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REVIEW ARTICLE

Multifaceted signaling regulators of chondrogenesis: Implications in cartilage regeneration and tissue engineering

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Abstract Defects of articular cartilage present a unique clinical challenge due to its poor self-healing capacity and avascular nature. Current surgical treatment options do not ensure consistent regeneration of hyaline cartilage in favor of fibrous tissue. Here, we review the current understanding of the most important biological regulators of chondrogenesis and their interactions, to provide insight into potential applications for cartilage tissue engineering. These include various signaling pathways, including fibroblast growth factors (FGFs), transforming growth factor β (TGF-β)/bone morphogenic proteins (BMPs), Wnt/β-catenin, Hedgehog, Notch, hypoxia, and angiogenic signaling pathways. Transcriptional and epigenetic

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Sox9;
TGFβ

regulation of chondrogenesis will also be discussed. Advances in our understanding of these signaling pathways have led to promising advances in cartilage regeneration and tissue engineering.

Introduction

Articular cartilage injury and repair is an issue of growing importance around the world. Kurtz et al predict that the number of total knee replacements will jump from 700,000 to 3.48 million annually by the year 2030.[1] Although common, articular cartilage defects continue to hold a unique clinical challenge due to its poor self-healing capacity, which is largely due to its avascular nature. Current surgical treatment options do not reliably produce hyaline cartilage tissue in favor of fibrous tissue, and therefore remain controversial. Deposition of fibrocartilage instead of hyaline cartilage can lead to poor durability and rapid decline in activity levels after 1 year. Therefore, there is a critical need for more effective cartilage regenerative therapies. The difficulty in obtaining a sufficiently large number of chondrocytes for implantation could be overcome by utilizing gene therapy to promote chondrogenesis from bone marrow-derived mesenchymal stem cells (MSCs). However, research regarding exogenous control over this multi-step process has yielded only limited clinically applicable results. For this reason, the ability to differentiate MSCs into chondrocytic cells, and subsequently implant these chondrocytes into cartilaginous defects is of significant therapeutic value.

MSCs are multipotent progenitor cells that can differentiate into several lineages, including bone, cartilage, fat, and muscle (Fig. 1). The proliferation and differentiation of mesenchymal cells to chondrocytes, or chondrogenesis, is a complex process that is regulated by a host of factors. These factors include intracellular proteins, receptor ligands, and transcription factors; and a disruption in signaling can result in defective chondrocyte production. Here, we review the current understanding of the most important biological regulators of chondrogenesis and how these regulators interact to provide insight into their potential for cartilage tissue engineering.

Fibroblast growth factor (FGF) signaling pathway

FGFs are a family of heparin-binding growth factors that are involved in the proliferation and differentiation of a variety of tissues. In general, FGFs are known to be positive regulators of chondrogenesis and they appear to be involved throughout the process. The most well studied and generally accepted function of the FGF family with regards to chondrogenesis is its role in the proper patterning of developing cartilage. The developing limb is divided into two components: the apical ectodermal ridge (AER) and the limb bud mesenchyme. The FGFs that are produced in the AER, zone of polarizing activity (ZPA), and dorsal ectoderm are necessary for early patterning events in the developing embryo.[2] The AER, which serves as the primary source of FGF2, FGF4, FGF8, and FGF9 in the developing embryo, is maintained by Wnt3 and FGF10 signaling.[3,4] The effect of FGF10 on the apical ectodermal ridge is the result of a positive feedback loop with FGF8. FGF2, 4, and 8 serve as important regulators of proximal-distal growth, and FGF2 has the additional function of priming MSCs for chondrocytic differentiation.[5,6] The latter effect is mediated by an increase in basal expression of prochondrogenic transcription factor Sox9 and inactivation of IGF-I and TGF-β signaling.[5,7]

Sequential expression of three FGFs has been shown to impact the progression of chondrocyte differentiation and maturation: FGF2, FGF9, and FGF18.[8–10] As previously stated, FGF2 administration is known to be sufficient to enhance chondrocyte proliferation speed and prepare chondroprogenitor cells for terminal differentiation; however, the mechanism by which FGF2 exerts these effects has been largely unknown until recently.[8] FGF2's ability to enhance chondrocyte proliferation speed is due to its ability to enhance TGFβ1 expression.[11] Likewise, FGF2-induced chondrocyte differentiation is a consequence of FGF2-mediated downregulation of TGF-β2 expression.[7] Given FGF2's interactions with TGF-β1 and TGF-β2, research sought to elucidate the impact of combining FGF2 with TGF-β3. It has now been determined that FGF2 is able to induce chondrocyte differentiation and enhance proliferation in the presence of TGF-β3, however these effects are accompanied by inhibition of chondrocyte hypertrophy.[12] In addition to its interactions with the TGF-β family of proteins, combination of FGF2 with Wnt3a or a combination of platelet-derived growth factor (PDGF), insulin, and TGF-β1 has been shown to be sufficient to support long-term production of fully functional cartilage.[13,14]

While FGF2 is known to have a major impact on priming chondroprogenitors for the chondrocyte differentiation program, FGF9 and FGF18 are regulators for early chondrogenic differentiation, augmentation of ECM production, and terminal chondrocyte hypertrophy.[9] FGF9 can be both prochondrogenic and proosteogenic depending on its expression level and the cell population in which it is expressed.[15] FGF9 has not only been found to promote chondrocyte hypertrophy at early stages but also to drive the vascularization of the growth plate prior to osteogenesis.[16] The multifunctionality of FGF9 is believed to be the result of its ability to upregulate expression of other prochondrogenic proteins, such as Sox9, Indian hedgehog (Ihh), and Col2a1. FGF9 also upregulates proosteogenic