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Review

# Signaling pathways effecting crosstalk between cartilage and adjacent tissues
# Seminars in cell and developmental biology: The biology and pathology of cartilage

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ABSTRACT

Endochondral ossification, the mechanism responsible for the development of the long bones, is dependent on an extremely stringent coordination between the processes of chondrocyte maturation in the growth plate, vascular expansion in the surrounding tissues, and osteoblast differentiation and osteogenesis in the perichondrium and the developing bone center. The synchronization of these processes occurring in adjacent tissues is regulated through vigorous crosstalk between chondrocytes, endothelial cells and osteoblast lineage cells. Our knowledge about the molecular constituents of these bidirectional communications is undoubtedly incomplete, but certainly some signaling pathways effective in cartilage have been recognized to play key roles in steering vascularization and osteogenesis in the perichondrial tissues. These include hypoxia-driven signaling pathways, governed by the hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF), which are absolutely essential for the survival and functioning of chondrocytes in the avascular growth plate, at least in part by regulating the oxygenation of developing cartilage through the stimulation of angiogenesis in the surrounding tissues. A second coordinating signal emanating from cartilage and regulating developmental processes in the adjacent perichondrium is Indian Hedgehog (IHH). IHH, produced by pre-hypertrophic and early hypertrophic chondrocytes in the growth plate, induces the differentiation of adjacent perichondrial progenitor cells into osteoblasts, thereby harmonizing the site and time of bone formation with the developmental progression of chondrogenesis. Both signaling pathways represent vital mediators of the tightly organized conversion of avascular cartilage into vascularized and mineralized bone during endochondral ossification.



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## 1. Introduction

All the long bones of the mammalian skeleton originate from cartilage templates via a complex multistep process termed endochondral ossification. During this process, mineralized bone is formed through the deposition of bone matrix by differentiated osteoblasts on top of a scaffolding cartilage mold generated by chondrocytes. This mold or cartilaginous bone template is formed during early fetal limb development by cells within the mesenchymal condensations that commit to the chondrocyte lineage under the influence of SOX9, the master transcriptional regulator of chondrogenesis [1]. Adequate longitudinal growth of the endochondral bones is absolutely dependent on precisely regulated proliferation, differentiation and matrix production by chondrocytes in the cartilaginous condensations and later in the growth plate. In addition, proper progression of bone development relies on a very stringent coordination between the processes of chondrocyte maturation in the growth plate, vascular expansion in the surrounding tissues, and osteoblast differentiation, recruitment, and bone-forming activity in the perichondrium and the developing bone center. The synchronization of these processes occurring in these adjacent tissues is regulated through vigorous crosstalk between chondrocytes, endothelial cells and osteoblast lineage cells. Studies using a growing number of genetically modified mouse models are increasingly providing insights in the molecular interplay regulating these complex interactions, some of which will be reviewed here (Fig. 1).

## 2. Chondrocyte-driven regulation of angiogenesis in surrounding tissues

During endochondral ossification, chondrocytes first generate cartilaginous templates of the forming bones from mesenchymal condensations, and subsequently create the growth plates that provide the prime engine for bone growth. These cartilaginous structures are rather unique tissues in that they are inherently avascular and physiologically hypoxic [2–4]. Hypoxia-driven pathways, governed by transcription factors called hypoxia-inducible factors (HIFs), are absolutely essential for the survival and functioning of chondrocytes in these challenging conditions [4] (see Fig. 2). HIF-mediated signaling has also been implicated in joint formation and the integrity of the adult articular cartilage [5,6]. One of the mechanisms by which HIF supports cartilage development is through the regulation of angiogenesis in the adjacent perichondrial tissues, mediated by the potent angiogenic stimulator vascular endothelial growth factor (VEGF) that is a direct transcriptional target of HIF (see Fig. 2). VEGF is also a key driver of the progressive conversion of the prefiguring cartilage into bone tissue during skeletal development and growth, a process that is driven by cartilage neovascularization and the concomitant infiltration of the future ossified region by osteoprogenitors (see Fig. 3). Here, we will go deeper into the studies performed to dissect the roles of hypoxia pathway components and VEGF family members during these key stages of the endochondral ossification program.

### *2.1. The multiple roles of the hypoxia-HIF-VEGF network in avascular cartilage*

#### *2.1.1. Hypoxia-inducible factors (HIFs)*

Cartilage, being an intrinsically avascular tissue, is highly dependent on cellular hypoxia-adaptation mechanisms. HIFs, the transcription factors that are the main orchestrators of the cellular responses to hypoxia, act as heterodimers consisting of an α-subunit which is regulated by oxygen (HIF-1α, HIF-2α/EPAS1, or the less characterized HIF-3α), and a β subunit that is constitutively expressed in an oxygen-independent manner. The best characterized member, HIF-1, is formed in hypoxic conditions by the subunits HIF-1α and HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator (ARNT)), which both contain basic helix-loop-helix-PAS domains that mediate heterodimerization and DNA binding [7–9].

In well-oxygenated conditions, the HIF-1α protein is hydroxylated on specific residues within its amino-acid sequence (prolines P402 and P564 in the oxygen-dependent degradation domain (ODD)), in an oxygen-dependent reaction executed by HIF prolyl-hydroxylase enzymes (PHD1-3, also known as Egln2, 1, and 3) [10]. Hydroxylated HIF-1α is recognized by the Von Hippel-Lindau protein (pVHL), which is part of an E3 ubiquitin ligase complex, leading to the ubiquitination and instant proteosomal degradation of HIF-1α in non-hypoxic conditions [7–9].

In hypoxia, represented by oxygen tension levels dropping below an estimated threshold of 5%, the hydroxylation and degradation of HIF-1α is inhibited. As HIF-1α is stabilized in these conditions, the protein can translocate to the nucleus and dimerize with HIF-1β. Along with nuclear co-factors such as p300 and CREB-binding protein (CBP), HIF-1α and HIF-1β form the transcriptional complex HIF-1. The complex can bind to hypoxia responsive elements (HRE), present in the promoter region of target genes, and induce transcription. More than a hundred putative HIF-1 target genes have been identified, several of which function in cell proliferation, differentiation, survival, resistance to oxidative stress, apoptosis, and extracellular matrix homeostasis, but most typically and abundantly involving genes regulating energy metabolism and

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