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Chapter 12

Mesenchymal Stem Cell in the Intervertebral Disc

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Abstract

Degeneration of the intervertebral disc (IVD) is a major spinal disorder that causes back pain. Nucleus pulposus (NP) in the central of IVD dehydrates and become more fibrous in the IVD degeneration. NP cells undergo apoptosis with the degeneration of extracellular matrix (ECM) components. To replenish the NP cells and core ECM, bone marrow mesenchymal stromal cells (BMSCs) have been highlighted in the regeneration of IVD degeneration. BMSCs differentiate into NP-like cells with the secretion of ECM components, which may not only replenish the number of NP cells but also stimulate NP reconstruction. This further maintains tissue homeostasis. Up to date, the disc progenitor cells (DPCs) have been identified with the characteristics of multidifferentiation and stem cell phenotype. These cells are involved in the IVD diseases and show regenerative potentials. However, the differences between the BMSCs and DPCs remain elusive, in particular, the cellular connection *in vivo*. As such, this chapter will discuss the findings of the two cell types and propose a novel concept in the understanding of the biology of IVD.

Keywords: low back pain, intervertebral disc, nucleus pulposus, progenitor cells, extracellular matrix

1. Introduction

Low back pain (LBP) is the second most common symptom in the United States. Of the US population, 85% people experience an episode of LBP at some point in their lifetime. For individuals under 45 years, LBP remains the most common cause of disability and is generally associated with a work-related injury. In 2005, an estimate of 85.9 billion dollars was spent in the related treatment of back and neck pain. The relevant statistics indicated that the healthcare expenditures increased 65% between 1997 and 2005 without evidence of improvement in health status.

2. The shielded structure and rigid environment of intervertebral disc

An intervertebral disc (IVD) is a cylindrical structure, comprising a well-hydrated central nucleus pulposus (NP), an annulus fibrosus (AF) consisting of firm and flexible collagenous lamellae which surrounds the NP, and cartilaginous endplates forming an interface between the disc and adjacent vertebrae (**Figure 1**).

During the development of mammals, the vertebral column derives from the aggregation of mesenchymal cells around the notochord [1]. Following segmentation, motion segments emerge with large number of cells accumulating in the developing AF but fewer cells in the rapidly growing vertebral bodies. The cells in the AF become highly orientated, laying down the disc matrix in a similar orientation to form the concentric annular lamellar structure [1, 2]. Notochordal cells are named by their typical morphology of the notochord (physaliferous), a population of large cells with small and densely packed nuclei and cytoplasmic matrix vacuoles in human nucleus pulposus, are presumed remnants of the embryonic notochord that guided formation of the spine and the nuclei polposi [1]. The abundance of notochordal cells within NP declines with age at a rapid rate which varies among different species; where, by early adulthood in the human and species including that of chondrodystrophoid dog, nucleus

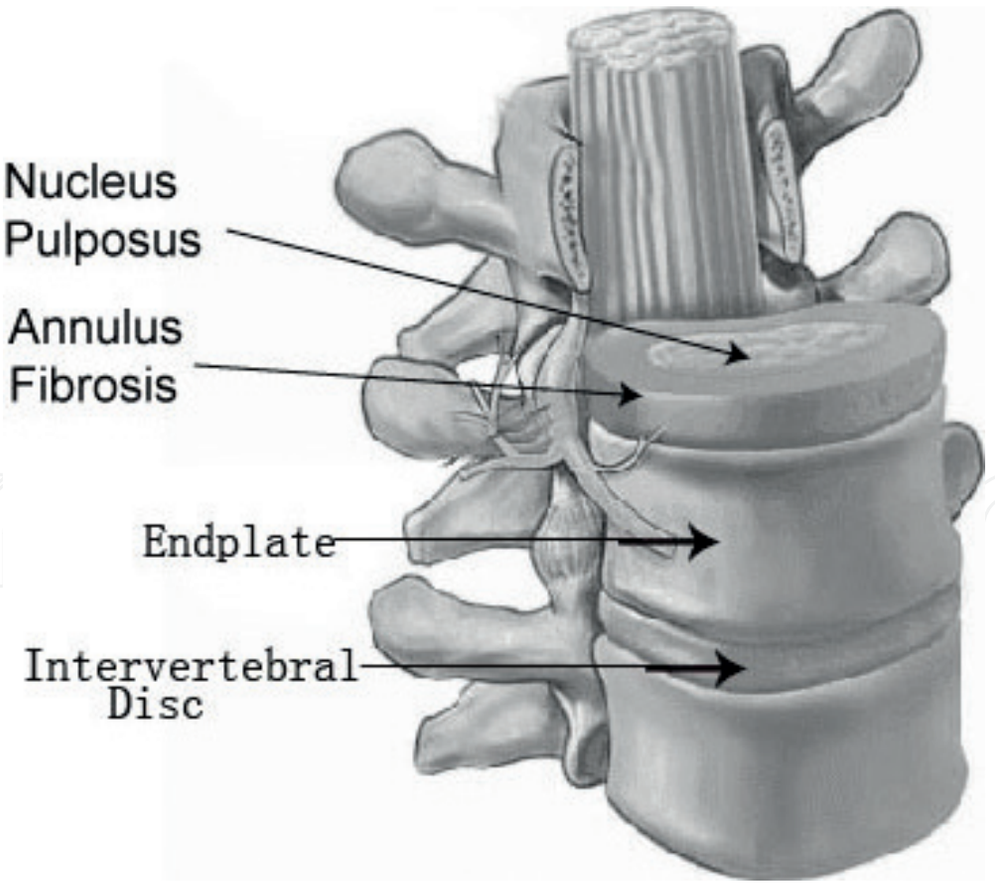

Figure 1. Schematic cross-section of an intervertebral disc.

becomes repopulated by chondrocyte-like cells that are thought to be originated from the adjacent endplate or inner AF regions [3]. All the previous results are solely based on the morphological detection and the existence of notochordal cells is believed to be significantly associated with aging. However, a recent study shows notochordal cells exist in human young and middle age by immunohistochemistry of notochordal cell markers. The occurrence of notochordal cells with immunohistochemical phenotype significantly correlates with granular matrix changes and cleft formation in the nucleus pulposus [4].

A network of microscopic blood vessels penetrates the endplates to principally provide nutrition for the disc and normally disappears around the time of skeletal maturity [3]. With a sparse vascular supply in the outer lamellae of the annulus, mature discs are totally reliant on diffusion of essential solutes across the endplates for nutrition and metabolic exchange [5]. The inner part of the IVD, particularly the NP, is completely avascular and aneural in the largest of the mature human lumbar IVD, where some cells can be 20 mm away from the nearest direct blood supply thereby making the NP severely hypoxic [5]. Mature IVD is composed of heterogeneous cell populations. A majority of the AF cells originate from the mesenchyme and exhibit many characteristics of fibroblasts and chondrocytes, such as the ability to synthesize the type I and II collagen and aggregating proteoglycans [3]. The morphology of AF cells may reflect their adaptation within the special biochemical and structural environment, as these cells appear ellipsoidal and align with the oriented collagen fibers within the lamellas [6]. Cells in the outer AF region display thin cytoplasmic projections that stain positive for both actin microfilaments and vimentin intermediate filaments, which have been associated with tissue regions subjected to compression [7]. Cells within the inner AF regions are often rounded, sparsely distributed, and surrounded by a pericellular matrix region rich in types III and VI collagen [7]. The NP is a gelatinous structure comprised primarily of aggrecan and type II collagen together with the small amounts of collagen type VI, IX, and XI. Cells are sparsely distributed in the NP and may also extend small cytoplasmic processes and, similar to chondrocytes, these cells highly express vimentin intermediate filaments, F-actin, and cytokeratins [7].

The most prominent feature of the IVD is its high content of extracellular matrix (ECM), which is substantially maintained by the cells within IVD, of which, the disc matrix is an elaborate structure of macromolecules that attract and hold water. The major structural components of the macromolecule are collagens and proteoglycans [8]. It is estimated that the ratio of type II collagen and the proteoglycan aggrecan in the AF is 1:20 [9]. Collagens provide firm and tensile strength whereas proteoglycans, through interactions with water, give the tissues stiffness, viscoelasticity and resistance to compression [8, 9]. Collagenous proteins comprise 70% of the outer annulus dry weight, but only account for 20% of NP [8, 9]. On the contrary, NP has a higher proteoglycan concentration, with up to 50% of the nucleus dry weight in adolescence. Given the co-existence of multiple matrix components and their high contents in IVD, the integrity of the IVD partially relies on the proper balance between the matrix synthesis and degradation, and the failure of which is suggested being a cause of the disc degeneration [9].

IVD degeneration is associated with the LBP. The IVD, especially the inner fibrosus (IF) and nucleus pulposus (NP), is virtually avascular and therefore highly hypoxic. At the cranial

and caudal ends of each disc are the cartilaginous endplates that separate the vertebral bone from the disc itself and are believed to be the major channel of nutrient diffusion in IVD. Recent studies have reported changes in tissue structure, various cellular parameters and composition of matrix macromolecules in degenerated discs. Disc degeneration is characterized by decreased water and proteoglycan content and loss of the gel-like appearance of NP. Disc degeneration is thought to be contributed by increased cell senescence and dysregulated cellular activities. The IVD has limited nutrient, oxygen supply, and constant high mechanical stress. These may lead to difficulty for IVD to regenerate itself in IVD degeneration and injuries.

3. The finding of disc progenitor cells

Adult tissue-specific stem cells are a rare heterogeneous population of multipotent cells that can be isolated from many different adult and fetal tissues, including bone marrow, muscle, fat, hair follicles, tooth root, placenta, dermis, perichondrium, articular cartilage, umbilical cord, lung, and liver [10]. These cells show extensive proliferation, produce differentiated progeny, and functionally repair damaged tissues [11]. Adult stem cells normally reside in a specific cellular microenvironment (niche) that constitutes a privileged setting for the support of self-renewal [12]. There are three general properties unique for all the stem cells, regardless of their source. Clonogenicity, the ability of a single cell to proliferate independently to form a colony, is a property commonly ascribed to stem cells, although many clonogenic cells are limited in their capacity for expansion *ex vivo* [13]. Secondly, stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation [13]. Differentiation is triggered by the signals inside and outside cells. The internal signals from genes are interspersed across long strands of DNA and carry coded instructions for all cellular structures and functions [13]. The external signals comprise physical contact with neighboring cells, chemicals secreted by other cells, and certain molecules in the microenvironment [14]. The interaction of signals during differentiation causes the cell's DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division [14]. Most adult stem cells are multipotent, capable of differentiating into at least three lineages (osteogenic, chondrogenic, and adipogenic) when cultured under defined *in vitro* conditions [14]. Thirdly, adult stem cells can go through numerous cycles of cell division while maintaining the undifferentiated state [15]. Stem cells are capable of dividing and renewing themselves for long periods. Unlike terminal stage cells, which do not normally replicate themselves, stem cells may replicate many times or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells [16]. If the resulting cells continue to be undifferentiated, like the original stem cells, the cells are said to be capable of self-renewal.

Bone marrow mesenchymal stromal cells (BMSCs) are composed of heterogeneous population of undifferentiated and committed cells [17]. The regenerative properties ascribed to BMSCs are characterized into three aspects: the plasticity to differentiate toward target cell types, the activation of the proliferation of resident cells, and the improvement of nutrient

supply via paracrine effects. One of the remarkable phenomena of IVD degeneration is a reduction of proteoglycan content, partially caused by the apoptosis/necrosis of the nucleus pulposus (NP) cells in the IVD. The biotherapeutic treatment, therefore, aims to replenish the local resident cells and structural extracellular matrix (ECM) within IVD [18, 19]. Three-dimensional (3D) cultures have been used to induce BMSCs to differentiate into chondrocyte-like cells. These include cell pellet, alginate bead, hydrogel, and engineered 3D scaffold [20–24]. Chondrocyte-like phenotype can also be obtained via a single monolayer coculture of BMSCs, either with NPC or annulus fibrosis (AF) cells with cell-cell contact [25, 26]. Importantly, the chondrocyte-like cells have been shown to possess NPC phenotype [27, 28]. Via intradiscal injection into degenerative IVD, BMSCs are able to survive and commence proliferation under severe hypoxic environment [29–32]. The production of ECM elevates in the NP post-transplantation, including aggrecan, collagen type, and glycosaminoglycans [33]. Animal studies have validated the effect of BMSCs. BMSCs are capable of replenishing NPCs and evoking their production of ECM components. This arrests the progressive decrease of disc height, as well as to partially maintain or even restore minimal disc height in mildly degenerative IVD [34]. Therefore, intradiscal transplantation of BMSCs shed some light on the maintenance of IVD homeostasis. However, the utility of BMSCs is still a subject of debate due to many unanswered questions. The method of transplantation, the choice of carrier, and the fate of BMSCs after delivery need further investigation. Notably, the intradiscal-delivered BMSCs have been found to leak from IVD and generate osteophytes [35]. Although embedding BMSCs in tissue-engineered scaffold before transplantation can alleviate the leakage issue, safety issues remain a concern [36].

IVD cannot self-repair and no cure is currently available for IVD degeneration. Various animal models have suggested the promising potential of mesenchymal stem cell (MSC) implantation to arrest IVD degeneration or even partially regenerate the disc [21, 37]. However, there are two major issues in MSC therapies: first, most studies are focused on the exogenous stem cells but the limitation is their potential immunogenicity. MSCs have indeed been shown to halt degeneration processes but are rarely able to completely regenerate the degenerative disc as the disc degeneration often continues after a certain period [37]. Besides, the therapy is invasive and therefore may potentially lead to complications such as infection and discitis. Second, all studies are carried out in quadruped animals and these models do not more closely resemble humans in terms of biomechanical loading in the spine, diffusion distances for nutrients and metabolites to the NP, age-related declination of notochordal cells, and the occurrence of age-related disc degeneration. In addition, most studies have been monitored only for relatively short time, in the range of weeks after treatments and their efficacy in long term remains elusive.

Recently, several studies have reported that cells derived from IVD tissue have multi-differentiation potential and possess mesenchymal stem cell-like features *in vitro*. NPCs express many MSC surface markers and are potent in differentiating into chondrogenic, osteogenic, and to some extent adipogenetic lineages [38, 39]. Similar multipotency of annulus fibrosus cells (AFCs) from scoliotic IVD was confirmed [40]. In degenerated and nondegenerated (scoliosis) IVD tissues, cells express stem/progenitor markers such as *OCT3/4*, *CD105*, *CD90*, *STRO-1* and *NOTCH1* [41]. A population of NPCs from nonchondrodystrophic canine IVD

possesses neurogenic differentiation potential *in vivo* and expresses stemness genes, including *Sox2, Oct3/4, Nanog, CD133, Nestin* and *NCAM* [42]. Interestingly, this subset of NPCs expresses higher level of *Nanog gene* compared to BMSCs and is negative in the expression of protein 0 and *Brachyury gene*, which are positive in unsorted NPCs [42]. However, these data were drawn from models of mice, rats, dogs, rabbits, and even Chinese hamsters [43]. Differences between the IVDs of human and these animals, however, are large and present at multiple levels. These include anatomical structure, cellular and biochemical components, mechanical loading, and age-related changes. Nonhuman primates are closely related to humans and have been shown to be excellent model organisms for many health and disease conditions in human. The structure of the spine of the primates, including baboon and the higher species Rhesus monkeys, is similar to that of human, except with some deviations in the number of the vertebra and the spine curvature. The monkeys spend much of their time in semi-erect and erect positions, possibly indicating the loading conducted through the vertebral column closely parallel to those encountered in humans [44, 45]. Histology and microscopic features of monkey IVD also suggest its high similarity to human IVD [45]. Furthermore, microarchitecture of glycosaminoglycans and collagens in the IVD of Rhesus monkey has also been shown to be similar to human IVD at the ultrastructural level [45]. More importantly, recent MRI studies have demonstrated that IVD degeneration develops in healthy monkeys at 5 years of age, the human age equivalent of 17.5 years [45]. With increasing disc degeneration, changes in disc height, MRI signals within NP and hyperostotic spondylotic can all be detected [46]. Such changes are also reported to correlate with radiographic and histopathologic changes [46]. Nonhuman primates, particularly Rhesus monkey, are also considered an advanced model to study IVD degeneration. Therefore, study on cells derived from normal IVD of Rhesus monkey further confirmed the existence of IVD disc progenitor cells (DPCs), which possess clonogenicity, multipotency, and differentiation after serial expansion *in vitro* and *in vivo* [47].

Thus, endogenous DPCs have become an enticing subject in the IVD study. However, whether IVD aging/degeneration is associated with or resulted from the diminishing of endogenous DPCs remains unknown. A study has identified a population of NPCs from mice and humans expressing tyrosine kinase receptor Tie 2, a novel surface marker of BMSCs, and disialoganglioside 2 (GD2), a hematopoietic stem cells (HSCs) surface protein [48]. $Tie2^{+}GD2^{+}$ NPCs are clonally multipotent and generate NP-like tissue in *in vivo* serial transplantation [48]. Importantly, $Tie2^{+}GD2^{-}$ NPCs are the precursor of $Tie2^{+}GD2^{+}$ NPCs and the frequency of these progenitor cells decreases with aging and the severity of degeneration of the IVD [48]. Interestingly, DPCs from healthy IVD possess higher differentiation capacity toward chondrogenic lineage and NP-like cells compared with DPCs from degenerative IVD [49]. Therefore, the number and functionality of DPCs are associated with the degenerative process. Further validation of this theory may promote understanding of the etiology of IVD degeneration and contribute to the development of novel biotherapies.

Taking together, DPCs, as an endogenous cell population, may be more suitable in the biotherapeutic treatment of IVD diseases and become a new target for IVD regeneration. However, before any therapeutic application or pre-clinical/clinical trial, several research gaps need to be addressed. First, the mechanism of hypoxia-induced $Tie2^{+}$ expression on $Tie^{+}GD2^{+}$ NPCs

awaits further elucidation. NPCs were sensitive to oxygen tension and hypoxia-inducible factors (HIF) reduce the susceptibility of hypoxic apoptosis of NPCs. Whether Tie2 couples with HIFs to resist hypoxic stress in NPCs is worthy of being studied. Second, the progenitor niche components of DPCs need to be identified. The fate of DPCs emerges with the pathological change of IVD. This suggests the existence of regulatory components within IVD, modulating the survival and self-renewal of these cells.

4. Conclusions

In conclusion, the studies of DPCs extend the current knowledge regarding the biology of endogenous IVD cells. Combined with tissue engineering and cell therapy, the application of DPCs would pave the way for the manipulation of IVD diseases and provide new hope that may contribute to IVD regeneration.

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Declaration of interest

All authors state that they have no conflicts of interest.

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