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Review

# Roles of osteoclasts in the control of medullary hematopoietic niches

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ABSTRACT

Bone marrow is the major site of hematopoiesis in mammals. The bone marrow environment plays an essential role in the regulation of hematopoietic stem and progenitor cells by providing specialized niches in which these cells are maintained. Many cell types participate to the composition and regulation of hematopoietic stem cell (HSC) niches, integrating complex signals from the bone, immune and nervous systems. Among these cells, the bone-resorbing osteoclasts (OCLs) have been described as main regulators of HSC niches. They are not limited to carving space for HSCs, but they also provide signals that affect the molecular and cellular niche components. However, their exact role in HSC niches remains unclear because of the variety of models, signals and conditions used to address the question. The present review will discuss the importance of the implication of OCLs focusing on the formation of HSC niches, the maintenance of HSCs in these niches and the mobilization of HSCs from the bone marrow. It will underline the importance of OCLs in HSC niches.



## Introduction

In adults mammals, hematopoietic stem cells (HSCs)[1] are maintained in the bone marrow (BM) preferentially in the endosteal region of the bone [1–4]. In mouse, HSCs are defined by their negative expression of lineage markers ($Lin^-$) and the co-expression of Sca1 and c-kit (LSK cells). However, LSK cells are heterogeneous and comprise different HSC subsets with distinct self-renewal and differentiation capacity [5,6]. Additional markers are therefore useful to identify the more primitive HSCs, such as the expression of the endothelial protein C receptor (EPCR) [7], CD49d [8–10] and the "signaling lymphocyte activation molecule" (SLAM) family of proteins [11], the absence of CD34 expression [12], or a low level of reactive oxygen species (ROS) [13]. HSCs reside in specialized niches, a concept that has first been proposed by Schofield in 1978 [14]. These niches regulate the fate of HSCs in terms of quiescence, proliferation, migration and differentiation. Analysis of the cellular components and the regulation of these niches was the object of many studies in the last decade (reviewed in [15,16]. The major components of the HSC niches include mesenchymal stromal cells (MSCs) [17–19], CXC12-abundant reticular (CAR) cells [20,21], osteoblasts [22–24] and endothelial cells [11,25,26]. In addition, glial cells [27], sympathetic nervous system [28,29], regulatory T cells [30], macrophages [31–33] and osteoclasts [34] were shown to contribute to the regulation of the HSC niches.

This variety of cellular components and regulators is probably related to the heterogeneity of HSCs and may reflect the existence of specialized niches for distinct HSCs. Initially, in murine mutant models displaying a selective increased or a depletion in the osteoblastic compartment, osteoblasts were shown to regulate the pool of HSCs [22–24]. Osteoblasts and the other different components of the niches share the expression of stromal cell-derived factor 1 (SDF-1/CXCL12) and stem cell factor (SCF/Kit-ligand, KL), two major factors for the maintenance of HSCs. Later, selective conditional deletion of the genes encoding these factors in the different BM cells involved in the niches suggested that osteoblasts and CAR cells mainly maintain early lymphoid progenitors, while endothelial cells and perivascular MSCs mainly support HSCs with long-term repopulating activity and are involved in HSC mobilization in the blood [35–37]. In agreement with these data, HSCs are mostly localized in the endosteum, a region which is enriched in sinusoid vessels [2,11,16,22,38]. Moreover, BM structures associating endothelial cells and perivascular osteoprogenitors have been reported to maintain HSPCs [39] and the ablation arteriolar niches relocalizes HSCs to sinusoidal niches in which they switch from a

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[1] *Abbreviations used:* HSCs, hematopoietic stem cells; BM, bone marrow; SLAM, signaling lymphocyte activation molecule; ROS, reactive oxygen species; MSCs, mesenchymal stromal cells; CAR, CXC12-abundant reticular; EPCR, endothelial protein C receptor; M-CSF, macrophage colony-stimulating factor; bFGF, basic fibroblast growth factor; HSPCs, hematopoietic and progenitor cells; CaRs, calcium-sensing receptors.

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quiescent to a proliferating status, indicating that this status is dictated by specific localization [37,40]. The variety of niche components supporting progenitors with distinct functional capacity and that probably require specific regulatory signals, could also explain the variety of cells involved in their regulation.

Among these cells with regulatory function, osteoclasts (OCLs) are monocytic cells responsible for bone resorption. They are located at the endosteal region of the bone and are induced by two main osteoclastogenic factors, the receptor activator of nuclear factor kappa-B ligand (RANK-L) and the macrophage colony-stimulating factor (M-CSF). Bone resorbing OCLs produce proteases that cleave factors important for the HSC niche [34], they release calcium from the bone that regulate HSC homing and maintenance [41] and growth factors such as transforming growth factor β (TGF β) or basic fibroblast growth factor (bFGF) that regulate the fate of HSCs [42,43]. OCLs are also tightly coupled with osteoblasts and regulate their differentiation and function as well as the phenotype of MSCs representing osteoblast progenitors [44–46]. They also control BM angiogenesis that is required for the colonization and egress of HSCs from the BM [47,48]. Their location, their production of growth factors and proteolytic enzymes as well as their coupling with osteoblasts and angiogenesis point OCLs as potential regulators of HSC niches. However, their exact role remains unclear and controversial: OCLs are necessary for the formation and maintenance of HSC niches [46,49,50] and for stress induced mobilization [34,51], but they have been reported to be dispensable for G-CSF induced HSC mobilization in mice lacking functional OCLs [52,53]. Moreover, in osteopetrotic mice, the presence of inactive OCLs has been reported to be associated with an hematopoietic BM content different from the one observed in the absence of OCLs [54–56]. Here, we will review the different processes in which the participation of OCLs to HSC niches has been investigated and we will show that these contradictory data can be explained by the different stimuli and contexts in which this question has been addressed.

## Establishment of hematopoiesis in the bone marrow

During ontogeny, hematopoiesis first appears in the mouse embryo in the aorta-gonad-mesonephros region [57,58]. Then, hematopoiesis shifts sequentially to the fetal liver, the spleen and the bone marrow [57–59] (reviewed in [60]). Irreversible cell tracing of embryonic HSCs showed that they colonize the BM cavity via the fetal liver confirming that they contribute to adult hematopoiesis [61].

The migration of HSCs to the BM is closely connected to endochondral bone formation at the late phase of fetal life. This process is initiated by a mesenchymal condensation and the patterning of the future bone by cartilage (Fig. 1) (reviewed in [62,63]. The chondrocyte proliferation allows the cartilage anlagen to elongate. In the center of the cartilage, the chondrocytes mature, become hypertrophic and produce a mineralized cartilage matrix (Fig. 1). Hypertrophic chondrocytes are involved in the differentiation of perichondrial cells into osteoblasts and in the formation of blood vessels through the production of angiogenic factors allowing the arrival of osteoclastic and hematopoietic cells. Hypertrophic chondrocytes undergo apoptosis and the remaining cartilage matrix serves as a scaffold for bone formation by osteoblasts [63]. Osteogenesis, angiogenesis and bone resorption are coupled processes [44,48,64,65] and this bone formation is associated with the development of vascularization and of marrow space (Fig. 1) both allowing the HSCs to colonize the BM in the newly established protective niches.

The dependence of BM hematopoiesis on endochondral ossification is supported by studies in genetically modified mice or in models of ectopic transplantation. In *Cbfa1* KO mice deficient in

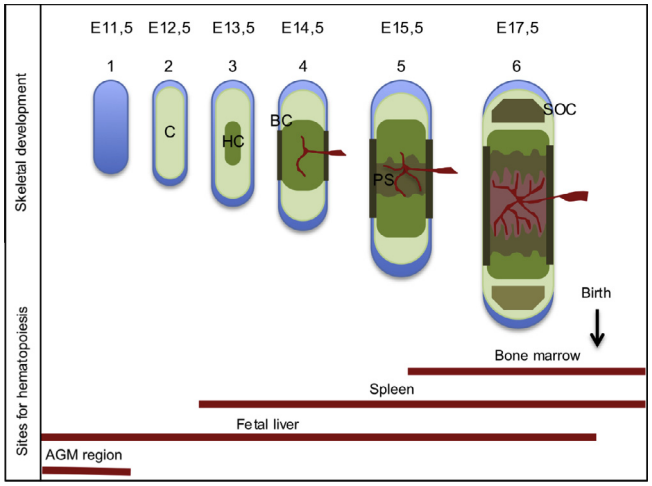


**Fig. 1.** Schematic diagram illustrating the link between the skeletal formation and hematopoiesis. (1) Endochondral ossification begins with mesenchymal condensation and (2) chondrocyte differentiation. (3) Then chondrocytes become hypertrophic in the center of the cartilage. (4) They induce the apparition of blood vessels and bone collar. (5) Vascularisation is accompanied by the development of bone primary spongiosa and the arrival of osteoclasts and hematopoietic cells. (6) Mineralized bone tissue replaces cartilage and the bone cavity is formed. HSCs can then colonize the bone marrow to establish the main site for hematopoiesis. The secondary ossification centers appear at the extremity of the bones. Red bars indicate the different sites of hematopoiesis in the mouse embryo during the skeletal formation. C = cartilage, HC = hypertrophic cartilage, BC = bone collar, PS = primary spongiosa, SOC = secondary ossification center.

endochondral bone formation, hematopoiesis was shown to be normal in the fetal liver until E17.5, but after this stage, the frequency of hematopoietic and progenitor cells (HSPCs) increases in the liver and spleen while no BM formation was observed [66]. Similar association between defect in BM hematopoiesis and impaired endochondral bone formation was found in *Collagen-X* deficient mice [67]. In an ectopic transplantation model of fetal bone cells in which the expression of Osterix, a factor essential for endochondral ossification, was inhibited, both osteogenesis and niche formation are severely reduced [68]. Subcutaneous transplantation of collagenous matrix in rats resulted in ectopic endochondral bone formation associated with the apparition of hematopoietic foci [69]. These observations strongly suggest that bone cells and bone remodeling are required for the generation of HSC niches.

## Requirement of OCLs for the formation of HSC niches in the bone marrow

The role of OCLs in the establishment of the BM niches remained unclear until recently. The absence of OCL activity in osteopetrosis results in BM failure, reduced or loss of BM hematopoiesis, and extramedullary hematopoiesis in the spleen and liver (Table 1) both in mice [70–77] and human [78–85]. These latest organs are the sites for fetal and perinatal hematopoiesis but they can maintain their hematopoietic function in case of the BM failure. Thus, the extramedullary hematopoiesis associated with osteopetrosis strongly suggests a link between OCL function and the HSC niches.

The implication of OCLs in the formation of HSC niches was suspected by the analysis of hematopoiesis in osteopetrotic mutant mice. *Oc/oc* mice are characterized by an absence of bone resorption due to inactive OCLs and by hematological defects [77,86–88]. The absence of bone resorption results in an alteration of the bone structure leading to a persistence of mineralized and unmineralized cartilage suggesting an impaired endochondral ossifica-