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Review

A reciprocal regulatory interaction between megakaryocytes, bone cells, and hematopoietic stem cells

Melissa A. Kacena *, Caren M. Gundberg, Mark C. Horowitz

Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, P.O. Box 208071, New Haven, CT 06520-0871, USA

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Abstract

A growing body of evidence suggests that megakaryocytes (MK) or their growth factors play a role in skeletal homeostasis. MK have been shown to express and/or secrete several bone-related proteins including osteocalcin, osteonectin, bone sialoprotein, osteopontin, bone morphogenetic proteins, and osteoprotegerin. In addition, at least 3 mouse models have been described in which MK number was significantly elevated with an accompanying marked increase in bone mineral density. Mice overexpressing thrombopoietin, the major MK growth factor, have an osteosclerotic bone phenotype. Mice deficient in transcription factors GATA-1 and NF-E2, which are required for the differentiation of MK, exhibited a strikingly increased bone mass. Importantly, recent studies have demonstrated that MK can stimulate osteoblast (OB) proliferation and differentiation in vitro and that they can also inhibit osteoclast (OC) formation in vitro. These findings suggest that MK play a dual role in skeletal homeostasis by stimulating formation while simultaneously inhibiting resorption.

Conversely, cells of the osteoblast lineage support hematopoiesis, including megakaryopoiesis. Postnatal hematopoiesis occurs almost solely in the bone marrow (BM), close to or on endosteal surfaces. This finding, in conjunction with the observed contact of OB with hematopoietic cells, has lead investigators to explore the molecular and cellular interactions between hematopoietic cells and cells of the OB lineage. Importantly, it has been shown that many of the cytokines that are critical for normal hematopoiesis and megakaryopoiesis are produced by OB. Indeed, culturing osteoblasts with CD34+ BM cells significantly enhances hematopoietic cell number by both enhancing the proliferation of long-term culture initiating cells and the proliferation and differentiation of MK. These data are consistent with cells in the OB lineage playing a critical role in the hematopoietic niche. Overall, these observations demonstrate the importance of MK–bone cell interactions in both skeletal homeostasis and hematopoiesis.

Keywords: Megakaryocytes; Osteoblasts; Osteoclasts; Megakaryopoiesis; Hematopoiesis; Skeletal homeostasis; Bone formation; Niche

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* Corresponding author. Fax: +1 203 737 2529.
E-mail address: melissa.kacena@yale.edu (M.A. Kacena).

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Introduction

Although cells of the hematopoietic and skeletal systems are in close juxtaposition in the BM, it was previously thought that they had little functional relationship. However, research has demonstrated that cell–cell and cell–cytokine interactions between these systems are critical for normal bone cell growth and development, skeletal homeostasis, and hematopoiesis, including megakaryopoiesis. A new paradigm has evolved in which regulatory interactions between skeletal and hematopoietic cells have been identified. This review will focus on the interactions between MK and bone cells, in the context of skeletal homeostasis and hematopoiesis.

The role of megakaryocytes in skeletal homeostasis

Bone-related proteins expressed/secreted by megakaryocytes

Evidence for a role of MK in bone formation comes from data indicating that MK express or secrete the bone matrix proteins osteocalcin, osteonectin, bone sialoprotein, and osteopontin [1–5]. Most recently, it was demonstrated that MK express BMP-2, -4, and -6 [6]. These data suggest that MK could, under the appropriate circumstances, such as high local concentrations, contribute to bone formation by the secretion of these proteins. MK have also been reported to stimulate the differentiation of osteoblasts (OB) as defined by enhanced expression of procollagen [7]. MK express several receptors that are known to be involved in the regulation of bone remodeling (such as estrogen receptors, NMDA-type glutamate receptors, and calcium-sensing receptors) [8–11]. MK also express specific growth factor receptors for transforming growth factor beta [TGFβ] receptors I and II, platelet-derived growth factor [PDGF], and vascular endothelial growth factor [VEGF] [12,13], all of which have shown to have marked effects on bone cells.

MK may also play a role in osteoclastogenesis as documented by the localization and expression of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) in MK [7,8,14–17]. The fact that MK express RANKL suggests that they may be an additional vector for OC induction, particularly during inflammatory responses.

In contrast, MK expression of OPG suggests that MK may also play a role in inhibiting osteoclastogenesis. Recent data by our laboratory demonstrate that, in vitro, MK and MK CM inhibit OC development by up to 10-fold [18]. We examined MK CM for known inhibitors of osteoclastogenesis by ELISA and could demonstrate that low levels of OPG were present [18]. However, Chagraoui et al. (2003) did not find OPG in MK CM, suggesting that the OPG, if secreted, was not detectable because it bound to the MK expressed RANKL [7,16,17]. Importantly, in our work, the addition of anti-OPG antibody failed to neutralize the ability of MK CM to inhibit OC formation, suggesting that MK-secreted OPG was not responsible for the inhibition of OC development. Next, we confirmed that OPG was not responsible for the MK-mediated inhibition of OC development by testing MK derived from OPG deficient mice. These experiments demonstrated that MK from OPG deficient and control mice inhibited OC formation equivalently. Finally, using tandem mass spectrophotometry, we demonstrated that there exists a factor or factors in MK CM that inhibit OC development, and while the identity of this inhibitory factor remains to be determined, it was not any of the major factors known to inhibit OC formation including OPG, interleukin-4 (IL-4), IL-10, IL-12, IL-13, IL-18, interferon gamma (IFN-γ), TGFβ, granulocyte–macrophage colony-stimulating factor (GM-CSF), OC inhibitory lectin (OCIL), calcitonin, amylin, and calcitonin-gene-related peptide [18].

Mouse models

There are three documented mouse models which are characterized by increased numbers of MK and a concomitant alteration in bone phenotype. It had been previously established that mice overexpressing TPO, the main MK growth factor, develop a phenotype characterized by increased numbers of MK, resulting in development of myelofibrosis and osteosclerosis by 9 months of age [19–22]. We have recently identified two novel mouse model systems in which loss of the transcription factors GATA-1 or NF-E2, which are restricted to cells in the hematopoietic lineage, develops phenotypes characterized by a marked increase in MK with a concomitant reduction or absence of platelets and a massive increase in trabecular and cortical bone [23–25].

GATA-1 and NF-E2 deficient mice

As shown in Fig. 1, MK arise from pluripotential hematopoietic stem cells and pass through a series of identifiable stages of differentiation, culminating in terminally differentiated MK that release platelets. The molecular dissection of the MK differentiation pathway has been greatly facilitated by the identification of transcription factors required for the cell's successful advance from stage to stage (Fig. 1). Loss of these specific factors precludes the cells from continued maturation and results in the accumulation of cells at the latest stage of differentiation prior to the arrest. The selective loss of two different transcription factors, GATA-1 and NF-E2, which were originally thought to be required exclusively for erythroid lineage development, has now been shown to play a critical role in MK differentiation. GATA-1 knock-down mice and NF-E2 knock-out mice exhibit a phenotype characterized by marked megakaryocytosis and thrombocytopenia [23,24].

The GATA family of zinc finger transcription factors is presently composed of six members, GATA-1 through GATA-6 in vertebrates. GATA is a single polypeptide chain with DNA binding activity in the C-terminal zinc finger [26].