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Recent Developments in Molecular and Cellular Spine Research

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INTRODUCTION

The intervertebral disc (IVD) is a soft-tissue-filled articulating joint between vertebrae and the functional unit of the spine. There are broadly two populations of cells within the disc (excluding the cartilaginous end plates): the mesenchyme-derived annulus fibrosus (AF) cells, and the notochord-derived nucleus pulposus (NP) cells. These are both distinct and unique populations within the body. The tissue composition is therefore also unique compared to any other soft tissue, and the gene expression, morphology, and signaling of the cells has signature characteristics which are increasingly well studied. The Spine Group at the 2014 Annual ORS Meeting, composed of several of the most active labs in the field, set out to create a consensus statement to codify what is known about healthy NP cells, hoping to advance research in the field. The result of their collaborative review was published in the Journal of Orthopedic Research in March 2015 and it laid forth several definitions for NP cells [1]. The review claims the key phenotypic markers are: stabilized expression of HIF-1α, GLUT-4, Sonic Hedgehog (Shh), Brachyury, KRT18/19, CA12, CD24 and maintaining an aggrecan/collagen II ratio > 20. Specifically they also went on to discuss methods for assessing microarray data and promoted the idea of using the above set of markers to assess cell differentiation.

While this comprehensive review was being compiled, a group in the Netherlands defined subpopulations among mature NP cells [2]. Using previously established sets of marker genes the NP cells were selected for and then made into 54 separate immortalized clones. The group reports generation of phenotypically distinct subclones from primary human NP (patients age 8-15) with subpopulations that differ by morphology, cell surface markers, and differentiation capacity. Many of the genes examined were included in the review above, but they also assayed for PAX1, PTN, and FOXF1. Within the subclones some responded to differentiation pressures and induced Sox9 and COL2A1, while some did not. Also, they noted some CD24- cells maintained a spheroid morphology possibly indicating the presence of a more immature NP cell. While efforts continue to be made to accurately describe the nucleus pulposus, the complexity of the cellular identity also seems to expand.

Phenotypic Maintenance Through Signaling

It is agreed that Shh is necessary for maintaining a healthy NP phenotype by inducing other ECM markers of the disc. Winkler et al. published that Wnt signaling is not only an activator of Shh in the developing mouse disc, but also that Wnt signaling can recapitulate Shh signaling and its down-stream benefits in adult/degenerative mouse discs [3]. This paper reports that the aging process of the NP is concurrent with a decrease in Shh expression, and that stimulating Shh with Wnt signaling can reverse the changes in expression seen in aging. Looking at this signaling pathway from the other extreme, Iwata et al. reported that an accumulation of B-catenin correlated with increased Runx2 transcription, and calcified deposits and degeneration in beagle IVDs [4]. The study further showed that Wnt pathway inhibition with LiCl led to a similar increase in Runx2 expression.

Whether precipitated by fluctuations in the Wnt pathway and Runx2 expression or not, ossification in the IVD is a hallmark of degenerative disc disease (DDD). There are many actors in the ectopic mineralization of a soft tissue: some are over-active tissue mineralizers while others are sub-functional inhibitors of mineralization. Wei et al. report the actions of Growth Differentiation Factor 6 (GDF6) in the latter category [5]. During human embryonic development GDF6 is highly expressed in soft tissues throughout the spine and as the vertebrae mineralize the expression of GDF6 locally decreases until it is only expressed in the soft tissue space itself. Furthermore, the overall expression of GDF6 falls with age. It is also known that GDF6 mutations

often lead to ectopic joint mineralization in animal models and human pathologies. This all indicates GDF6 is at least a marker of soft-tissue maintenance, and likely a regulator of the NP tissue phenotype, as was suggested by Clarke et al. in 2014 regarding the differentiation of adipose-derived MSCs [6].

Inflammatory Cytokines

Besides ossification of soft tissue, the other hallmark of DDD is the expression of inflammatory cytokines. Much has been done to establish some of the pleiotropic effects of these stimuli in the disc space. Wang et al. describe the expression of catabolic MMP3 driven by TNFα and IL-1β in rat and human NP cells [7]. The study puts forth that cell-surface proteoglycan syndecan-4 must interact with the MAPK/NF-κB axis to induce catabolic gene expression. Further they show that inhibiting syndecan-4 blocks the up-regulation of MMP3, and TGFβ actively counters the effect of TNF stimulation. While this study examined both cytokines it spent more time decrypting the effects of TNFα.

Meanwhile, Christine Le Maitre's group released two papers which indicate IL-1β is the master regulator of the catabolic process in the IVD. Phillips et al. reported minimal effects treating primary hNP with IL-16, CCL2, CCL3, CCL7, and CXCL8, yet they noted dramatic ECM remodeling and expression of other cytokines when challenged with IL-1β [8]. This study highlights the variety of cytokine and chemokine receptors present on the NP cell surface and how they could all be employed for autocrine and paracrine signaling, but that IL-1β exerts the greatest regulatory potential.

Similarly Binch et al. examined the mechanism of vascular invasion in DDD [9]. The study identified neurotropins in IVD tissue, found that degenerative human discs contained nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) receptors, and that BDNF was more highly expressed in degenerative samples; both vascular endothelial growth factor (VEGF) and the neuropeptide Substance P were higher in infiltrated discs. TNFα induced expression of Neurotropin-3, whereas IL-1β-induced NGF, substance P, VEGF and pleiotropin in hNP cells in alginate culture. While neurotropin induces NGF signaling in some cells [10], it has also been shown to have anti-inflammatory effects by inhibiting NF-κB and JNK in liver cells [11]. Inducing expression of VEGF and NGF reinforces the role of IL-1β as a master regulator of DDD, as these processes allow vascular and neural invasion of the disc space.

More so as IL-1β increases substance P, Kepler et al. confirm that substance P in turn up-regulates cytokines through activation of p38 and MAPK (though not NF-κB) [12]. Further, this group showed that NKIR (a substance P antagonist) is able to suppress expression of IL-1β, IL-6, and IL-8 in the presence of substance P in hNP alginate bead culture. This then neatly ties together a system of inflammatory activation, catabolic regulation, and degeneration.

Artificial Disc Replacements

Finally, in clinical practice the discs in need of intervention are too far degenerated for the subtleties of these signaling pathways. To this end, there have been several exciting developments in the artificial IVD field. Vadala et al addressed the issue creating a reproducible model system for testing hydrogel NP replacements [13]. They validated a reliable method done by mechanical transpedicular arthroscopic nucleotomy which they found to be superior to enzymatic digestion of the nucleus as it preserved the AF without damage and created a consistent cavity volume as assessed by μCT.

Rosenweig et al. presented exciting results using a 3D printer to fabricate an ABS or PLA scaffold seeded with articular chondrocytes or NP cells which allowed them to produce ECM and maintain individual phenotype over a 3-week culture period [14]. Their use of large-pore thermoplastics allowed them to recapitulate the native mechanical stiffness, and eventually fill the scaffold with cell-produced GAGs.

Finally, there was further advancement from the lab of James Iatridis, continuing work with genepin cross-linked fibrin (FibGen) scaffolds as a sealant for AF tears [15]. Having already established the negative effects of TNF a in the disc and determining the efficacy and kinetics of anti-TNF treatment the group applied this knowledge to the functional scaffold gel [16, 17]. Using the scaffold/sealant as a drug-delivery system for anti-inflammatory infliximab (anti-TNFα mAB) they assessed efficacy in culture. The addition of collagen hollow spheres (CHS) in the FibGen allowed a slow and controlled release of the infliximab, which provided sustained depression of IL-1β, IL6, IL8 and TNFα for 20 days in culture, more effective than drug or CHS alone. This is in addition to the already demonstrated effects the FibGen has of restoring the biomechanical properties of the AF. This successful delivery of a drug in the polymer marks an exciting direction in IVD replacement that combines mechanics and therapeutics, a combination of increasing interest [18].

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