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Regulatory Role of Hypoxia Inducible Factor in the Biological Behavior of Nucleus Pulposus Cells

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Intervertebral disc (IVD) degeneration is implicated as a major cause of low back pain. The alternated phenotypes, reduced cell survival, decreased metabolic activity, loss of matrix production and dystrophic mineralization of nucleus pulposus (NP) cells may be key contributors to progressive IVD degeneration. IVD is the largest avascular structure in the body, characterized by low oxygen tension *in vivo*. Hypoxia-inducible factor (HIF) is a master transcription factor that is induced upon hypoxia and directs coordinated cellular responses to hypoxic environments. This review summarizes relevant studies concerning the involvement of HIF in the regulation of biological behaviors of NP cells. We describe current data on the expression of HIF in NP cells and further discuss the various roles that HIF plays in the regulation of the phenotype, survival, metabolism, matrix production and dystrophic mineralization of NP cells. Here, we conclude that HIF may be a promising target for the prevention and treatment of IVD degeneration.

Key Words: Hypoxia inducible factor, intervertebral disc degeneration, nucleus pulposus

INTRODUCTION

Low back pain is a major public health problem that leads to intense discomfort and loss of function, resulting in considerable economic loss and health care expenditures.[1-3] It is reported that the lifetime prevalence of low back pain ranges from 30% to 85%, with a point prevalence of 20% to 40% in Western developed countries.[4] The etiology of low back pain remains unclear, but intervertebral disc (IVD) degeneration has been implicated as one of the major causes.[5,6] IVD is a specialized complex that separates the vertebra and functions to provide load bearing and allow for flexibility of the spinal column.[7,8] Healthy human IVDs are composed of the peripheral annulus fibrosus (AF), the central nucleus pulposus (NP), and the superior and inferior cartilaginous endplates. The NP is populated by NP cells which play a critical role in the generation and maintenance of the IVD matrix.[9] Histopathological observations have indicated that significant degenerative changes occur in the NP during the degeneration process;[10,11] alternated phenotypes, reduced cell survival, decreased metabolic activity, loss of matrix produc-

tion and dystrophic mineralization of NP cells may be key contributors to progressive IVD degeneration.

Current treatment modalities for symptomatic IVD degeneration are mainly targeted at the symptoms of pain and neurological deficits, without treating the degenerative problems or repairing the biomechanical function of the degenerative disc.[12] Thus, a better understanding of the regulation of the phenotypes, survival, metabolism, matrix production and dystrophic mineralization of NP cells may help to develop prevention and treatment strategies for IVD degeneration.

The IVD is the largest avascular structure in the body.[13] A limited number of blood vessels infiltrate the superficial region of the cartilaginous endplate and the outer third of the AF, but none of these vessels infiltrate the NP.[14,15] Necessary nutrients, including oxygen and glucose, reach the NP predominantly through diffusion which imposes a hypoxic state on the NP cells.[16,17] In addition, this hypoxic state is enhanced by the loss of cartilaginous endplate permeability during IVD degeneration.[18] Hypoxia is an important cellular stress with significant pathological implications in many disease processes, such as cerebral ischemia, cancer and chronic degenerative disorders.[19,20] Hypoxia-inducible factor (HIF) is a transcription factor that initiates a coordinated cellular cascade in response to a low oxygen tension environment, including the regulation of numerous enzymes in response to hypoxia.[19,21-23] Recently, the expression of HIF in NP cells has been reported by many groups.[10,15,18,24-31]

Based on these previous studies, this review will discuss the regulatory role of HIF in the biological behaviors of NP cells. Additionally, we will summarize current data on the expression of HIF in NP cells and further discuss the various roles HIF plays in the regulation of the phenotypes, survival, metabolism, matrix production and dystrophic mineralization of NP cells.

HIF IN NP CELLS

In response to hypoxic conditions, cells respond to low oxygen tension by up-regulating the synthesis of HIF proteins.[22,32] The HIF family of proteins comprises several distinct HIF proteins, HIF-1, HIF-2, and HIF-3, each of which consist of an α-subunit and a constitutively expressed β-subunit known as aryl hydrocarbon receptor nuclear translocator.[33,34] Transactivation of HIF target genes involves the dimerization of the two subunits and binding to an enhancer element, the hypoxia response element (HRE) in the target genes.[35,36] Under normoxic conditions, α-subunits cannot be detected as they undergo rapid degradation, but under hypoxic conditions they are stabilized, accumulated and translocated to the nucleus where they dimerize with β-subunits to bind to HREs and activate the expression of numerous hypoxia response genes.[35,36]

Among the three HIF isoforms, HIF-1 and HIF-2 play an important role in the regulation of the biological behaviors of NP cells.[34,37] Risbud, et al.[24] examined the expression of HIF-1α in rat, human, and sheep NP cells under both hypoxia and normoxia (2% and 21% oxygen) and found that NP cells consistently expressed functionally active HIF-1α protein under hypoxia; Agrawal, et al.[25] observed HIF-2α expression in rat NP cells. Furthermore, they found that the protein and mRNA levels of HIF-2α were similar under both normoxic and hypoxic conditions, though there was a significant increase in HIF-2α transactivation under hypoxic conditions.

Prolyl-4-hydroxylase domain (PHD) proteins are members of the 2-oxoglutarate/iron dependent dioxygenase superfamily and include PHD1, PHD2 and PHD3.[38] Previous studies have shown that in many cell types the degradation of HIF-1α and HIF-2α is primarily mediated by oxygen-dependent proteasome and catalyzed by PHDs.[26,39] However, in NP cells, the degradation of HIF-1α and HIF-2α is mainly mediated through 26S proteasome, irrespective of oxygen tension. Moreover, among all PHDs, only PHD2 controls limited HIF-1α degradation in an oxygen-dependent manner, while the degradation of HIF-2α is largely independent of PHD activity.[26]

HIF-1 AS A PHENOTYPIC MARKER OF NP CELLS

Currently, the phenotype characteristics of NP cells have not been clearly defined.[10] Using western blot and immunohistochemistry analysis, Rajpurohit, et al.[10] demonstrated that the expression of HIF-1α was only found in NP cells, but not in AF and cartilaginous endplate cells, while HIF-1β expression levels were significant higher in NP cells than that in AF cells and cartilaginous endplate cells. Based on these data they suggested that the difference in the expression of the two HIF-1 isoforms provides a phenotypic signature that could be used to distinguish NP cells from neighboring AF cells and cartilaginous endplate cells.

In another study, Richardson, et al.[18] detected the expres-

sion of HIF-1α in normal and degenerate human IVDs and found that HIF-1α was only expressed in NP cells. These results further provide support that HIF-1 may be a phenotypic marker of NP cells.

HIF PROMOTES NP CELL SURVIVAL IN IVDS

NP cell survival is crucial for the homeostasis and function of the IVD.[40] The chemical microenvironment of IVD is harsh and is characterized by low oxygen tension.[16] However, *in vitro* studies indicated that NP cells could survive at low oxygen tension without a significant loss of cell viability.[41,42] Thus, NP cells may develop some mechanisms to ensure their survival in the hypoxic environment of the IVD.

It is known that the Fas and Fas ligand (FasL) system delivers a death signal that rapidly commits cells to apoptosis.[43] Notably, Fas and FasL are coexpressed in the disc cells of herniated lumbar IVD tissues.[43] Galectin-3 (gal-3), a member of a growing family of β-galactoside-binding animal lectins, is involved in the regulation of cell adhesion and apoptosis.[44] Zeng, et al.[27] analyzed the interaction of HIF-1α with the gal-3 promoter in rat NP cells and found that the inhibition of HIF-1α down-regulated the promoter activity of gal-3, sequentially enhancing Fas/FasL-mediated NP cell apoptosis. They further confirmed that HIF-1α combined with gal-3 HRE and that site-directed mutagenesis of HRE completely blocked hypoxic induction of gal-3 promoter activity. These data suggest that HIF-1α induces the expression of gal-3 and sequentially inhibits Fas/FasL-mediated apoptosis of NP cells.

Vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) play crucial roles in both physiological and pathological angiogenesis.[45] Several lines of evidence suggest that VEGF serves as a survival factor in normal IVD tissue. Fujita, et al.[40] found that NP cells expressed both VEGF-A and mbVEGFR-1 (a membrane-bound form of VEGF-A receptor) and treatment of NP cells with the VEGF-A antagonist VEGFR-1-Fc led to NP cell apoptosis, suggesting that VEGF-A/VEGFR-1 cascade mediates an anti-apoptotic function in NP cells. Agrawal, et al.[25] found that under hypoxic conditions, HIF-1α and HIF-2α regulated the promoter activity and expression level of cited2, a p300 binding protein. The forced expression or suppression of cited2 would result in corresponding changes in the expression of VEGF in NP cells. Based on these studies, we concluded that HIF-1 and HIF-2 may also serve to enhance NP cell survival in the specialized microenvironment of the IVD via HIF-1 and HIF-2 mediated VEGF expression.

HIF MAINTAINS THE METABOLIC ACTIVITIES OF NP CELLS

In the presence of low oxygen tension, the energy metabolism of NP cells almost completely relies on anaerobic glycolysis.[46,47] HIF plays an important role in the maintenance of the metabolic activities of NP cells.[10,24,29] HIF-1 serves as a key transcription factor that regulates the expression of a number of genes involved in glycolysis as well as mitochondrial energy metabolism.[48,49] One of the important genes involved in promoting anaerobic glycolysis of NP cells is hypoxia responsive glucose transporter (GLUT).[50] In all mammalian cells, GLUT is an integral membrane protein that presents on the cell surface and serves to transport glucose down its concentration gradient by diffusion.[51,52]

Richardson, et al.[18] analyzed the expression of GLUT-1, GLUT-3, GLUT-9 and HIF-1α in normal and degenerate human IVDs. The results indicated that HIF-1α, GLUT-1, GLUT-3 and GLUT-9 were coexpressed in normal human IVDs and an increase in HIF-1α expression was associated with increases in GLUT-1, GLUT-3 and GLUT-9 expression in NP cells. However, there was no correlation between the expression of HIF-1α and GLUT-1, 3 or 9 in AF cells. Interestingly, they also observed that the expression of GLUTs increased as IVD degeneration progressed.

Taken together, HIF-1 maintains the metabolic activities of NP cells under a hypoxic environment in IVDs, mainly via the regulation of GLUT-1, GLUT-3 and GLUT-9 expression.

HIF PROMOTES EXTRACELLULAR MATRIX SYNTHESIS OF NP CELLS

Although disc cells constitute only 1% of the adult disc tissue by volume, these cells are responsible for maintaining the extracellular matrix of the disc,[53] which is key to the function of IVDs.[54] The homeostasis of extracellular matrix in IVDs is biologically regulated by the active maintenance of a balance between the anabolism and catabolism of disc cells. Degenerative disorders of the IVD are characterized by disequilibrium between extracellular matrix repair and degen-

erative processes.[55]

The disc extracellular matrix is predominantly composed of proteoglycans and collagens, and extracellular matrix compositions of AF and NP are distinct.[56] Proteoglycans are abundant in the NP, which permits the IVD to withstand compressive loads.[29] The collagen network provides the tensile properties for the spine to bend and flex. Agrawal, et al.[29] reported that HIF-1α promotes aggrecan (the major proteoglycan) synthesis directly by inducing its mRNA and protein expression and, possibly, indirectly, through lineage specification as well as the promotion of sulfation reactions.

Another component of the disc extracellular matrix is glycosaminoglycan, which is critical to preservation of the gelatinous nature of the NP.[57] Glucose is known as an important factor for the synthesis of this large molecule.[18] As mentioned above, HIF-1 regulates the expression of a number of GLUTs, which could promote NP cells to uptake glucose across the plasma membrane. In this aspect, HIF-1 reportedly promotes the synthesis of glycosaminoglycans. On the contrary, there are some disagreements about the role of HIFs in glycosaminoglycan synthesis. Gogate, et al.[30] measured the effects of HIF-1α and HIF-2α on the promoter activity of β-1, 3-glucuronyltransferase-1 (GlcAT-1), a key enzyme in chondroitin sulfate (the major glycosaminoglycan) synthesis in NP cells. They found that HIF-1α and HIF-2α suppressed the promoter activity of GlcAT-1 through interactions with one or more HREs. These data indicate that HIFs serve as transcriptional repressors of GlcAT-1 in NP cells.

HIF REGULATES DYSTROPHIC MINERALIZATION OF NP CELLS

In normal healthy individuals, mineralization is restricted to hard tissues, which form the skeleton and dentition.[58,59] Within these specialized tissues, mineralization is highly controlled in both growth and development, as well as in normal adult life.[59] However, dystrophic mineralization, resulting from aging, injury and disease, is a common problem observed in soft tissues.[58,59] It is known that dystrophic mineralization could lead to a number of diseases, including calcification of joint cartilage resulting in osteoarthritis and mineralization of the cardiovascular tissues resulting in exacerbation of atherosclerosis and blockage of blood vessels.[59] Moreover, IVD calcification has been considered to cause or at least promote the process of IVD degeneration.[60] In all cases, cells in the soft tissues play an important role in regulating mineral deposition. Cells may regulate crystal nucleation by synthesizing a mineralization competent matrix, releasing matrix vesicles, providing degeneration products and cell death.[58,59,61] Conversely, cells also synthesize inhibitors that serve to prevent dystrophic mineralization.[61] Surprisingly, although the disc contains both fibrous proteins and a hydrated extracellular matrix, there are no calcified deposits in the normal NP tissue. The local control of dystrophic mineralization in the NP is necessary to prevent dystrophic mineralization of the disc.[62]

It has been suggested that the deposition of mineral salt is regulated by ANK, a multi-pass transmembrane channel that controls the transport of inorganic pyrophosphate, a powerful inhibitor of dystrophic mineralization.[63] Moreover, numerous studies have shown that mutations in ANK could result in abnormal dystrophic mineralization in joints and bone.[64,65]

Oxemic status may influence ANK expression which may be mediated by HIF-1α.[63] Skubutyte, et al.[31] examined the expression and localization of ANK in the IVDs of mature and neonate rats and found that the expression of ANK in the NP was significantly higher in mature rats than in neonate rats. Furthermore, they found that when the expression of HIF-1α or HIF-2α was silenced in NP cells, ANK expression was induced under hypoxia at both the mRNA and protein level. In addition, forced expression of HIF-1α or HIF-2α caused suppression of ANK reporter activity in NP cells. Taken together, these studies suggest that HIF controls the dystrophic mineralization of NP cells through the suppression of ANK expression.

CONCLUSION

In summary, a better understanding of the regulatory roles of HIF in the biological behavior of NP cells would shed new light on the prevention and biological repair of IVD degeneration. Based on the above discussion, it is reasonable to predict that HIF may be a potential target for the prevention and treatment of IVD degeneration, given that HIF could modulate the biological behavior of NP cells in regards to degeneration. Development of a HIF-targeted drug to enhance cell survival, maintain metabolic activities, stimulate extracellular matrix synthesis and control dystrophic mineralization in NP cells may lead to better management of IVD degeneration.

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