Review

The lumbar intervertebral disc: From embryonic development to degeneration

Pauline Colombier\(^a, b\), Johann Clouet\(^a, b, c, f\), Olivier Hamel\(^a, b, d, g\), Laurent Lescaudron\(^a, b, e\), Jérôme Guicheux\(^a, b, h, *\)

\(^a\) INSERM UMR571, LIOAD, Groupe Skeletal Tissue Engineering and Physiopathology (STEP), 1, place Alexis-Ricordeau, 44042 Nantes, France
\(^b\) Université de Nantes, UFR Odontologie, 1, place Alexis-Ricordeau, 44042 Nantes, France
\(^c\) Université de Nantes, UFR Sciences Biologiques et Pharmaceutiques, 9, rue Bias, 44035 Nantes, France
\(^d\) Université de Nantes, UFR Médecine, 1, rue Gaston-Veil, 44035 Nantes, France
\(^e\) Université de Nantes, UFR Sciences et Techniques, 2, chemin de la Houssinière, 44300 Nantes, France
\(^f\) CHU de Nantes, Pharmacie Centrale, 85, rue Saint-Jacques, 44093 Nantes, France
\(^g\) CHU de Nantes, Service de Neurotraumatologie, 1, place Alexis-Ricordeau, 44000 Nantes, France
\(^h\) CHU de Nantes, Pôle Hospitalo-Universitaire 4 OTONN, 1, place Alexis-Ricordeau, 44000 Nantes, France

ABSTRACT

Lumbar intervertebral discs (IVDs) are prone to degeneration upon skeletal maturity. In fact, this process could explain approximately 40% of the cases of low back pain in humans. Despite the efficiency of pain-relieving treatments, the scientific community seeks to develop innovative therapeutic approaches that might limit the use of invasive surgical procedures (e.g., spine fusion and arthroplasty). As a prerequisite to the development of these strategies, we must improve our fundamental knowledge regarding IVD pathophysiology. Recently, several studies have demonstrated that there is a singular phenotype associated with Nucleus pulposus (NP) cells, which is distinct from that of articular chondrocytes. In parallel, recent studies concerning the origin and development of NP cells, as well as their role in intervertebral tissue homeostasis, have yielded new insights into the complex mechanisms involved in disc degeneration. This review summarizes our current understanding of IVD physiology and the complex cell-mediated processes that contribute to IVD degeneration. Collectively, these recent advances could inspire the scientific community to explore new biotherapeutic strategies.

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1. Introduction

Lumbar intervertebral discs (IVDs) are complex anatomical structures that are essential for the mobility of intervertebral joints. They also participate in anchoring vertebrae together and distributing the pressure that results from movement of the entire trunk. Notably, the functional roles of IVDs in absorption and load distribution are directly related to their unique structure (Fig. 1).

In most mammals, the first signs of IVD degeneration begin to appear upon skeletal maturity in the Nucleus pulposus (NP) [1]. Up until this time, two cell types populate the NP: chondrocyte-like cells and notochordal cells. It is now accepted that notochordal cells are largely responsible for maintaining homeostasis [2–5]. Thus, the loss of these cells during skeletal maturation might constitute one of the first changes that occur in the cascade of degenerative events. Although this degeneration arises during the natural aging process, pathological degeneration can also occur, which progresses in an accelerated and brutal manner. Nevertheless, in this review, we only focus on the mechanisms of development, maturation, and degeneration associated with normal aging.

2. Intervertebral disc physiology

2.1. Embryonic development

In humans, the formation of the three embryonic layers occurs in the third week of gestation. During this phenomenon, called gastrulation, epiblast cells (future ectoderm) invaginate at the Hensen’s node and colonize the mesoblastic space to form the notochord (chordal mesoderm). The development of the notochord is dependent on the expression of several genes, including Forkhead box A2 (Foxa2), Brachyury (T), and Notochord homolog (Noto). In addition, a part of maturing somites known as the sclerotome gives rise to the vertebrae, endplates, and Annulus fibrosus (AF) under the action of the Sonic hedgehog (Shh) factor and members of the transforming growth factor (TGF) family (Fig. 2). The Shh-dependent expression of Paired box 1 and 9 (Pax1/9) controls the vertebral endochondral ossification process [6], whereas TGF-β is involved in the differentiation of the sclerotome into AF cells [7].

* Corresponding author. INSERM U791, 1, place Alexis-Ricordeau, 44042 Nantes cedex 1, France. Tel.: +33 2 40 41 29 82; fax: +33 2 40 08 37 12.
E-mail address: jerome.guicheux@inserm.fr (J. Guicheux).

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Notochordal cells die by apoptosis when the sclerotome condenses and proliferates to form vertebral bodies. However, the NP is generated by their proliferation at the intervertebral level. Recent studies have demonstrated that all of the NP cells arise from the notochord [8,9].

2.2. The endplates

Like articular cartilage, the endplates are composed of subchondral bone and a thin layer of hyaline cartilage (approximately 1 mm in humans) on which the fibers of the AF anchor. Notably, these cartilage endplates (CEPs) are composed of only chondrocytes, which synthesize an extracellular matrix (ECM) that is rich in type II collagen and proteoglycans (PGs). In these plates, the ratio of PG to type II collagen is approximately 2:1, which is similar to articular cartilage, and the water content is 50–60% [10]. PGs are macromolecules that are composed of a protein body covalently linked to sulfated polysaccharide chains, called glycosaminoglycans (GAGs). These sulfated GAGs carry an overall negative charge that is responsible for retention of water molecules, efficiently allowing hydration of the extracellular matrix (ECM).

CEPs are also the site of a microscopic network of blood vessels that are responsible for nutritional intake during development and growth of IVDs [11]. Metabolites diffuse through pores present in the growth plates based on their size and charge. Only positive ions (e.g., sodium, calcium) or neutral molecules, such as glucose and oxygen, can diffuse [12].

2.3. The Annulus fibrosus

The AF is composed of fibroblasts (approximately 9000 cells/mm³), which mainly synthesize type I collagen fibers. The ECM of the AF is organized into 15–25 concentric lamellae oriented at 65° relative to the vertical plane. These lamellae are interconnected by PG aggregates and lubricin, as well as type VI collagen fibers [13,14]. Lubricin, known for its lubricant role within diarthrodial joints, is probably involved in the reduction of friction between adjacent lamellae of the AF [14].

The AF can be divided into two distinct areas: the outer AF and inner AF. The inner AF, which is also known as the transition zone, contains poorly organized ECM that is composed of type II collagen, PGs, and water. In contrast, the outer AF is highly organized and is rich in type I collagen, whereas type II collagen and PGs are virtually undetectable in this region [15]. Moreover, the outer AF has a higher resistance to tension than the inner AF. Collectively, the fibrous structure of the AF yields important mechanical properties that limit NP protrusion.

2.4. The Nucleus pulposus

The NP contains approximately 3000 cells/mm³ and is composed of several cell types embedded in a matrix that is rich in both type II collagen and PGs (PG to type II collagen ratio is 27:1). The main PG within the NP is aggrecan, which contains ~30 chains of sulfated GAGs, contributing to a negative charge that fosters the hyper-hydrated state of the NP. Notably, this water content, along with type II collagen fibers, allows the NP to be elastic and deform under stress. Notochordal and chondrocyte-like cells synthesize the matrix components of the NP. It is now accepted that these chondrocyte-like cells have a phenotype distinct from that of articular chondrocytes [16,17]. Moreover, the presence of a third cell type, displaying progenitor properties similar to those of mesenchymal stem cells (MSCs), was recently described by Sakai et al. [18]. However, considering the characteristics of notochordal cells, it is likely that these progenitor cells are actually notochordal cells. Cells of the NP are highly specialized and survive in a very hypoxic environment (1% of O₂). For this reason, the hypoxia inducible transcription factors–1 and –2 (HIF-1 and HIF-2), which are key cellular regulators of the hypoxic response, were found to be constitutively active in NP cells [19]. Indeed, Agrawal et al. have demonstrated that the promoter of the aggrecan gene responds to HIF-1. Thus, constitutive activity of HIF-1 might be partly responsible for the large production of aggrecan by NP-resident cells, independently of oxygen conditions. They also demonstrated that the expression of some glucose transporters (GLUT1 and 2) were under the control of HIF-1 [20]. Therefore, NP cells possess a unique metabolism that allows them to be functionally and constitutively adapted to their environment, which is low in oxygen and nutrients.

Recent studies have shown the importance of notochordal cells in the synthesis of functional ECM and in the survival of chondrocyte-like cells. Erwin et al. have demonstrated that notochordal cells synthesize growth factors, such as connective tissue growth factor (CTGF)/CCN2, stimulating the proliferation of chondrocyte-like cells and the synthesis of type II collagen and aggrecan [4]. It was also found that the secretome of notochordal

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Fig. 1. Schematic representation of the structure and composition of the IVD, indicating proteoglycan content as well as type I and II collagen in the NP and AF. AF: Annulus fibrosus; CEP: cartilage endplate; NP: Nucleus pulposus.

Fig. 2. Schematic representation of the resegmentation phenomenon of the sclerotome during the formation of the V and AF. AF: Annulus fibrosus; NT: notochord; SC: sclerotome; Shh: sonic hedgehog; TGFs: transforming growth factors; V: vertebra.
cells had an anti-apoptotic effect on chondrocyte-like cells [5]. In fact, the uncharacterized factors secreted from notochordal cells have the ability to inhibit the activation of the apoptotic caspases-3 and -9 and favor the expression of aggrecan and type II collagen. Furthermore, similar to HIF-1 regulation in NP cells, it was reported that oxygen tension can control the expression of CTGF/CCN2 by notochordal cells [21]. Thus, this mechanism not only controls the hypoxic response in NP cells, but also the amount of CTGF/CCN2 for maintaining PG production.

Taken together, these data highlight the fundamental role of notochordal cells in the survival and activity of chondrocyte-like cells, which subsequently maintains NP homeostasis. Therefore, the gradual disappearance of notochordal cells during skeletal maturation and aging constitutes a primary event initiating degeneration of the NP.

3. The intervertebral discs aging

So far, it has been challenging to understand the mechanisms involved in disc aging. Thus, even though tissue remodeling during aging has been relatively well described, the mechanisms contributing to age-related changes in the discs remain poorly defined.

3.1. Cell modifications

Cellular changes, which mainly involve the NP, begin to occur upon skeletal maturity. The first major alteration involves a gradual disappearance of notochordal cells, an event that might be explained by their differentiation into chondrocyte-like cells. Indeed, after birth, the NP consists mainly of notochordal cells, which differentiate progressively in chondrocyte-like cells. In fact, it was recently reported that the activation of the Wingless integration site (Wnt)/beta-catenin signaling pathway controls self-renewal and maintenance of notochordal cells during the formation and growth of IVDs [22]. Nevertheless, recent studies have also implicated this pathway in the production of enzymes that degrade ECM during disc aging in humans [23]. Thus, this apparent dichotomy in the role of the Wnt/beta-catenin signaling pathway is of primary interest with regard to disc development and degeneration.

In parallel with the disappearance of notochordal cells during aging, the chondrocytes of CEPs begin to engage their hypertrophic conversion and synthesize type X collagen. Ultimately, the ECM begins to calcify and becomes impermeable, which not only blocks the diffusion of nutrients, but also the removal of metabolites within the NP [24]. These events obstruct tissue homeostasis, primarily by inducing acidification and restricting the supply of oxygen and nutrients. The chondrocyte-like cells residing within the NP are therefore exposed to significant metabolic stress, which may induce cell death. As concerning AF cells, they present membrane mechanoreceptors and are very sensitive to mechanical stresses. It has been described that these cells undergo a massive apoptotic cell death in response to overload [25]. In this context, NP cell apoptosis could be initiated through several caspase-dependent pathways [26]. Aging can result in caspase activation by either the death receptor-mediated extrinsic pathway [27] or the intrinsic pathway, which is linked to mitochondrial activity and the endoplasmic reticulum, especially during the most advanced stages of degeneration [25,28].

Despite the fact that the NP has a low capacity for spontaneous repair, its resident cells have the potential to protect themselves from cell death through the senescence and autophagy pathways. Senescence involves cell cycle arrest at the G1 phase and entry of cells into the non-proliferative G0 phase. Two distinct senescence programs have been described, which include replicative senescence (i.e., telomere shortening during division) and senescence due to oxidative stress and/or mitochondrial defects [29]. Upon entry into the G0 phase, cells no longer respond to environmental cues (whether anabolic or catabolic), which can permit evasion of programmed death. Interestingly, it has been found that NP cells experience replicative senescence during aging [30].

The second mechanism of cell protection involves autophagy, a process that allows cells to generate their own energy under conditions of high stress, such as prolonged nutrient deficiency [31]. In the NP, it is widely accepted that nutrient intake decreases during aging. In this context, autophagic vesicles have been observed in NP-resident cells [32]. Notably, the number of autophagosomes, as well as autophagic flow, was found to increase with aging. This strongly suggests that NP cells have the potential to compensate for nutrient deprivation by generating their own energy when exposed to intense stress.

Despite these protective mechanisms utilized by NP cells during the aging process, they gradually die, resulting in significant alterations in the ECM.

3.2. Tissue modifications

Similar to cellular changes, tissue remodeling in the NP is also an early step involved in the aging process. ECM integrity within IVDs is based on a balance between anabolism and catabolism. This equilibrium, which is affected by the loss of notochordal cells, is even further disrupted by senescence and apoptosis of chondrocyte-like cells. This results in the uncontrolled synthesis of enzymes that degrade ECM components by the NP cells, including matrix metalloproteinases (MMPs) and A desintegrin and metalloproteinase with thrombospondin repeats (ADAMTS) [33]. These enzymes are capable of degrading both collagen and PGs, leading to dehydration and progressive disorganization of ECM. Under these conditions, it has been observed that the ECM takes on a grainy appearance and displays cracking and tearing. It has also been demonstrated that over time, type II collagen in the NP is replaced by type I collagen. Moreover, the anatomical border between the NP and AF becomes less defined, with the NP gradually becoming fibrous, losing its capacity to absorb and deform under stress.

Degradation of matrix components is later initiated in the AF, resulting in destabilization of its structure. During this process, the collagen fibers become thinner and more irregular, eventually leading to cracking [34].

Along with these structural changes, key players in the inflammatory response (i.e., interleukin-1 [IL-1] and tumor necrosis factor [TNF]) have been found to participate in tissue remodeling within IVDs. Indeed, these cytokines can induce the expression of genes coding for MMPs [35]. In fact, it was found that NP cells were capable of secreting IL-1, which can stimulate the synthesis of nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and brain-derived neurotrophic factor (BDNF) [36]. Secretion of these growth factors could partly explain the innervation and vasculatization observed during aging of the NP. It has also been suggested that NGF production correlates with innervation by nociceptive nerves, which might contribute to discogenic pain during pathological IVD [37].

Disc aging is a physiological process that is defined by a series of changes occurring at both cell and tissue levels (Table 1). IVDs gradually lose integrity, no longer providing their biomechanical role. However, advances in our knowledge regarding the pathophysiological degeneration of IVDs have offered new prospects for treatments. For example, several cellular processes that could be targeted in novel therapeutic strategies are now well acknowledged, such as blocking apoptosis, inhibiting inflammation, or promoting cellular protection. Repopulating the NP with functional cells might also constitute a relevant strategy [38]. In this regard,
intradiscal injection of suitable biomaterials along with adult stem cells (e.g., undifferentiated or differentiated into chondrocyte-like cells and/or notochordal cells) could represent a promising approach [39]. This type of therapy would delay the loss of homeostasis as well as the associated adverse consequences.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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