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The skeletal vascular system – Breathing life into bone tissue

Steve Stegen, Geert Carmeliet *

Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, 3000 Leuven, Belgium
Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, 3000 Leuven, Belgium

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ABSTRACT

During bone development, homeostasis and repair, a dense vascular system provides oxygen and nutrients to highly anabolic skeletal cells. Characteristic for the vascular system in bone is the serial organization of two capillary systems, each typified by specific morphological and physiological features. Especially the arterial capillaries mediate the growth of the bone vascular system, serve as a niche for skeletal and hematopoietic progenitors and couple angiogenesis to osteogenesis. Endothelial cells and osteoprogenitor cells interact not only physically, but also communicate to each other by secretion of growth factors. A vital angiogenic growth factor is vascular endothelial growth factor and its expression in skeletal cells is controlled by osteogenic transcription factors and hypoxia signaling, whereas the secretion of angiocrine factors by endothelial cells is regulated by Notch signaling, blood flow and possibly hypoxia. Bone loss and impaired fracture repair are often associated with reduced and disorganized blood vessel network and therapeutic targeting of the angiogenic response may contribute to enhanced bone regeneration.

1. Introduction

A functional vascular network is a prerequisite for normal tissue development, homeostasis and repair. Skeletal blood vessels supply different cell types in the bone environment with oxygen and nutrients, but also serve as a source for hormones, growth factors and calcium and phosphate, the building blocks for matrix mineralization. In addition, skeletal and endothelial cells interact reciprocally by paracrine signaling. Indeed, skeletal cells secrete angiogenic factors whereas endothelial cells produce angiocrine factors that regulate skeletal cell behavior. Finally, the bone marrow vascular system serves as a specialized microenvironment that promote maintenance of stem and progenitor cells. This strong link between blood vessels and skeletal tissue is not only observed during bone development, where there is a close connection between angiogenesis and osteogenesis, but also during aging and in different skeletal pathologies that are associated with altered vasculature. Increasing insight into the molecular and cellular processes orchestrating the angiogenic cascade may help to develop novel treatments for bone healing, especially for clinical situations with a limited angiogenic host response. In this review, we first discuss the current knowledge of developmental skeletal angiogenesis and its regulation by angiogenic growth factors. We here focus on the role of vascular endothelial growth factor, a pivotal angiogenic factor, and its regulation by hypoxia signaling. We refer the reader for information on other angiogenic regulators to some excellent recent reviews [1,2]. In the second part, we discuss the importance of the vascular system during bone pathology and repair.

2. Bone development: close interaction between osteogenesis and angiogenesis

Bones are highly vascularized tissues, whether they are formed through endochondral or intramembranous ossification. Both bone-forming processes are tightly coupled to angiogenesis, the growth of new blood vessels from existing ones. During endochondral ossification, mesenchymal cells condense and form clusters within avascular regions [3,4]. Osteochondroprogenitor cells in the central part of the condensation differentiate into chondrocytes, which start to proliferate and form a cartilaginous template for future bone deposition. Chondrocytes in the center of this cartilage anlage stop to proliferate, become hypertrophic and secrete pro-angiogenic factors (Fig. 1A). Concurrently, mesenchymal cells in the outer layer of the cartilage template (*i.e.* the perichondrium) differentiate to osteoprogenitors, which also produce pro-angiogenic factors. Blood vessels are attracted to first invade the perichondrium and then the region of hypertrophic chondrocytes.

Abbreviations: BMP, bone morphogenetic protein; COL2, type II collagen; CXCL9, C-X-C motif (CXC)-chemokine; Dll4, delta-like 4; EC, endothelial cell; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; MMP, matrix metalloproteinase; mTORC1, mechanistic target of rapamycin complex 1; PHD, prolyl hydroxylase domain protein; PlGF, placental growth factor; POC, primary ossification center; Runx2, Runt-related transcription factor 2; SOC, secondary ossification center; T2DM, type 2 diabetes mellitus; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

* Corresponding author at: Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, O&N1 Herestraat 49 box 902, 3000 Leuven, Belgium.
E-mail address: geert.carmeliet@kuleuven.be (G. Carmeliet).

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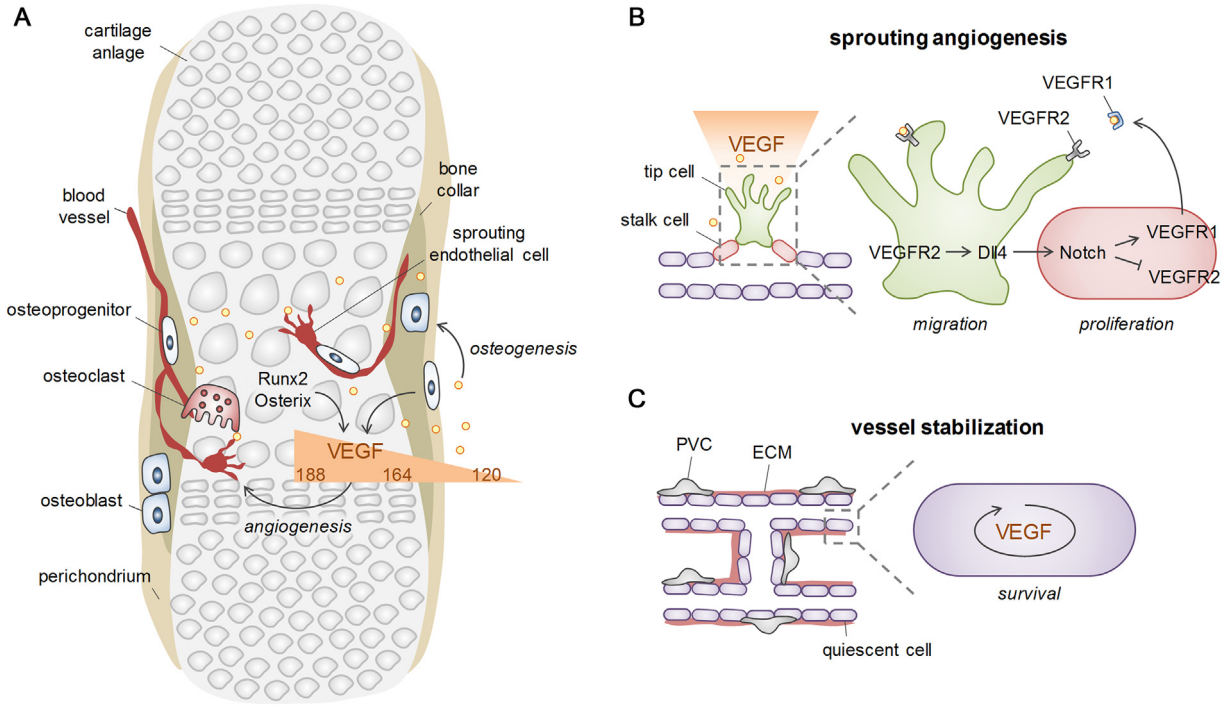

Fig. 1. Angiogenesis during early bone development. (A) During embryogenesis, the initiation of neovascularization of the cartilage anlage is coordinated by the induction of vascular endothelial growth factor (VEGF) expression in perichondrial osteochondroprogenitors and hypertrophic chondrocytes in the cartilage template, mainly by transcription factors such as Runx2 and Osterix. The combined secretion of three VEGF isoforms (VEGF120, VEGF164 and VEGF188) results in a VEGF gradient that controls guided sprouting angiogenesis. Blood vessel invasion is accompanied by osteoclastic cartilage resorption and osteoprogenitors that move to the nascent primary ossification center. (B) Sprouting angiogenesis in general is triggered by VEGF that induce sprouting and migration of a few endothelial cells, the tip cells, by activating VEGF receptor 2 (VEGFR2) signaling. Tip cell VEGFR2 activation also increases expression of the Notch ligand Delta-like 4 (Dll4) to initiate Notch signaling in neighboring endothelial cells. In these so-called stalk cells, Notch signaling regulates proliferation, but inhibits tip cell behavior by downregulating VEGFR2, while upregulating VEGFR1. (C) Vessel stabilization relies on the recruitment of perivascular cells (PVCs) and deposition of extracellular matrix (ECM) by the quiescent ECs, which depend on (autocrine) VEGF signaling for survival.

Concomitantly with the invasion of blood vessels into the cartilage template, Osterix-positive osteoprogenitors from the perichondrium move along [5]. The cartilage is degraded by the invading osteoclasts and replaced by trabecular bone, formed by osteoblasts, and a bone marrow cavity in which the hematopoietic bone marrow cells reside [3,4]. By these coordinated actions, the primary ossification center (POC) is formed. The epiphyseal growth plates that are thereby formed at both ends of the long bones further mediate longitudinal bone growth. The hypertrophic chondrocytes and cartilage matrix at the chondro-osseous junction are continuously replaced by trabecular bone, which is associated with angiogenic growth in the metaphysis and capillary invasion of the hypertrophic chondrocyte region. Simultaneously, the epiphyseal growth plate expands in size and because of its avascular nature the chondrocytes in the center become hypoxic and start to produce pro-angiogenic factors. Comparable to the formation of the POC, blood vessels, osteogenic cells and osteoclasts invade the epiphyseal cartilage and form the secondary ossification center (SOC) [3,4].

Whereas most skeletal elements are formed through endochondral ossification, most craniofacial bones and part of the clavicle are formed by intramembranous ossification, in which mesenchymal cells directly differentiate into osteoblasts [4,6]. These cells produce pro-angiogenic factors that attract blood vessels, which further promote osteogenesis. In this review, we will mainly focus on the link between endochondral ossification and angiogenesis.

During endochondral ossification, the invasion of blood vessels into the avascular cartilage template to form the POC is through a process resembling sprouting angiogenesis [7]. This highly branched vasculature further expands during the longitudinal and radial growth of the long bones. In early postnatal life, the vascular network obtains its characteristic organization, consisting of arteries, a dense capillary network and a large central draining vein. Typical for bone, two types of capillaries are present that form a single network [8]. From the arteries, the blood circulates first to capillaries present at the endosteum and in the metaphysis, close to the growth plate where they display a columnar structure, interconnected by distal loops or arches that are juxtaposed to the hypertrophic chondrocytes. These capillaries are surrounded by Osterix-positive osteoprogenitors and are also called type H vessels because of their **h**igh expression of CD31 and endomucin. The type H capillaries are connected through transition vessels to the highly branched sinusoidal vessels in the diaphysis that are surrounded by hematopoietic cells. These so-called type L vessels are characterized by **l**ow expression of CD31 and endomucin, and drain into the central vein [8]. This vascular organization has several consequences. First, it may affect the oxygen and nutrient delivery to the local microenvironment. Indeed, the metaphysis and endosteal region are considered to be less hypoxic than the diaphysis [8,9], although conflicting data exist [10]. This regional hypoxia may be due to the serial organization of the two capillary systems and thus reduced delivery of oxygen in the diaphysis. On the other hand, the high number of metabolically active hematopoietic cells in this region may lead to high oxygen consumption rate and thereby contribute to low oxygen levels. Second, the blood flow and shear rate are higher in the arteriolar capillaries than in the sinusoids [11,12]. Third, the endosteal capillaries display a tight endothelial blood-bone marrow barrier, whereas the sinusoids are more fenestrated and the vascular wall is thus more permeable [11]. The slower blood flow and higher permeability of the sinusoids promotes leucocyte trafficking primarily at this site, whereas quiescent hematopoietic stem cells (HSCs) mainly reside near endosteal capillaries, indicating the importance of vascular permeability in modulating HSC quiescence and leukocyte trafficking.

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