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Review

Endochondral ossification: How cartilage is converted into bone in the developing skeleton

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Abstract

Endochondral ossification is the process by which the embryonic cartilaginous model of most bones contributes to longitudinal growth and is gradually replaced by bone. During endochondral ossification, chondrocytes proliferate, undergo hypertrophy and die; the cartilage extracellular matrix they construct is then invaded by blood vessels, osteoclasts, bone marrow cells and osteoblasts, the last of which deposit bone on remnants of cartilage matrix. The sequential changes in chondrocyte behaviour are tightly regulated by both systemic factors and locally secreted factors, which act on receptors to effect intracellular signalling and activation of chondrocyte-selective transcription factors. Systemic factors that regulate the behaviour of chondrocytes in growth cartilage include growth hormone and thyroid hormone, and the local secreted factors include Indian hedgehog, parathyroid hormone-related peptide, fibroblast growth factors and components of the cartilage extracellular matrix. Transcription factors that play critical roles in regulation of chondrocyte gene expression under the control of these extracellular factors include Runx2, Sox9 and MEF2C. The invasion of cartilage matrix by the ossification front is dependent on its resorption by members of the matrix metalloproteinase family, as well as the presence of blood vessels and bone-resorbing osteoclasts. This review, which places an emphasis on recent advances and current areas of debate, discusses the complex interactions between cell types and signalling pathways that govern endochondral ossification.

Keywords: Chondrocyte; Hypertrophy; Cell death; Extracellular matrix; Bone development

Contents

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMP, bone morphogenetic protein; DDR2, discoidin domain receptor-2; FGF, fibroblast growth factor; FGFR3, fibroblast growth factor receptor-3; GH, growth hormone; HDAC4, histone deacetylase-4; IGF, insulin-like growth factor; IGFR1, insulin-like growth factor receptor-1; Ihh, Indian hedgehog; MEF2C, myocyte enhancer factor-2C; MMP, matrix metalloproteinase; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; STAT, signal transducer and activator of transcription; T3, triiodothyronine; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor

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1. Introduction

Most bones develop through a process known as endochondral ossification, the initial stage of which is the formation of a cartilage model. During foetal development and postnatal growth, this model is gradually replaced by bone. Cartilage models are formed through condensation of mesenchymal cells, followed by their differentiation into chondrocytes and secretion of typical cartilage extracellular matrix components (Fig. 1A). The cartilage model once formed is invaded first at its centre and later at each end by a mixture of cells that establish the primary and secondary (respectively) centres of ossification (Fig. 1B–D). These centres of ossification gradually encroach on the remaining cartilage, ultimately replacing it completely (except at the articular surfaces) by the time skeletal maturity is achieved (Fig. 1E). The importance of the cartilage model lies not only in its provision of a mechanically stable template for bone formation, but also in its role as the source of longitudinal bone growth. The dynamic events that occur in growth cartilage leading to its replacement by bone will be the subject of this review. A number of recent reviews have described the roles of specific groups of molecules in endochondral ossification; the aim of this review is to provide an overview of the complex molecular and cellular interactions underlying the process of endochondral ossification, with an emphasis on recent advances and current areas of debate.

2. Morphology of growth cartilage

Cartilage that participates in endochondral ossification within developing and growing bones will be referred to in this review as growth cartilage. Growth cartilage is found in two locations at each end of a developing long bone: the growth plate and the articular-epiphyseal growth cartilage, which drive expansion of the primary and secondary centres of ossification, respectively (Fig. 1D). There are differences in the spatial organization of chondrocytes between the two locations, but these will not be discussed here (Byers & Brown, 2006).

No matter what the location or stage of development, chondrocytes in growth cartilage are arranged in morphologically distinct zones, which reflect changes in the functional state of the cells (Figs. 1 and 2A). The zone furthest from the ossification front is the zone of resting chondrocytes. Adjacent to this is the zone of proliferation; round proliferating chondrocytes become flattened as they are packed into multicellular clusters. Following proliferation, chondrocytes pass through a transition stage in which they are known as 'pre-hypertrophic' chondrocytes. These cells then undergo hypertrophy, increasing their volume dramatically, at the same time secreting extracellular matrix, which eventually becomes mineralised; chondrocyte proliferation and matrix secretion between them cause the elongation of the bone. Hypertrophic chondrocytes then die, and as they do so, the transverse septa of cartilage matrix surrounding them are broken down, leaving vertical septa largely intact, but allowing entry of the invading cells of the ossification front: blood vessels, osteoclasts (multinucleate bone-resorbing cells), and precursors of osteoblasts (bone-forming cells) and bone marrow cells. The osteoclasts assist in the removal of cartilage matrix, and the differentiating osteoblasts use the remnants of cartilage matrix as a scaffold for the deposition of bone matrix.

Chondrocytes in growth cartilage are usually considered to be a uniform population, but ultrastructural studies demonstrated many years ago that two types of cells, 'light' and 'dark', can be observed in growth cartilage (Fig. 2B–D; Hwang, 1978; Wilsman, Farnum,