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On Bone-forming Cells and Blood Vessels In Bone Development

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Abstract

Replacement of non-vascular cartilage by bone and bone marrow is a critical step in bone development. In a recent issue of *Developmental Cell*, Maes *et al.* (2010) report that a distinct population of immature precursors of bone-forming cells migrate into the cartilage in intimate association with invading blood vessels.

The development of most bones, such as bones in the limbs and spine, proceeds via a two-stage process known as endochondral ossification. The architectural modelling of a bone takes place in the location of the future bone via assembly of a template. The template consists of hyaline cartilage, a non-vascular tissue composed of chondrocytes dispersed within a complex extracellular matrix. As the template grows and takes on the shape of the future bone, chondrocytes in the central region of the template stop proliferating, express a characteristic set of transcription factors, cytokines and matrix molecules, increase in size (become hypertrophic) and activate apoptotic cell death mechanisms. These changes set in motion a process that results in the conversion of the non-vascular cartilage template to an organ with compact bone tissue on the surface (cortical bone) and spongy (trabecular) bone tissue and highly vascularized bone marrow inside. During the past 25 years a number of significant studies have provided insights into the molecular mechanisms regulating differentiation of chondrocytes and osteoblasts from mesenchymal precursor cells and signals that control penetration of blood vessels into hypertrophic cartilage during endochondral ossification. However, the origin of the osteoblastic cells that produce the spongy/trabecular bone (also referred to as the primary spongiosa) in which hematopoietic bone marrow niches are established, has remained uncertain. Many cellular sources have been considered, including perichondrial cells on the cartilage surface, hypertrophic chondrocytes, pericytes associated with blood vessels penetrating into the cartilage, and progenitor cells circulating in the blood. Experimental support for the notion that osteoblasts may be generated from all these sources under various conditions exists, but no study providing definitive identification of the major origin of the cells that form the primary spongiosa during bone formation has been published, until now. In a recent study, Maes *et al.* (2010) advance the field by presenting compelling data in support of the conclusion that osteoblasts in the primary spongiosa are derived from immature precursors that invade into the cartilage in intimate association with blood vessels (Figure 1).

Differentiation of osteoblasts from mesenchymal cells requires the transcription factor Osterix (Osx) (Nakashima *et al.* 2002). Osterix is expressed at an early stage in the osteoblastic lineage, before cells reach the fully differentiated stage and start synthesizing extracellular components such as collagen type I. Taking advantage of the difference in

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timing of Osterix and collagen I expression, Maes *et al.* (2010) generated two transgenic mouse lines in which Cre recombinase (controlled either by the Osterix or a Collagen I promoter) could be activated in a tamoxifen-dependent manner to induce expression of β-galactosidase/LacZ in osteoblastic lineage cells. By controlling the timing of exposure of developing transgenic embryos in the two lines to a pulse of tamoxifen relative to the timing of endochondral ossification in the limbs, the investigators labelled early (Osx/LacZ+) and late stage (Col1/LacZ+) osteoblastic lineage cells as they differentiated in the perichondrial regions of developing bones. Electron microscopy suggested that about 60% of the Osx/LacZ+ cells were immature cells (large nuclei and sparse endoplasmic reticulum) and about a third of these cells were found in pericyte-like fashion along endothelial cells in blood vessels. The remaining Osx/LacZ+ cells were classified as early osteoblasts. In contrast, Col1/LacZ+ cells appeared to be fully differentiated osteoblasts, engaged in the process of forming cortical bone. Not surprisingly, further analyses indicated that some of the immature Osx/LacZ+ cells serve as precursors of the mature osteoblasts. However, the intimate association with blood vessels raised the possibility that they could have additional fates and functions.

Remarkably, as the two cell populations were followed during endochondral ossification, 70 % of Osx/LacZ+ cells were found in the spongy/trabecular bone region *inside* the developing bone, whereas Col1/LacZ+ cells remained predominantly on the *outside*, in the cortical region. Thus, a major subset of Osx/LacZ+ cells serves as precursors of the osteoblasts that form the primary spongiosa. Maes *et al.* (2010) also examined whether hypertrophic chondrocytes make a contribution to trabecular bone formation during endochondral ossification, a possibility that has long been considered, by following the fate of hypertrophic chondrocytes. They found that although some of the chondrocyte-like cells at the cartilage/perichondrial junction may contribute to bone formation, the majority of the hypertrophic chondrocytes do not become osteoblasts in the central region of the primary spongiosa, at least in mice (and, as pointed out by Maes *et al.*, in the “time span of our studies”).

An important question raised by these data is the mechanism by which only immature osteoblastic cells are recruited from the perichondrium into the primary ossification center together with vascular endothelial cells and osteoclasts. Based on previous studies (Zelzer *et al.* 2001; Zelzer *et al.* 2004) demonstrating that hypertrophic chondrocytes express high levels of VEGFA, a potent stimulator of both endothelial and osteoclastic migration, it seems likely that the mechanism includes VEGF-associated activities. One possibility suggested by the present study is piggybacking of immature osteoblast precursors on vascular endothelial cells as the vessels sprout and extend from the perichondrial region towards the hypertrophic cartilage inside the forming bone. Interestingly, Maes *et al.* (2010) provide preliminary data suggesting that Osx-positive cells express high levels of VEGF and of Angiopoietin-1 and PDGRβ, molecules that play a role in interactions between endothelial cells and pericytes. Finally, it is possible that immature osteoblastic progenitors differ from mature osteoblasts in that they, like vascular endothelial cells and osteoclasts, are able to respond to migratory signals, such as VEGF.

This study also provides a basis for addressing problems that go beyond the realm of bone formation. For example, with the osteoprogenitor population responsible for the formation of the trabecular bone *inside* endochondral bones defined and sorting techniques for the isolation of these cells from mixed populations available, the question of whether or how these cells and their extracellular matrix products contribute to the formation of hematopoietic stem cell niches, may now be addressed using the subcapsular kidney assay that Chan *et al.* (2009) recently used to demonstrate that endochondral ossification is required for hematopoietic stem cell niche formation. Given that Nestin-positive

mesenchymal stem cells in bone marrow were recently reported to constitute an essential component in the hematopoietic stem cell niche (Mendez-Ferrer *et al.* (2010), it will be important to examine whether they interact with or are related to these Osterix-expressing immature cells that give rise to the osteoblastic cells forming the primary spongiosa in endochondral bone.

Selected Reading

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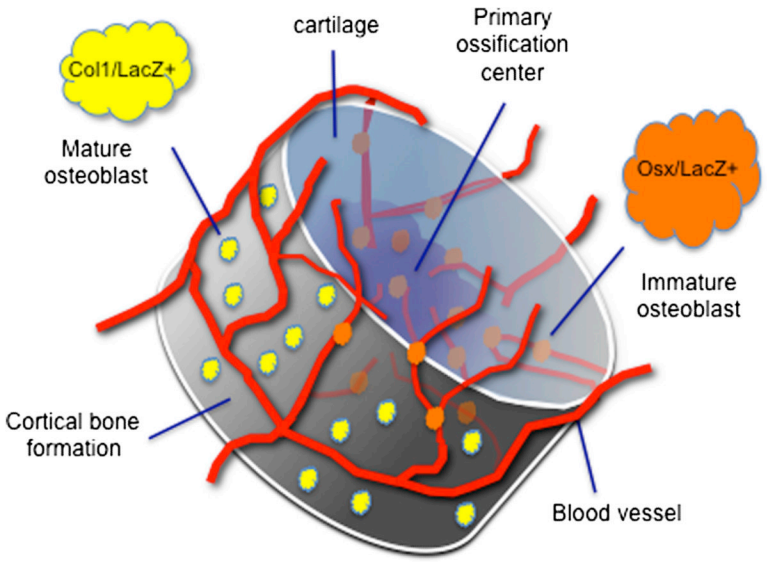

Figure 1.
Schematic drawing, incorporating the findings of Maes *et al.* (2010), of the middle portion of a developing endochondral bone. Mature osteoblasts on the outside form cortical bone, while immature precursors invade the cartilage and reach the primary ossification center in association with blood vessels.