Review

The cartilage extracellular matrix as a transient developmental scaffold for growth plate maturation

James Melrose[a,b,c], **Cindy Shu**[a], **John M. Whitelock**[b] and **Megan S. Lord**[b]

a - Raymond Purves Bone and Joint Research Laboratory, *Kolling Institute, Northern Sydney Local Health District, St Leonards, NSW 2065, Australia*
b - Sydney Medical School, *Northern, The University of Sydney, Royal North Shore Hospital, St Leonards, NSW 2065, Australia*
c - Graduate School of Biomedical Engineering, *University of New South Wales, Sydney, NSW 2052, Australia*

Correspondence to Megan S. Lord: *m.lord@unsw.edu.au*
http://dx.doi.org/10.1016/j.matbio.2016.01.008
Edited by Marian Young

Abstract

The cartilage growth plate is a specialized developmental tissue containing characteristic zonal arrangements of chondrocytes. The proliferative and differentiative states of chondrocytes are tightly regulated at all stages including the initial limb bud and rudiment cartilage stages of development, the establishment of the primary and secondary ossification centers, development of the growth plates and laying down of bone. A multitude of spatio-temporal signals, including transcription factors, growth factors, morphogens and hormones, control chondrocyte maturation and terminal chondrocyte differentiation/hypertrophy, cell death/differentiation, calcification and vascular invasion of the growth plate and bone formation during morphogenetic transition of the growth plate. This involves hierarchical, integrated signaling from growth and factors, transcription factors, mechanosensory cues and proteases in the extracellular matrix to regulate these developmental processes to facilitate progressive changes in the growth plate culminating in bone formation and endochondral ossification. This review provides an overview of selected components which have particularly important roles in growth plate biology including collagens, proteoglycans, glycosaminoglycans, growth factors, proteases and enzymes.

Introduction

The cartilage growth plate is a highly specialized developmental tissue. The earliest stages of long bone development occur through mesenchymal condensations of pre-chondrocytes which establish the limb buds (Fig. 1A) [1]. Initially single chondrocytes are contained within lacunae (Fig. 1B) and subsequently proliferate axially and lay down a cartilaginous extracellular matrix (ECM) that forms the cartilage rudiments which are a transient developmental scaffold with characteristic columnar arrangements of cells parallel to the long-axis of the developing limb surrounded by a perichondrium (Fig. 1C) [2]. The proliferating cells gradually undergo hypertrophy and expansion in cellular volume [2,3]. As was shown using time-lapse two-photon laser scanning microscopy of live avian embryonic cartilage [4], the chondrocytes undergo up to a 10-fold increase in intracellular volume and 2.7-fold increase in ECM volume accompanied by a 5-fold increase in metabolic activity [5]. The increase in cell size associated with hypertrophy is not simply due to increased hydration from the degradation and endocytosis of the condensed pericellular hyaluronan (HA) layer around these cells [6,7], but also an increase in the number and size of organelles and vacuoles in the hypertrophic chondrocyte. Hypertrophic cells synthesize increased levels of type X collagen and alkaline phosphatase in preparation for calcification of this tissue (Fig. 1D). The alkaline phosphatase is contained in vesicles which also contain calcium and are secreted by the hypertrophic cells. The matrix vesicles also contain matrix

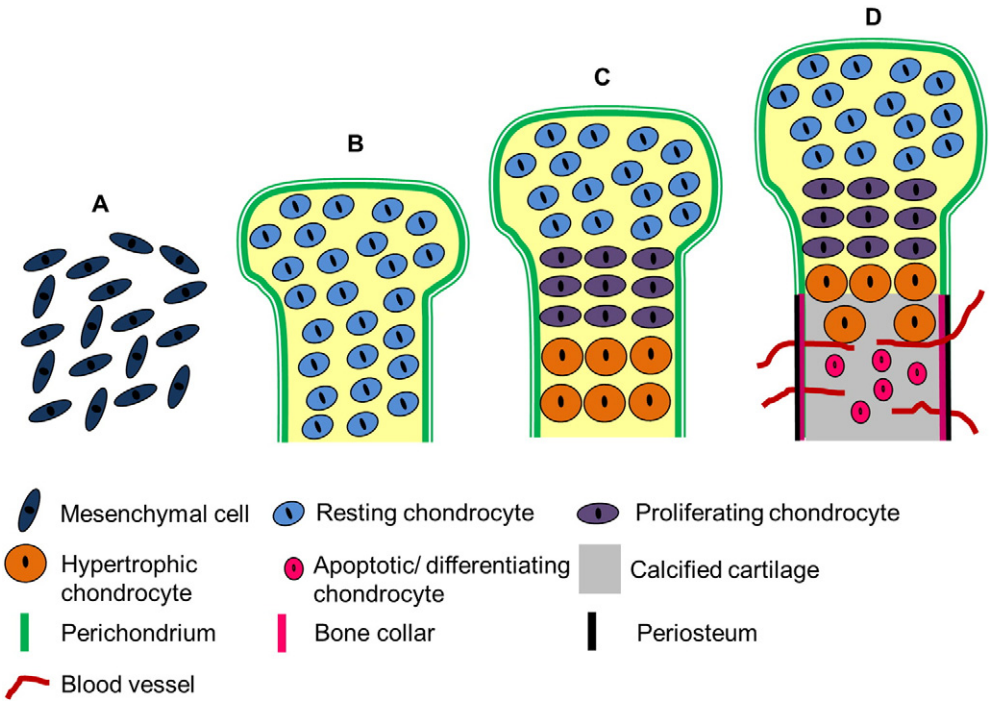

Fig. 1. Schematic depiction of the sequential stages in establishment of the growth plate: (A) mesenchymal cell condensation followed by (B) chondrogenic differentiation to form the cartilage template and cells located at the periphery form the perichondrium. (C) Development of proliferative and hypertrophic zones of chondrocytes. (D) Formation of the periosteal bone collar, calcification of the cartilage rudiment and vascularization into the perichondrium followed by the influx of osteoprogenitors to form osteoblasts and/or the differentiation of chondrocytes prior to the replacement of the calcified hypertrophic cartilage by bone.

metalloproteinases (MMPs) which remodel the ECM into a structure amenable to hydroxyapatite and calcium deposition. Osteoblasts differentiate from precursors in the perichondrium forming a bone collar (Fig. 1D) [8]. Blood vessels invade through this bone collar into the hypertrophic cartilage leading to vascularization and formation of the primary and secondary ossification centers from osteoblast precursors derived from the perichondrium and these are the fore-runners of the distal and proximal growth plates comprising trabecular bone (Fig. 1D) [9,10]. Until recently, it was accepted that hypertrophic chondrocytes undergo cell death prior to invasion by blood vessels and the formation of new bone. However, there is now evidence that some hypertrophic chondrocytes become osteogenic cells in fetal and postnatal endochondral bones [11].

The proliferative and differentiative states of chondrocytes are tightly regulated at all stages [12]. A multitude of spatio-temporal signals from growth factors/morphogens and mechanosensory cues originating in the ECM regulate these developmental processes. Specific transcription factors (Sox9, runt-related transcription factor 2 (Runx2), WNT, Indian hedgehog homolog (Ihh) and PTH-rP), growth factors (members of the fibroblast growth factor (FGF) family, transforming growth factor (TGF)-β superfamily including the morphogens bone morphogenic proteins (BMP) and vascular endothelial growth factors (VEGF)) and hormones (for example thyroid hormone) [13–18] coordinate sequential changes in chondrocyte morphology, proliferation, terminal differentiation and matrix assembly. These facilitate progressive changes in the growth plate culminating in bone formation and elongation of long bones in a process known as endochondral ossification [3,19,20]. The perichondrium surrounds the cartilage rudiments during early stages of limb/joint formation and remains at the surface of the permanent articular cartilages [1]. It is a source of perichondrial precursors that contribute to chondrocytes, osteoblasts, stromal cells and bone marrow stromal/mesenchymal progenitor cells [10,21] and is involved in the regulation of chondrocyte differentiation, such as through the expression of Patched, the receptor for Ihh [22]. Although the magnitude of the forces experienced by cells in the fetal growth period is small in comparison to those experienced postnatally, the rudiment growth plate