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Degeneration and regeneration of the intervertebral disc: lessons from development

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Degeneration of the intervertebral discs, a process characterized by a cascade of cellular, biochemical, structural and functional changes, is strongly implicated as a cause of low back pain. Current treatment strategies for disc degeneration typically address the symptoms of low back pain without treating the underlying cause or restoring mechanical function. A more in-depth understanding of disc degeneration, as well as opportunities for therapeutic intervention, can be obtained by considering aspects of intervertebral disc development. Development of the intervertebral disc involves the coalescence of several different cell types through highly orchestrated and complex molecular interactions. The resulting structures must function synergistically in an environment that is subjected to continuous mechanical perturbation throughout the life of an individual. Early postnatal changes, including altered cellularity, vascular regression and altered extracellular matrix composition, might set the disc on a slow course towards symptomatic degeneration. In this Perspective, we review the pathogenesis and treatment of intervertebral disc degeneration in the context of disc development. Within this scope, we examine how model systems have advanced our understanding of embryonic morphogenesis and associated molecular signaling pathways, in addition to the postnatal changes to the cellular, nutritional and mechanical microenvironment. We also discuss the current status of biological therapeutic strategies that promote disc regeneration and repair, and how lessons from development might provide clues for their refinement.

Introduction

Low back pain affects up to 85% of people at some point during their lives, resulting in healthcare and related costs in the United States of $100 billion every year (Andersson, 1999; Katz, 2006). Degeneration of the intervertebral discs is strongly implicated as a cause of low back pain (Bogduk, 1991; Freemont, 2009). The intervertebral discs are partially movable joints that connect each of the vertebral bodies in the spine, functioning both to transfer loads and impart mobility. The etiology of disc degeneration has proven challenging to characterize because it is poorly defined and its progression is closely linked to aging (Adams and Roughley, 2006; Urban and Roberts, 2003). Disc degeneration is perhaps best defined as a cascade that begins with changes to the cellular microenvironment within the substructures of the disc that progresses over decades to structural breakdown and functional impairment (Freemont, 2009; Urban and Roberts, 2003).

Current treatments for discogenic low back pain are predominantly conservative, involving, for example, physiotherapy and anti-inflammatory medications (Mirza and Deyo, 2007). In cases in which surgical intervention is warranted, the current gold standard is spinal fusion (Mirza and Deyo, 2007); however, fusion seeks only to alleviate painful symptoms without restoring disc mechanics or structure, recurrent episodes of pain are common and adjacent levels of the spine can experience accelerated degeneration requiring additional surgery (Ghiselli et al., 2004; Hanley et al., 2010). More recently, disc arthroplasty (artificial disc replacement) has been used to restore mobility; however, these implants do not recapitulate the mechanical function of the native joint, are subject to wear and failure, and resection is a complex surgical procedure (Hanley et al., 2010). There is, therefore, a strong need for therapies that both alleviate painful symptoms and restore disc structure and mechanical function by directly addressing the underlying biological causes of disc degeneration.

Although disc degeneration is not commonly present until adulthood (Miller et al., 1998), changes to the cellular microenvironment of the disc begin within just a few years of birth (Boos et al., 2002). Developmentally, the disc is a unique structure formed from cells of at least two disparate embryonic origins: the notochord and the somites. These lineages give rise to a tissue that is complex and specialized in terms of its microstructure, mechanical function and cell types. In this Perspective, we begin by providing a short overview of disc degeneration and current treatment strategies. We then provide a detailed review of embryonic development of the disc and the subsequent postnatal changes that precede and potentially predispose the disc to clinically significant degeneration later in life. We conclude with a synergistic discussion, examining how an understanding of the mechanisms that underlie development might influence therapeutic strategies

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for repair and regeneration of degenerate discs, and suggest directions for future research.

Structure and function of the intervertebral disc

The intervertebral disc consists of multiple, structurally distinct anatomical regions (Fig. 1). The central nucleus pulposus (NP) contains large quantities of the proteoglycan aggrecan, which aggregates along chains of hyaluronan (Urban, 1996). The glycosaminoglycan side chains of these proteoglycans carry a fixed negative charge and generate an osmotic swelling pressure within an irregular meshwork of collagen II fibrils. The NP is contained peripherally by the annulus fibrosus (AF), which has a heterogeneous composition and architecture (Humzah and Soames, 1988). The highly organized outer regions of the AF consist of distinct lamellae, which are composed of bundles of collagen I fibers oriented at oblique angles that alternate within each consecutive lamella to form an angle-ply structure (Cassidy et al., 1989; Marchand and Ahmed, 1990). In the inner AF, there is a transition to collagen II that, together with increasing proteoglycan concentration, gives rise to a less fibrous and less organized structure (Humzah and Soames, 1988). Two thin endplates of hyaline cartilage extend superiorly and inferiorly over the inner AF and NP to interface with the vertebral bodies, and function to regulate nutrient diffusion between the disc and the vertebral bodies (Rajasekaran et al., 2004; Urban et al., 2004). In the outer regions of the AF, collagen fibers anchor directly into the vertebral bone.

The foremost function of the intervertebral disc is mechanical: it transfers loads, dissipates energy and facilitates joint mobility. The NP and AF structures act synergistically to distribute and transmit loads between the vertebral bodies (Heuer et al., 2008; Johannessen et al., 2006; O'Connell et al., 2007a). When the disc is compressed, hydrostatic pressure is generated within the NP, which is constrained peripherally by the AF, generating tensile circumferential stresses within the lamellar structure (Heuer et al., 2008; O'Connell et al., 2007a). Compressive loads are also supported directly by the inner AF, which is rich in proteoglycans (Roughley et al., 2006; Vresilovic et al., 2006). The angle-ply structure and nonlinear properties of the AF facilitate both joint mobility and stability in multiple modalities, including bending and rotation, and combinations thereof (Guerin and Elliott, 2007; Heuer et al., 2008; Schmidt et al., 2007).

The adult disc is almost completely avascular (Nerlich et al., 2007), so resident cells must survive and function in an environment that is low in nutrients and oxygen (Urban et al., 2004). Cell density in the adult disc is very low, averaging 9000 cells/mm^3 in the AF and 4000 cells/mm^3 in the NP. These values are considerably less than other cartilaginous tissues (Maroudas et al., 1975). Cells in the outer AF are fusiform fibroblast-like cells, whereas those in the inner AF are more rounded (Bruehlmann et al., 2002). Cells in the NP begin as large vacuolated cells that resemble those of the embryonic notochord; however, early in postnatal life there is a transition to smaller, less metabolically active cells (Urban et al., 2000). The potential significance of this transition in NP cell phenotype will be a focus of this Perspective.

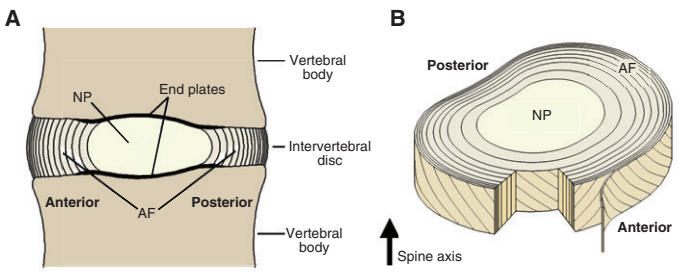

Fig. 1. Schematic representations of the adult intervertebral disc. (A) Mid-sagittal cross-section showing anatomical regions. (B) Three-dimensional view illustrating AF lamellar structure.

Pathogenesis of intervertebral disc degeneration

With advancing age comes pronounced changes in the composition of the disc extracellular matrix (Antoniou et al., 1996; Roughley, 2004). Decreasing aggrecan content in the NP leads to reduced hydration (Buckwalter, 1995), leading in turn to impaired mechanical function (Boxberger et al., 2006; Costi et al., 2008). A less hydrated, more fibrous NP is unable to evenly distribute compressive forces between the vertebral bodies. The forces are instead transferred non-uniformly to the surrounding AF (Adams et al., 1996), which can result in altered AF mechanical properties (Acaroglu et al., 1995; O'Connell et al., 2009) and progressive structural deterioration, including the formation of circumferential and radial tears (Vernon-Roberts, 1988). On occasion, radial tears can progress to a posterior radial bulge or herniation of NP material (Vernon-Roberts, 1988), resulting in painful symptoms. Decreased disc height is also commonly associated with advanced disc degeneration (Videman et al., 2003) and results in painful compression of surrounding structures. Examples of discs with advancing degrees of degeneration, as visualized by magnetic resonance imaging, are shown in Fig. 2.

Multiple interdependent factors, including altered mechanical loading (Stokes and Iatridis, 2004), reduced nutrient supply (Urban et al., 2004) and hereditary factors (Battie and Videman, 2006), have been implicated in the initiation and progression of the degenerative cascade. Changes to disc extracellular matrix composition with age are attributable to alterations in function and increased death of the cells that make up the disc (Zhao et al., 2007). The cellular microenvironment of the disc becomes progressively more hostile, and is characterized by upregulated levels of proinflammatory cytokines and associated catabolic enzymes (Le Maitre et al., 2007). This is in part due to a reduction in the diffusion of nutrients through the endplates that accompanies thinning and calcification; the reasons for these endplate changes are not well understood (Rajasekaran et al., 2004; Urban et al., 2004). Mechanical loading might also play a direct role in the progression of disc degeneration. Cell survival and matrix synthesis are both sensitive to compressive stress (Maclean et al., 2004; Walsh and Lotz, 2004). Although some mechanical stimulation is necessary to induce nutrient diffusion and to promote matrix synthesis, excessive loading can result in localized tissue injury that is slow to repair and alters strain distribution throughout the extracellular matrix of the entire disc (Stokes and Iatridis, 2004). Finally, hereditary factors also play a role in an individual's susceptibility to disc degeneration (Battie and Videman, 2006). Twin studies suggest that genetics predispose individuals to disc degeneration (Sambrook et al., 1999). Population studies for candidate genes and genome-wide assays are advancing this idea, although the fact that disc degeneration is a multi-factorial

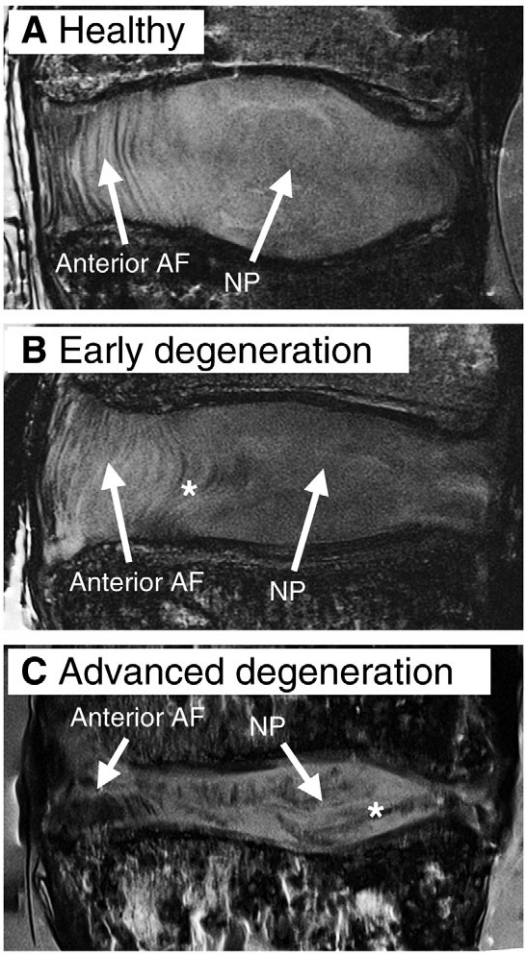

Fig. 2. Magnetic resonance images illustrating different stages of human lumbar disc degeneration. (A) A healthy disc exhibiting distinct AF lamellae (AF) and central NP region (NP). (B) A disc exhibiting early stages of degeneration, including moderate height reduction, decreased NP signal intensity and inward bulging of AF lamellae (*). (C) A disc exhibiting advanced stages of degeneration, including severely reduced height, large fissure (*) and generalized structural deterioration. Images obtained using 7T Siemens scanner and a turbo spin echo sequence at 200 μm isotropic voxel resolution.

process and because large sample sizes are needed for such studies, genetic analyses have been challenging (Chan et al., 2006). Additionally, undertaking gene analyses (PCR or microarray) for thousands of individuals becomes prohibitively expensive.

Models of intervertebral disc degeneration

The development and application of model systems in which to study the pathogenesis of disc degeneration and evaluate associated treatments has proved extremely challenging (Alini et al., 2008). Reasons for this include the slow progression of the condition, multifactorial underlying causes and a poor understanding of the circumstances under which degenerative changes are associated with painful symptoms. Nevertheless, the scarce availability of primary human degenerate disc tissue, and the almost non-existence of healthy tissue for comparison in in vitro studies, means that model systems, despite their limitations, are indispensable for investigating the molecular and cellular pathways that maintain healthy discs and that characterize the degenerative cascade. Indeed, the discs of both large and small animals possess anatomical and biomechanical traits that make them suitable models for studying the human condition (O'Connell et al., 2007b; Beckstein et al., 2008). Techniques for initiating degenerative changes in such model systems include AF injury (Elliott et al., 2008; Yoon et al., 2008), mechanical overload (Iatridis et al., 1999; Kroeber et al., 2002) and enzymatic treatment to reduce NP glycosaminoglycan content (Boxberger et al., 2008; Hoogendoorn et al., 2007). There are also a few animals that develop disc degeneration spontaneously, such as the sand rat and chondrodystrophoid dog (Gruber et al., 2002; Hansen, 1952).

Current treatments for disc degeneration

The broad objectives of any treatments for disc degeneration should be both to alleviate painful symptoms and to restore mechanical function. Biological treatment strategies, when appropriately targeted, have the potential to effectively satisfy both objectives. Depending on the stage of degeneration (Fig. 2) during which treatment strategies are designed to act, they can be classified as regenerative or reparative. In general, regenerative strategies, such as cell, gene and protein therapy, are more amenable to early-stage degeneration that is localized to the NP (Fig. 2B) (Leung et al., 2006; Masuda, 2008; Sakai, 2008; Sobajima et al., 2004; Wallach et al., 2003). Repair strategies are more appropriate for more advanced stages of degeneration that are characterized by structural degradation of both the NP and AF (Fig. 2C) (Kandel et al., 2008; Nerurkar et al., 2010; O'Halloran and Pandit, 2007). In this section we provide a brief overview of current strategies for disc regeneration and repair.

Regeneration

Regenerative strategies for the treatment of disc degeneration are focused on reviving or healing extant disc tissue. This can be done either by altering the phenotype of cells native to the ailing disc or by introducing new cell populations. Injection of growth factors such as bone morphogenetic protein 7 (BMP-7), transforming growth factor-β (TGFβ), growth/differentiation factor 5 (GDF-5) and others into the disc has been widely studied as a means to stimulate extracellular matrix production and cell proliferation (Masuda, 2008). In certain animal models of disc degeneration, treatments have successfully diminished or even reversed degeneration-like characteristics (Masuda, 2008). However, the translation of such treatments to human application and clinical use is hampered by the inability to accurately recreate the progressive, life-long degenerative transformation of the disc in an animal model (Alini et al., 2008). Moreover, the potential success of anabolic factors injected directly into the disc might be limited, both owing to the short biological half-life of the factors and their rapid diffusion away from the delivery site. Alternatively, cell populations within the disc can be manipulated through gene therapy approaches, which involve the delivery of genes into cells through viral-vector-mediated gene transfer (Sobajima et al., 2004). Finally, a more recent regenerative approach under investigation is cell therapy, whereby cells are delivered locally to the degenerated disc. The purpose of these cells is to either provide signaling cues that ameliorate the effects of disc degeneration, or adopt and/or

maintain disc-like phenotypes themselves, producing extracellular matrix intended to re-establish healthy disc function (Leung et al., 2006; Sakai, 2008).

Repair

Reparative strategies are focused on either augmenting or replacing degenerate disc tissue to re-establish healthy disc function. Although there are several non-biological reparative methods available, a recent focus in this area is on tissue engineering. The appeal of tissue engineering strategies is that, unlike non-biological materials that can wear with time, cell-generated tissues retain their capacity for remodeling and growth. The prevailing paradigm of tissue engineering is that cells on a biomaterial substrate or scaffold can be coaxed into forming new tissue when the appropriate stimuli (biological or physical) are provided. Hydrogels such as alginate-, collagen- and hyaluronan-based gels, among others, have been shown to support the survival of mature NP cells and to be conducive to matrix deposition (O'Halloran and Pandit, 2007). Although NP tissue engineering has been a particular focus over the years, interest has more recently turned to the AF and to whole disc composite tissues (Bowles et al., 2010; Mizuno et al., 2006; Nerurkar et al., 2010; Nesti et al., 2008). Most of these studies have used either disc cells or mesenchymal stem cells. Although experiments involving in vitro formation of disc-like structures have been used to make significant advances, important challenges remain to be addressed, including translation of these technologies to large animal models for pre-clinical trials and meeting in vivo nutritional requirements.

Disc development: embryogenesis and postnatal growth

In contrast to the challenges encountered in establishing model systems that accurately recapitulate the complex cascade that leads to symptomatic disc degeneration, model systems have recently facilitated a rapid expansion of our understanding of intervertebral disc development. For example, mice have been used to fate map cell populations, and other small and large in vivo models have been used to study structural maturation and extracellular matrix patterning. In addition, many in vitro models have been used to study microenvironmental mediators of cell phenotype and matrix synthesis. These model systems have enabled the identification of growth factors that stimulate extracellular matrix synthesis during embryonic tissue formation (Pelton et al., 1990; Dahia et al., 2009; Baffi et al., 2006; Baffi et al., 2004; Sohn et al., 2010), transcriptional programs involved in cell differentiation and matrix patterning (Peters et al., 1999; DiPaola et al., 2005; Wallin et al., 1994; Barrionuevo et al., 2006; Smits and Lefebvre, 2003), and the unique gene expression signatures that identify the resident cell phenotypes (Minogue et al., 2010; Chen et al., 2006). Understanding how these factors function during normal disc development could contribute to the successful advancement of regenerative and reparative treatment strategies for disc degeneration. Furthermore, model systems have been used to uncover multiple postnatal changes within the disc microenvironment, including a transition in cell phenotype (Peacock, 1952; Trout et al., 1982a; Hunter et al., 2004), vascular regression (Nerlich et al., 2007) and altered matrix synthesis (Cappello et al., 2006; Aguiar et al., 1999; Erwin et al., 2006; Erwin and Inman, 2006), which, by altering the mechanical and nutritional microenvironment, might together predispose the disc to degenerative changes later in life. Within this context, in the following sections we review in detail the embryonic formation of the disc tissue structures, the associated molecular signaling pathways and important aspects of subsequent postnatal growth.

Embryogenesis

Embryonic development of the vertebral column centers on the notochord, a rod-like mesoderm-derived structure (Fleming et al., 2001; Stemple, 2005). For development of the intervertebral disc, the notochord is important both as a signaling center that mediates cell migration, differentiation and survival, and as the structure that physically gives rise to the NP (Choi et al., 2008; Peacock, 1951; Walmsley, 1953). Embryonic morphogenesis of the disc, as well as key molecules implicated in this process, are illustrated schematically in Fig. 3.

The AF and NP regions of the disc arise concurrently along distinct developmental pathways. At approximately 30 days fetal gestation in the human (12 days in the mouse), cells of the sclerotome migrate medially from pairs of paraxial somites (Fig. 3A) to condense around the notochord (Fig. 3B) (Hunter et al., 2003a; Peacock, 1951). This condensation adopts a metameric pattern of more condensed and less condensed regions (Fig. 3C), which later give rise to the AF and vertebral bodies, respectively (Aszodi et al., 1998). Cells in the future AF region adopt a fibroblastic morphology. These cells align and orient to form the template for matrix deposition that later defines the AF angle-ply lamellar structure (Fig. 3E) (Rufai et al., 1995). AF cell organization is mediated by cytoskeletal actin filaments. Stress fibers form within these cells with an alignment that presages the mature collagen organization of the AF (Hayes et al., 1999).

Concurrently with AF morphogenesis, the notochord contracts within the forming vertebral body rudiments while simultaneously expanding within the intervertebral regions to form the NP (Aszodi et al., 1998; Pazzaglia et al., 1989; Peacock, 1951). This process of notochord 'involution' is illustrated schematically in Fig. 3D, and histologically in Fig. 4. Biomechanical forces have been proposed to play a role in this transformation: swelling of the pre-vertebral chondrogenic condensations might constrict the notochord in these regions, inducing notochord cell migration towards the intervertebral anlagen (Aszodi et al., 1998). This hypothesis is supported by observations in collagen-II-deficient mice, in which vertebral body formation is impaired and the notochord persists as a continuous rod-like structure. The alternating regions of notochordal narrowing and expansion that occur during normal morphogenesis are absent and, consequently, no NP is formed (Aszodi et al., 1998).

Molecular signaling

Disc embryogenesis occurs in response to a coordinated series of molecular signals that originate from the cells of the notochord and the floor plate of the neural tube (Placzek, 1995). Sonic hedgehog (Shh) is a signaling molecule that performs diverse roles in regulating skeletal morphogenesis by providing positional information and directing cell differentiation (Ehlen et al., 2006; McMahon et al., 2003). Patterning of the somites is regulated by opposing gradients of Shh and Wnt signaling, with Shh being specifically responsible for definition of the sclerotome (Ehlen et

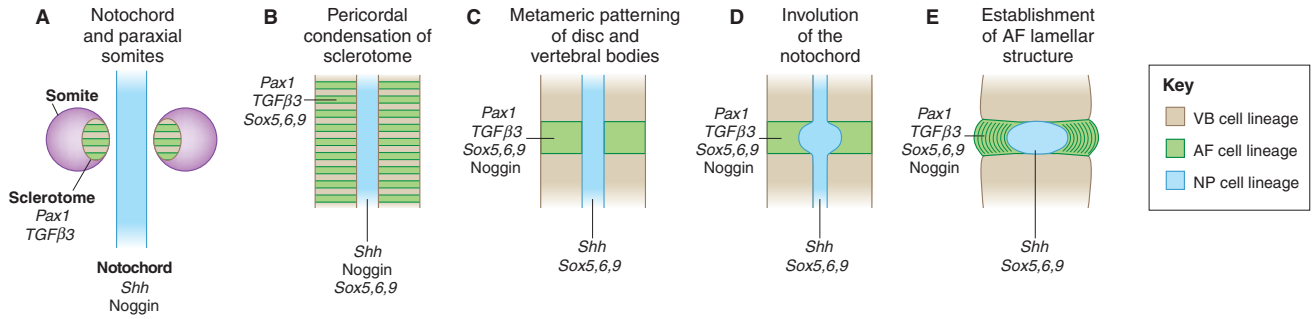

Fig. 3. Schematic representation of embryonic morphogenesis of the mammalian intervertebral disc. Colors represent origins and fates of cell populations. Also indicated are key morphogens and transcriptional regulators implicated in the growth and differentiation of the disc structures at each developmental stage. (A) The notochord adjacent to pairs of paraxial somites, which contain sclerotome cells. (B) Sclerotome cells condense around the notochord. (C) Cells adopt a metameric pattern of more condensed (green) and less condensed (brown) regions that give rise to the disc and vertebral bodies, respectively. (D) The notochord contracts within the vertebral body rudiments and expands within the future intervertebral disc to form the NP. (E) Basic structures of the disc are established, and AF cells adopt orientations and alignments that form the template for the lamellar structure. VB, vertebral body.

al., 2006). Shh operates synergistically with noggin, a BMP antagonist, during induction of the sclerotome (McMahon et al., 1998). Noggin is initially expressed by cells of the notochord (McMahon et al., 1998) before becoming localized to the developing AF where it remains until birth, potentially acting to block BMP signaling that originates from the vertebral bodies (DiPaola et al., 2005).

Pax genes encode transcription factors that regulate proliferation, differentiation, apoptosis, cell migration and stem cell maintenance. In particular, Pax expression is important for specifying and maintaining tissue boundaries (Frost et al., 2008; Wallin et al., 1994), and as such might be responsible for delineating the more and less condensed regions of cells that will give rise to the discs and vertebral bodies, respectively (Smith and Tuan, 1994). Of the Pax gene family, *Pax1* and *Pax9* specifically have been implicated in the development of the intervertebral disc (Peters et al., 1999). In their absence, both intervertebral discs and vertebral bodies fail to develop; in their place forms an irregular, cartilaginous rod that is interrupted by ventral extensions of the neural arches (Peters et al., 1999). *Pax1* expression in the sclerotome is mediated by Shh and noggin signaling that originates from the notochord (Fan and Tessier-Lavigne, 1994; Furumoto et al., 1999; McMahon et al., 1998). Prior to formation of the disc and vertebral body anlagens, almost all sclerotome cells express *Pax1*. Following disc formation, *Pax1* is localized solely to the disc anlagen (the precursor of the AF) surrounding the notochord as it transforms into the NP (DiPaola et al., 2005; Wallin et al., 1994). There is also evidence that *Pax1* mediates signaling from the sclerotome back to the notochord: in *Pax1* mutants, notochords are enlarged and have increased rates of cell proliferation (Wallin et al., 1994). As such, it has been

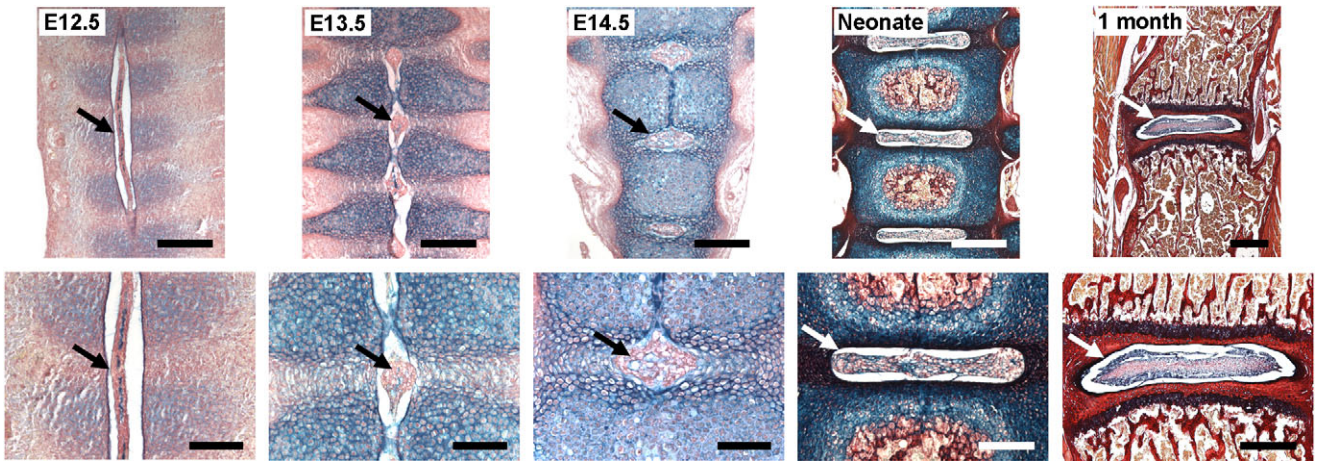

Fig. 4. Stages of notochord transformation into the NP in the mouse embryo. Embryos were stained with Alcian Blue (which marks glycosaminoglycans) and Picrosirius Red (which marks collagen). At embryonic day (E)12.5, the notochord (arrow) runs uninterrupted the length of the vertebral column. Sclerotome cells have condensed perichordially, and metameric patterning of future disc and vertebral body condensations is apparent. At E13.5, the notochord has begun to contract within the vertebral body regions and expand within the disc regions (arrow). At E14.5, the notochord has virtually disappeared from the vertebral bodies, persisting solely in the locations of the future NPs (arrow). In the neonate, lateral expansion of the NP has occurred (arrow) and primary ossification centers are present in the vertebral bodies. At postnatal age 1 month, vertebral bodies are fully ossified and the NP (arrow) contains a glycosaminoglycan-rich extracellular matrix surrounded by the collagenous AF. Scale bars: E12.5, E13.5 and E14.5: 200 μm (top) and 100 μm (bottom); neonate: 400 μm (top) and 200 μm (bottom); 1 month: 500 μm (top) and 400 μm (bottom).

suggested that *Pax1*, as a mediator of notochordal cell proliferation, regulates contraction and expansion of the notochord as it transforms into the NP.

Members of the Sox (Sry-related high-mobility-group box) gene family perform diverse functions during development (Schepers et al., 2002; Wegner, 2009). Among these, *Sox5, Sox6* and *Sox9* are specifically implicated in chondrogenesis (Schepers et al., 2002), and all three seem to be important for disc development. *Sox5* and *Sox6* are expressed in both sclerotome-derived and notochordal cells (Smits and Lefebvre, 2003). In mice lacking both *Sox5* and *Sox6*, the notochordal sheath fails to form, probably due to associated downregulation of genes encoding cartilage matrix components such as collagen II and aggrecan (Smits and Lefebvre, 2003). The absence of this sheath results in downstream consequences, including: notochordal cell apoptosis during perichordal condensation of the sclerotome; aberrant removal of notochordal cells from the intervertebral regions compared with the vertebral regions; and, ultimately, failure of the NP to form. Additionally, there is delayed and impaired differentiation of cartilage in the inner AF (Smits and Lefebvre, 2003). *Sox9* is expressed in all primordial cartilage during embryogenesis, coincident with collagen II expression (Bi et al., 1999), including in the sclerotome and notochord (Barrionuevo et al., 2006). Although mice lacking the *Sox9* gene initially develop a notochord, it later disintegrates (Barrionuevo et al., 2006), perhaps due to lack of matrix formation within the notochordal sheath. Absence of the notochord and associated signaling henceforth impairs development of the sclerotome (Barrionuevo et al., 2006).

TGFβ signaling has also been implicated in the metameric patterning of the discs and vertebral bodies. TGFβ signaling is important for regulating cell proliferation and differentiation, and extracellular matrix production during skeletal development, with different TGFβ isoforms exhibiting tissue-specific expression profiles (Millan et al., 1991). TGFβ3 has been shown to be strongly localized to the perichordal condensations that give rise to the AF and vertebral bodies. As condensation advances, this expression pattern becomes localized to the disc anlagen, showing clear demarcation with respect to the adjacent vertebral bodies (Pelton et al., 1990). Conditional deletion of *TGFβ* receptor 2 (*TGFβ-r2*) in cells expressing collagen II results in incomplete formation of the NP and inner AF, and partial mineralization of the disc region. Cells in the AF exhibit a more chondrogenic phenotype and are poorly organized. This suggests that *TGFβ-r2* plays a role both in defining the boundaries between the disc and the vertebral bodies, and in defining and maintaining AF cell phenotype and organization (Baffi et al., 2006; Baffi et al., 2004; Sohn et al., 2010). There is evidence that disc cells continue to respond to TGFβ signaling during postnatal growth (Dahia et al., 2009).

Postnatal vascular regression

Poor nutritional supply to the cells of the avascular intervertebral disc has been implicated in the pathogenesis of degeneration with age. In humans, during the early postnatal years, blood vessels that have penetrated the AF and cartilage endplates from as early as 35 weeks gestation begin to recede, eventually leaving the disc as a completely avascular structure (Nerlich et al., 2007; Urban and Roberts, 1995). Possible reasons for vascular regression include decreased nutrient requirements following the initial period of rapid growth or, more likely, the inability of circulatory pressure to compete with large physiological stresses in the surrounding extracellular matrix. Following regression, there is evidence that the pathways followed by blood vessels are never fully remodeled into the surrounding microarchitecture, and that they remain as translamellar bridging elements (Melrose et al., 2008; Smith et al., 2010); this idea is consistent with the fact that the disc has a poor ability to remodel and repair (Buckwalter, 1995). It is likely that these vascular remnants influence AF mechanics in response to radial and shear deformations; however, it is unclear whether this influence would assist or impair the function of the disc.

Changing NP cell phenotype

As discussed above, altered cellularity is a hallmark of disc degeneration. In fact, changes to the resident cell populations, and specifically to those of the NP, begin to occur very early in life. Soon after birth, the cells that populate the NP exhibit morphological characteristics that are similar to the cells that populate its notochord precursor (Peacock, 1952; Wolfe et al., 1965; Trout et al., 1982a; Trout et al., 1982b). Because of these similarities, these cells have classically been referred to as 'notochordal-like' in reference to their putative origin. Notochordal-like cells in the postnatal NP are large (30-40 μm in diameter), frequently appear in clusters and possess actin-filament-bounded intracellular vacuoles that occupy more than 25% of the cell area (Hunter et al., 2003b; Hunter et al., 2004). These vacuoles are a trait common to cells of the embryonic notochord; here, they seem to contain secreted glycosaminoglycans, enabling the cells to generate an osmotic swelling pressure that contributes to the elongation and straightening of the notochord (Adams et al., 1990).

In the first 10 years following birth, the number of notochordal-like cells declines, and eventually these cells disappear (Peacock, 1952; Trout et al., 1982a; Hunter et al., 2004). Concurrently, a second population of smaller cells appears, classically referred to as 'chondrocyte-like' owing to apparent phenotypic and morphological similarities with cartilage chondrocytes (Urban and Roberts, 1995). Hereafter, these cells will be referred to as 'mature NP cells' because they should not be confused with cartilage chondrocytes. In comparison with notochordal-like cells, the mature NP cells are smaller (~10 μm in diameter) and lack intracellular vacuoles (Hunter et al., 2004).

The rate at which the transition between cell types occurs varies by species: in humans, notochordal-like NP cells persist only for the first few years of life and have long disappeared by skeletal maturity. In other species, such as rats, these cells persist well beyond skeletal maturity (Hunter et al., 2003a; Hunter et al., 2004). The origin of mature NP cells has been the subject of debate (Risbud et al., 2010; Shapiro and Risbud, 2010): whereas some studies suggest that these cells are recruited from adjacent tissues such as the endplates (Kim et al., 2003), there is a solid and growing body of experimental evidence that all cell types within the adult NP are descended directly from the embryonic notochord (Risbud et al., 2010). A recent study used tamoxifen-inducible Shh-Cre-ERT2 mice to fate map descendents of embryonic notochord cells, exploiting the fact that these cells express *Shh*, whereas those of the sclerotome do not (Choi et al., 2008). The results clearly demonstrated that all cell types resident in the adult mouse NP are descended directly from the notochord.

Notochordal-like cells and mature NP cells exhibit some gene-expression profile similarities, and also some differences. Although both cell types have been found to express the typical chondrogenic markers aggrecan, collagen II and *Sox9* to a similar extent (Chen et al., 2006; Kim et al., 2009; Sive et al., 2002), the relative expression of collagen I, as well as biglycan, decorin and lumican, which are small leucine-rich proteoglycans that are important for collagen fibrillogenesis, is higher for mature NP cells than notochordal cells (Chen et al., 2006). Expression of these factors is consistent with the extracellular matrix changes that are observed with aging and a transition to a more fibrous structure. Putative markers of mature NP cells – such as cytokeratins 8, 18 and 19, snaptosomal-associated protein 25 (SNAP-25), cadherin-2 and sclerostin domain-containing protein 1 (SOSTDC1) – are all also expressed by notochordal-like cells, in many cases at higher levels (Minogue et al., 2010). There is some evidence, however, that the overall number of cells expressing these markers decreases after 30 years of age (Weiler et al., 2010). Expression of the embryonic notochordal cell marker brachyury (T) by both notochordal-like and mature NP cells has been suggested to indicate their common notochordal lineage (Minogue et al., 2010).

The factors that underlie the transition in NP cell type are not well understood; however, changes to both the mechanical and nutritional microenvironment have been implicated. Mechanical forces within the disc increase considerably after birth, particularly with the onset of bipedal locomotion. It is possible that this additional mechanical stress induces apoptosis or chondrogenic-like differentiation of notochordal cells. Indeed, compressive loading has been shown to mediate chondrogenic differentiation of other cell types, such as mesenchymal stem cells (Huang et al., 2010). Compressive loading in a rabbit model decreased the number of notochordal-like cells and increased the number of mature NP cells (Guehring et al., 2010). Cells of the NP must survive and function in a low nutrient and low oxygen environment, which becomes progressively moreso following postnatal vascular regression. Furthermore, as a result of their higher metabolic activity, the nutritional requirements of notochordal-like cells are significantly greater than those of mature NP cells (Guehring et al., 2009). A low nutrient environment might therefore induce notochordal-like cell apoptosis or differentiation towards a cellular phenotype with a lower nutrient demand (Rastogi et al., 2009).

Matrix synthesis

A key factor in early disc degeneration is the decrease in NP proteoglycan content. Notochordal-like cells have been shown to synthesize matrix in a manner distinct from mature NP cells (Cappello et al., 2006). Proteoglycans synthesized by notochordal-like cells are evenly distributed between the inter- and pericellular regions, compared with mature NP cells, in which the majority of proteoglycans are intercellular. Additionally, the rate at which proteoglycans migrate to the intercellular regions is significantly greater for notochordal-like cells than for mature NP cells (Cappello et al., 2006).

The matrix-producing potential of notochordal-like cells in vitro seems to be enhanced in a low oxygen environment that replicates that of the native tissue (Erwin et al., 2009). Notochordal-like cells can influence the matrix-synthesis behavior of mature NP cells (Aguiar et al., 1999; Erwin et al., 2006; Erwin and Inman, 2006). Co-culture of bovine mature NP cells and canine notochordal-like cells results in significantly higher levels of proteoglycan synthesis than for either of these cell types in isolation (Aguiar et al., 1999). Canine notochordal-like cells have been shown to secrete connective tissue growth factor (CTGF) in culture (Erwin et al., 2006). Bovine mature NP cells cultured in notochordal-like cell conditioned medium exhibit increased expression of aggrecan in a dose-dependent manner, a result replicated by recombinant CTGF-supplemented medium (Erwin and Inman, 2006). Notochordal-like cells also seem to have the capacity to mediate the matrix-production characteristics of non-NP cell types. Mesenchymal stem cells cultured in media conditioned using porcine notochordal-like cells synthesize higher quantities of glycosaminoglycans than those cultured either without conditioned media or in the presence of TGFβ3 (Korecki et al., 2010).

Improving treatments for disc degeneration: lessons from development

Through identification of important growth factors and transcriptional regulators that are present during the progressive conversion from notochord and sclerotome cells to mature NP and AF cells, respectively, it might be possible to target and modulate specific genes associated with cell survival, differentiation and matrix deposition to advance biological therapies for disc degeneration. For example, the expression of *TGFβ3* in perichordal condensations of AF progenitor cells during AF morphogenesis suggests that this molecule is a promising candidate for restoration of AF architecture and function following degeneration-associated tears and fissures. Indeed, organ culture studies of rat intervertebral discs identified that TGFβ3 improved cell viability and matrix retention in vitro (Risbud et al., 2006). In addition, application of TGFβ3 to adult bovine AF cells cultured on electrospun nanofibrous polymer scaffolds resulted in robust extracellular matrix production and improved mechanical properties in vitro (Nerurkar et al., 2007). This approach was recently employed to generate nanofibrous biologic laminates that replicated the angle-ply form and mechanical function of the native AF (Nerurkar et al., 2009).

The transcription factor *Sox9*, which is associated with chondrogenic differentiation and collagen II synthesis, is expressed throughout the disc structures during embryogenesis (Barrionuevo et al., 2006) and in the newborn AF (Gruber et al., 2005). A decrease in *Sox9* expression has been associated with disc degeneration (Gruber et al., 2005), suggesting that it could also be a potential therapeutic target. Indeed, preliminary studies investigating the use of *Sox9* gene therapy support this idea: transduced cells from degenerate human discs showed enhanced collagen II production (Paul et al., 2003). These results support the future therapeutic potential of this and other transcriptional regulators, although associations between disc degeneration and altered expression of many of these factors remain to be elucidated.

Recent advances in notochordal cell biology have demonstrated that these cells, through secretion of factors such as CTGF, can enhance matrix synthesis by other cell types, and even direct mesenchymal stem cells towards an NP-cell-like phenotype (Korecki et al., 2010). Such cellular conditioning has the potential

to optimize the phenotype of cells used in both NP cell therapy and in NP tissue engineering.

As our understanding of the physical forces associated with disc development improves, it will be possible to tailor therapeutic approaches such that AF and NP tissue engineering might become distinguished from ligament and cartilage tissue engineering, respectively. At present, however, little is known about the role of these forces, either in the segmentation of the notochord and its transformation into the NP, or in the generation of an angle-ply alignment in the AF. Most NP tissue engineering endeavors have relied on entrapping cells within hydrogel scaffolds to maintain the rounded cell morphology present in vivo (O'Halloran and Pandit, 2007; Yang and Li, 2009). Additionally, motivated by the large swelling pressure in the notochord and later in the NP, studies have shown that hydrostatic pressure can modulate the phenotype and matrix production of mature NP cells when encapsulated in hydrogels (Hutton et al., 1999; Kasra et al., 2006). On the basis of the observation that cell alignment in the developing AF precedes ordered matrix deposition, some tissue engineering strategies have focused first on aligning cells, either through the use of micro-grooved channels (Johnson et al., 2006) or aligned scaffolds (Mauck et al., 2009). Furthermore, studies have demonstrated that, similarly to the process in the developing AF, alignment of AF cells results in deposition of aligned collagen as well (Johnson et al., 2006; Nerurkar et al., 2008). In the future, it might be possible to design and implement deformational loading bioreactors that are specifically tailored to recapitulate the physical environment of the developing spine.

Future directions

Despite many years of research, the etiology of discogenic low back pain remains poorly understood, and palliative therapies do not restore healthy disc structure or mechanical function. Development of the intervertebral disc involves the coalescence of different cell types under the direction of complex molecular interactions. The resulting structures must function synergistically in an environment that is subjected to continuous mechanical perturbation and in the presence of poor nutrient supply, throughout the lifetime of an individual. It is likely that early postnatal changes, including vascular regression, altered NP cell phenotype and altered extracellular matrix composition, despite representing a necessary adaptation to a changing biochemical and biomechanical microenvironment, set the disc on a slow but relentless course towards degeneration. Future work in this area should build on current understanding in order to establish the mechanisms by which postnatal changes affect disc degeneration, as well as establishing the direct consequences of altered extracellular matrix synthesis and molecular signaling on this process. Improved understanding of these factors will lay the foundation for the emergence of exciting new regenerative or reparative biological treatments for this debilitating condition.

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COMPETING INTERESTS

The authors declare no competing or financial interests.

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