescence, caveolin-1, a marker of stress-induced premature senescence (SIPS), has also been identified in the disc. Its expression is correlated with increases in $p16^{INK4A}$, but not with age, suggesting a potential role for SIPS in degeneration (Heathfield et al. 2008). Interestingly, various stressors such as reactive oxygen species (Homma et al. 1994; Chen et al. 1995), mechanical loading (Martin et al. 2004) and the presence of cytokines such as IL-1 (Dai et al. 2006), all thought to play a role in the processes leading to disc degeneration, have been reported to induce SIPS, suggesting that this type of senescence may contribute to disc degeneration. However, more work is required to confirm these links.

Expression of cell senescence markers in the study by Le Maitre et al. was also shown to be correlated with changes in expression of two proteolytic enzymes, MMP 13 and ADAMTS 5, which are known to be upregulated in degenerate tissue. This finding indicated that a link may exist between senescence and induction of matrix catabolism (Le Maitre et al. 2007a). As well as inhibiting proliferation, studies in other tissues show that senescent cells adopt an altered phenotype, described as the senescence-associated secretory phenotype (SASP) (Freund et al. 2011). Such cells secrete a range of pro-ageing and catabolic factors, most notably IL-1, which are found to be elevated in the degenerate intervertebral disc. Studies on senescent chondrocytes show similar increases in cytokines, as well as MMPs (including MMP 13) and other proteolytic enzymes. Likewise, these cells exhibit a decreased response to anabolic stimuli that is characteristic of cells from degenerate discs. While more work is clearly needed to elucidate a potential causative link between senescence and disc degeneration, it is clear that the increase in senescence in degeneration and the phenotype adopted by the senescent cell suggest that this process may be important in disease progression.

Box 11.2: Elucidation of Cellular Phenotype Is Central to Improving Understanding of the Degenerative Process and Development of Novel Therapies

The adult nucleus pulposus is populated by cells routinely described as 'chondrocyte-like' based on their rounded morphology and expression of SOX-9, type II collagen and aggrecan, although controversy has long surrounded their origin and exact phenotype. However, recent microarray studies from our group and others have begun to elucidate the true phenotype of these cells. They reveal interesting gene signatures, the significance of which are yet to be fully understood. However, the expression of some of these genes (such as FOXF1) lends weight to the growing body of evidence from developmental biology studies and other sources that the adult human nucleus pulposus is populated, at least in part, by notochordally derived cells. Notochordal cells produce higher levels of proteoglycans than mature disc cells and animals which retain their notochordal cells have a gelatinous nucleus pulposus which does not show signs of degeneration. How the novel marker genes change during degeneration and their potential role in the pathogenesis of disease has yet to be fully elucidated. However, the elucidation of the nucleus pulposus phenotype has important implications for the development of novel stem cell-based regenerative medicine therapies as it allows researchers to understand the end point of differentiation and avoid generation of chondrocyte-like cells which may not produce a correctly functioning extracellular matrix.

11.8 Response to Mechanical Load

The human intervertebral disc is exposed to a number of physical stresses, including compressive loading, which predominantly affects the nucleus pulposus, and stretch, shear and torsion which mainly affect the annulus fibrosus. Using pressure-sensitive needles inserted into the nucleus pulposus, Nachemson et al. demonstrated that loads experienced within the human disc ranged from 250N when lying down to 1900N when lifting a 10 kg weight with a bent spine (Nachemson 1981). However, further analysis of this data, taking into consideration the tensile forces exerted by muscles in the back, suggests that loading may be as high as 9000N when lifting. It has been suggested that this combination of compression and flexion is responsible for disc prolapse, with over-flexion combined with moderate load being more detrimental than excessive load with moderate flexion (Hutton and Adams 1982). Following on from Nachemsons research, Wilke et al. demonstrated a

load of between 0.1 MPa when lying prone and 2.3 MPa when lifting a 20 kg weight with a flexed spine (Wilke et al. 1999); however, this study again did not take into consideration the tensile forces exerted by the musculature in the back.

The investigations mentioned above indicate that cells in the disc experience substantial loads, and predictably, these loads are thought to have a profound effect on cell behaviour. Indeed, mechanical-loading studies using in vivo animal models and in vitro cell culture techniques have demonstrated that the type, magnitude, frequency and duration of loading are paramount in determining cell response (MacLean et al. 2004, 2005; Wang et al. 2007; Wuertz et al. 2009; Korecki et al. 2009; Sowa et al. 2011). In particular, while moderate loads and low-frequency loading both promote anabolic responses, high-magnitude, high-frequency as well as sustained static loads all elicit a catabolic or anti-anabolic response in disc cells. Using human intervertebral disc cells, Neidlinger-Wilke et al. reported increased matrix protein expression (type I collagen and aggrecan), but no change in the expression of matrix-degrading enzymes (MMPs 1, 2, 3 and 13) after low-magnitude compressive loading; high-magnitude loads led to decreased matrix protein expression with increased matrix-degrading enzyme expression (mainly MMP 3) (Neidlinger-Wilke et al. 2006). Similarly, Handa et al. found that load influenced proteoglycan synthesis and MMP gene expression in human nucleus explants, with low loads promoting matrix anabolism and high loads leading to matrix catabolism (Handa et al. 1997).

While the effect of mechanical load on nondegenerate disc cells has been well documented, studies into the response of degenerate nucleus pulposus cells remain limited. To address this need, recent investigations have compared loading responses of nondegenerate and degenerate human nucleus pulposus. With physiological loads, Le Maitre et al. showed that nondegenerate nucleus pulposus cells produced an anabolic response, while degenerate cells remained unresponsive (Le Maitre et al. 2008). This finding suggests an alteration in mechanotransduction pathways between normal and degenerate cells. In support of this notion, Gilbert et al. showed differences in response to cyclic tensile strain between nondegenerate and degenerate human annulus fibrosus cells (Gilbert et al. 2010, 2011). Accordingly, while cyclic tensile strain applied at 1 Hz to nondegenerate annulus fibrosus cells resulted in a decrease in catabolic gene expression, the same strain caused a decrease in anabolic gene expression by degenerate annulus fibrosus cells. In the latter cells, there was evidence of an altered mechanotransduction pathway which appeared to be independent of cytokine involvement (Gilbert et al. 2010, 2011). While the implications of these changes require further elucidation, the profound effects of mechanical forces on cell behaviour cannot be ignored, and the possibility exists that force plays a fundamental role in the initiation or progression of disc degeneration. For a further discussion of the effects of force on cells, see Chap.7.

11.9 Clinical Implications: Relevance of Understanding the Cell Biology and Pathogenesis of Intervertebral Disc Degeneration for Development of Novel Therapeutic Agents

Current clinical interventions for back pain are predominantly aimed at relieving symptoms rather than treating the underlying disorder. In many cases, this involves regular administration of pain-relieving pharmaceuticals, e.g. non-steroidal anti-inflammatories, or application of more novel therapies, such as transcutaneous electrical nerve stimulation, physical manipulation, exercise therapy or behavioural therapies (Bogduk 2004). However, despite widespread use, the efficacy of these interventions is still questionable (van der Roer et al. 2005). In patients who are unresponsive to conservative therapies, but have identifiable imaging deficits and clinical symptoms of back pain, fusion surgery is the ultimate end point (Errico 2005). This approach removes the source of pain, but due to alterations in spinal biomechanics, it reduces mobility and can cause problems at adjacent motion segments (Hilibrand and Robbins 2004). One alternative to spinal fusion is whole intervertebral disc or nucleus pulposus transplantation, using either autologous or allogeneic tissues (Katsuura and Hukuda 1994; Luk et al. 1997). These procedures have been successfully performed, but require complicated surgery, and issues have arisen regarding loss of tissue integrity, tissue instability and immunogenicity (Alini et al. 2002). While tissue transplantation does not seem a feasible alternative, an increasing range of disc replacement devices are currently being investigated. These include devices such as the prosthetic disc nucleus (PDN) device or whole-disc replacements such as Charite and ProDisc (Jin et al. 2003; Guyer et al. 2009; Delamarter et al. 2011). Large-scale trials are ongoing with these devices, and with the whole-disc replacement, a significant reduction in pain score has been demonstrated. However, complications including device migration, extrusion and failure are all issues and studies have so far failed to show improved outcomes compared to fusion (Errico 2005; Di et al. 2005; Lindley et al. 2010). For a more detailed discussion of these devices, see Chaps. 13 and 14.

This lack of clinically successful long-term treatment for discogenic back pain has led researchers to investigate both biological modulators of disc cell function and novel cell-based tissue engineering and regenerative medicine therapies.

In line with the increase in knowledge surrounding the control of disc matrix anabolism and tissue degradation during degeneration, the utility of a number of biologically

active agents has been evaluated. These modulators include cytokine inhibitors, such as IL-1Ra, to inhibit matrix degradation (Le Maitre et al. 2006a) and growth factors, such as OP-1, to promote matrix restoration (Masuda et al. 2006). Given the growing understanding of nerve ingrowth into the degenerate disc, it should also be possible to use biological modulators to prevent or inhibit migration of nerves into the disc and thereby block transmission of discogenic pain. Of course, while biological agents provide a mechanism to inhibit the progression of early-stage degeneration, they may not be sufficient to regenerate tissue at later stages of degeneration. At this late stage, pain is the driver behind an individual seeking clinical help, and thus, identification of a suitable cohort of early-stage patients may be difficult. However, a clearer understanding of genetic predisposition may enable individuals to be screened and interventions targeted prior to the development of symptomatic back pain.

The other area where biological modulators may be beneficial is in combination with cell-based tissue engineering therapies, where they may be used to stimulate cell differentiation or matrix formation. For intervertebral disc tissue engineering, the use of nucleus pulposus cells would initially appear to be the obvious choice, with disc cell reimplantation showing promising results in both animal models and small-scale human safety trials (Meisel et al. 2006). However, the alterations in disc cell phenotype and function during degeneration raises questions about the applicability of using autologous cells, isolated from degenerate discs, for such therapies. Problems also surround the acquisition of autologous cells from discs adjacent to the degenerate level, as the local damage caused by removing tissue from those regions has been shown to lead to degeneration at an accelerated rate (Nomura et al. 2001). The use of allogeneic cells from young, healthy donors also poses immunogenicity risks. For these reasons, the focus has shifted to the use of autologous adult stem cells, derived from either bone marrow or adipose tissue, which have been shown to be capable of differentiating into nucleus pulposus-like cells and producing an nucleus pulposus-like extracellular matrix both in vitro and in vivo (Sakai et al. 2005; Richardson et al. 2006a, b, 2008a; Box 11.2). The potential value of these therapies is that they may be able to regenerate disc tissue and restore long-term functionality. However, for the therapy to be successful, it is important to take into account the environment into which these cells will be implanted. Thus, these studies must be conducted under conditions which closely mimic the complex microenvironment of the degenerate disc. This microenvironment includes increased levels of catabolic cytokines, with low levels of nutrients and a low pH, all factors which are known to affect cell function and may have a profound effect on the ability of stem cells to survive, differentiate or secrete matrix.

To aid cell survival and function following implantation, advanced biomaterials are required which can be implanted using minimally invasive procedures. These materials must be deformable and able to withstand the loads experienced by the spine; they must be able to support or promote cell survival, differentiation and matrix formation; and finally, they can biodegrade over a suitable timescale to non-toxic by-products. At present, no 'ideal' biomaterials exist, but the field is developing rapidly and cell-based regenerative medicine therapies appear likely to revolutionise the treatment of discogenic back pain over the coming decades.

11.10 Summary of Critical Concepts Discussed in the Chapter

- Disc degeneration is a complex, multifactorial process, in which disc cells themselves play a fundamental role. While there are strong genetic predeterminants, a clear predisposition is difficult to detect.
- During degeneration, there is a cell-driven loss of proteoglycans from the extracellular matrix, which results in gross morphological, biological and biomechanical changes within the spine and the development of clinical back pain.
- Members of the MMP and ADAMTS families are responsible for breakdown of the extracellular matrix, including an imbalance between ADAMTSs and TIMP that could lead to aggrecan degradation.
- In the disease state, the loss of aggrecan, with the shift to versican production, reduces the disc water content and the shift to collagen type I production results in a more fibrous tissue, less capable of withstanding load.
- Expression levels of NGF and BDNF are increased in individuals with symptomatic disc degeneration. The disc cells express the high-affinity NGF and BDNF receptors and the low-affinity NGF/BDNF receptor $p75^{NTR}$, as well as SP, suggesting autocrine signalling.
- Interplay between catabolic cytokines and neurotrophins, neurotrophin receptors and chemorepellant molecules may guide nerve ingrowth during degeneration.
- The predominant catabolic cytokines are IL-1 and TNF-α. During degeneration, interleukin family members, interferon gamma (IFN-γ), TNF-α, PGE2 and NOx are increased.
- TGF-β, along with BMPs 2 and 14, can stimulate MSC differentiation towards nucleus pulposus-like cells in vitro.
- A reduction in essential nutrients drives degeneration. With tissue hypoxia, an increase in lactate production reduces the pH, which in turn reduces matrix synthesis and can cause cell death. The effects of low oxygen and low pH may be cumulative and promote disc cell death.

- During 3 and 10 years of age, identifiable notochordal cells disappear, and the highly hydrated, gelatinous extracellular matrix converts to a more fibrous, cartilaginous nucleus pulposus.
- It is probable that apoptosis plays a critical role in the pathogenesis of disc disease. Autophagic changes have also been identified in the degenerate disc. While autophagy can lead to cell death, it can also maintain cell viability during periods of stress.
- Senescent cells are present in the disc, adopt a senescence-associated secretory phenotype and secrete pro-ageing and catabolic factors.
- Disc loading has a profound effect on cell behaviour. Moderate loads and low-frequency loading promote anabolic responses; high-magnitude, high-frequency and static loads elicit a catabolic response.

The increasing understanding of the processes involved in degeneration is leading to the development of novel strategies which have the potential to revolutionise medical intervention in discogenic back pain.

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