REVIEW

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Bone Vasculature and Bone Marrow Vascular Niches in Health and Disease

Junyu Chen,[1] Michelle Hendriks,[2,3] Alexandros Chatzis,[1] Saravana K Ramasamy,[2,3] and Anjali P Kusumbe[1]

[1]Tissue and Tumor Microenvironments Group, The Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK
[2]Institute of Clinical Sciences, Imperial College London, London, UK
[3]MRC London Institute of Medical Sciences, Imperial College London, London, UK

ABSTRACT

Bone vasculature and bone marrow vascular niches supply oxygen, nutrients, and secrete angiocrine factors required for the survival, maintenance, and self-renewal of stem and progenitor cells. In the skeletal system, vasculature creates nurturing niches for bone and blood-forming stem cells. Blood vessels regulate hematopoiesis and drive bone formation during development, repair, and regeneration. Dysfunctional vascular niches induce skeletal aging, bone diseases, and hematological disorders. Recent cellular and molecular characterization of the bone marrow microenvironment has provided unprecedented insights into the complexity, heterogeneity, and functions of the bone vasculature and vascular niches. The bone vasculature is composed of distinct vessel subtypes that differentially regulate osteogenesis, hematopoiesis, and disease conditions in bones. Further, bone marrow vascular niches supporting stem cells are often complex microenvironments involving multiple different cell populations and vessel subtypes. This review provides an overview of the emerging vascular cell heterogeneity in bone and the new roles of the bone vasculature and associated vascular niches in health and disease. © 2020 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: ANGIOGENESIS; BONE; BONE MARROW VASCULAR NICHES; BONE VASCULATURE; CHONDROCYTE; OSTEOBLAST; OSTEOCLAST

Introduction

Mammalian skeletons contain an intricate network of blood vessels that supply a plethora of blood to the bone, around 10% to 15% of resting cardiac output.[1] In addition to being a transport network, blood vessels in bone play multiple roles in the maintenance of bone homeostasis during physiological and pathological conditions. Recent evidence shows the presence of heterogenic blood vessel subtypes and specialized vascular microenvironments in the bone. For instance, a specific subtype of blood vessels termed type H blood vessel, defined by high expression of CD31 and endomucin, was identified to support osteoprogenitors in the coupling of angiogenesis and osteogenesis[2–4] during developmental bone formation, bone repair, bone modeling, and bone remodeling.[5,6] Functional interactions of blood vessels with the skeletal tissue are regulated by complex intercellular crosstalk between bone lineage cells and vascular cells. In addition, endothelial cells (ECs) and perivascular cells release crucial factors in a paracrine or juxtracrine mode, also termed "angiocrine signals," to regulate the behavior of neighboring cells in the complex bone microenvironment.[7,8] Vascular cells have also been identified to be part of niches that control the maintenance and functions of hematopoietic stem cells (HSCs) and derived hematopoietic progenitors in the bone.[4,9] Overall, blood vessels play a critical role in regulating specialized microenvironments that are directly associated with the bone and blood physiology.

In this review, we provide an overview of the distribution and identification of blood vessel subtypes in the bone, their functions during physiological processes, and contributions in pathological conditions such as osteoporosis, osteoarthritis, osteonecrosis, bone metastasis, and bone malignancies. Finally, we discuss the importance of vasculature in bone tissue engineering to explore potential relevance in therapy and repair.

Pattern and Diversity of Blood Vessels in Bone

Blood vessels are distributed throughout the bone tissue except in cartilaginous areas such as the growth plate.[10–14] Similar to most other tissues, the bone vasculature consists of arteries from

Received in original form June 1, 2020; revised form July 21, 2020; accepted August 5, 2020. Accepted manuscript online August 26, 2020.
Address correspondence to: Anjali P Kusumbe, PhD, Kennedy Institute of Rheumatology, University of Oxford, Roosevelt Drive, Headington, Oxford OX3 7FY, UK. E-mail: anjali.kusumbe@kennedy.ox.ac.uk
Journal of Bone and Mineral Research, Vol. 35, No. 11, November 2020, pp 2103–2120.
DOI: 10.1002/jbmr.4171

which oxygenated blood enters the bone and veins by which blood exits, connected through a network of capillaries.[13,15] Long bones such as the tibia and femur have been reported to have several types of arteries surrounding and penetrating the bone.[12,16] The principal nutrient artery (PNA) as historically reported penetrates the cortical bone near the middle of the tibia's length in the diaphysis, slightly closer to the distal end.[17,18] In the diaphysis, the PNA splits into ascending and descending central arteries, branching into arterioles as they run toward the metaphysis.[16] A more recent study suggests that murine long bones have 16 nutrient arteries.[15] Periosteal arteries and Haversian arteries run along the bone's outer surface and in the cortex, respectively. They are bridged by Volkmann's arteries, the particular configurations of which vary from species to species.[14] Haversian and Volkmann's arteries are accompanied by nerves, and Haversian arteries further converge at the metaphysis.[12] Nutrient arteries supply the medulla and the inner two-thirds of the cortex; the other one-third of the cortical blood supply is derived from the periosteal system.[12,16,18] Finally, epiphyseal and metaphyseal arteries enter the bone at its ends. Metaphyseal arterioles join the blood supply from the central artery in the metaphysis. Epiphyseal arterioles supply capillaries in the epiphysis, which form a completely separate network.[12,16] Flat bones such as the cranial bones are perfused primarily by periosteal arteries, though they also have nutrient and metaphyseal arteries. The configuration of the blood supply in irregular bones such as mandible is not well-studied but appears to vary.[12,16] The mandible, for instance, is supplied by three arteries—the lingual, facial, and inferior alveolar—which provide periosteal and internal blood supplies in varying configurations, including nutrient arteries, with possible independent vascular domains perfusing different regions of the mandible.[12,19]

The bone capillary network fills the marrow cavity and consists mostly of dense, fenestrated, and highly branched sinusoidal vessels. However, the linear columnar arrangement of capillaries is predominant in the metaphysis and endosteum, where most of the arteriolar blood supply is directed.[2,10,14,20] These columnar vessels are interconnected at their distal ends, adjacent to the growth plate in the metaphysis, by structures termed as loops or arches.[2,3,10] Sinusoidal and columnar vessels are interconnected, forming a single vascular network. The main capillary network drains into a large central vein found in the diaphysis of long bones.[3,10] Smaller veins branch off the central venous sinus and exit the bone into periosteal veins.[13] There are also two exit sites for the central vein at the two ends of the bone.[15] The epiphyseal capillaries drain separately into a smaller vein.

The linear or columnar vessels in the metaphysis and endosteum can be distinguished and identified by high expression levels of the cell surface markers endomucin and CD31, also known as Pecam1. Due to the high expression of these cell surface markers, these vessels are termed "type H" vessels, as opposed to the type L diaphyseal sinusoidal vessels, which express low levels of these markers (Fig. 1).[2,4,10,12] Because type H vessels are[2] fed directly by arterioles, type H vessels exhibit higher partial pressure

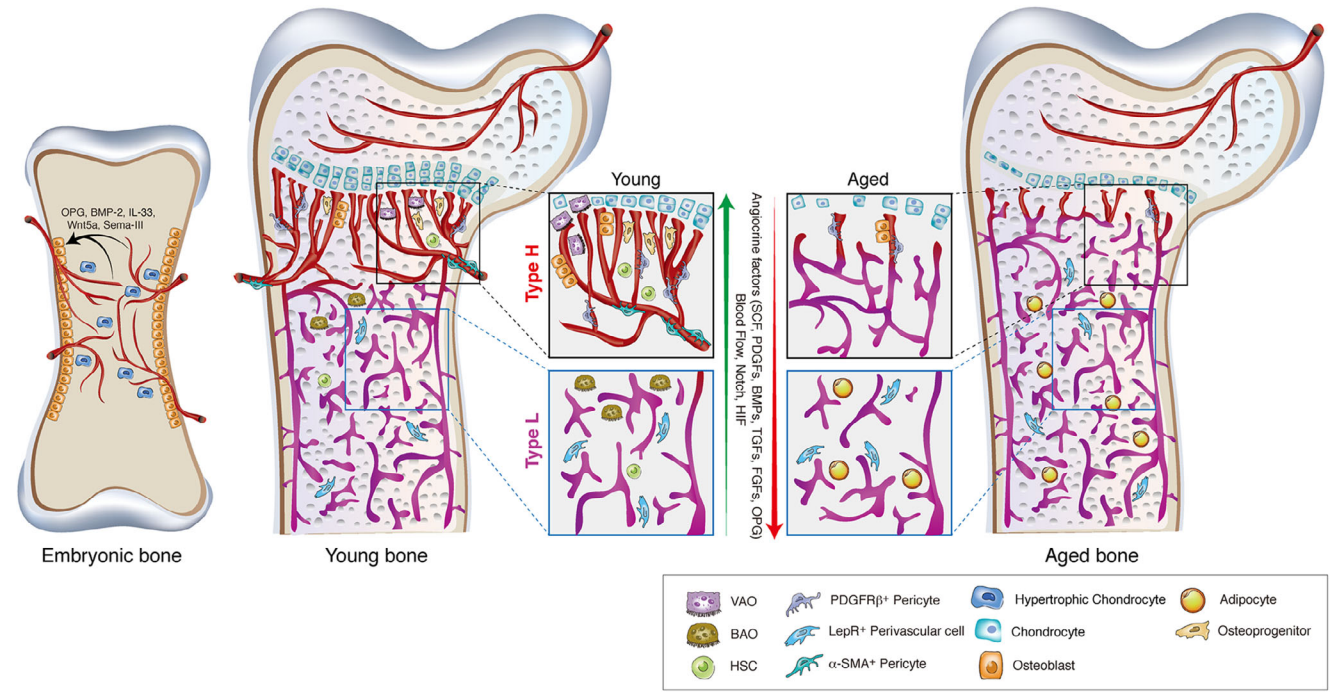

Fig 1. Schematics showing blood vessel organization and niche microenvironments in long bones during development, homeostasis, and aging. During the embryonic development, hypertrophic chondrocytes at the primary ossification center secrete pro-angiogenic factors that stimulate blood vessel invasion, and blood vessels produce angiocrine factors to promote bone formation. In the young bone, type H vessels with the columnar organization and arterial connections are found in the metaphysis, and sinusoidal type L vessels are located at the diaphysis. Higher magnification insets show cells perivascular to the type H vessels and the interactions of type H vessels and type L vessels with other cell types in the bone. BAO = bone-associated osteoclast; BMP = bone morphogenetic protein; FGF = fibroblast-derived growth factor; HIF = hypoxia-inducible factor; HSC = hematopoietic stem cell; IL-33 = interleukin-33; LepR = leptin receptor; OPG = osteoprotegerin; PDGF = platelet-derived growth factor; SCF = stem cell factor; TGF = transforming growth factor; VAO = vessel-associated osteoclast.

of oxygen (pO_2) and blood velocity than type L vessels.[4,21] The differing properties of type H and type L vessels have functional consequences in terms of generating tissue microenvironments. For instance, the lower permeability of type H vessels and nearby arterioles creates an environment low in reactive oxygen species (ROS).[12] Because of differential gene expression profiles, different capillary subtypes support distinct perivascular cell types, which further impacts the local microenvironment.[4]

Recently the presence of transcortical vessels (TVCs) in bone has been shown.[15] Three-dimensional X-ray microscopy and light-sheet fluorescence microscopy showed 100s of small TCVs of either arterial or venous features traversing cortical bone. These arterioles and venules are directly connected in the endosteum, providing a short "hyperloop" for blood flow in and out of the bone cavity.[15]

Distribution and Heterogeneity of Perivascular Cells in the Bone

Blood vessels consist of an innermost layer of ECs, outside of which perivascular or mural cells are located.[10] The types of perivascular cells in bone vary with vessel subtype. Pericyte coverage of capillary walls varies between species, ranging from 51% in humans to 71% in mice; this may be reduced when large numbers of hematopoietic cells are being egressed.[14] Perivascular cells of type L vessels are leptin receptor (LepR)-expressing stromal cells or C-X-C motif chemokine 12, CXCL12-expressing cells termed as CXCL12 abundant reticular (CAR) cells. The CAR cell subsets can act locally as cytokine-secreting cells and establish perivascular microniches.[22] The perivascular cells of type H vessels express platelet-derived growth factor receptor β (PDGFRβ), Nestin, and NG2. Arterioles have perivascular cells similar to those found with type H vessels, and larger arteries have perivascular cells that express smooth muscle actin (α-SMA).[10] However, it is not completely clear the extent to which different perivascular cell types in bone overlap or are entirely distinct.[10]

Many perivascular cell types exhibit characteristics of mesenchymal progenitors and can differentiate into osteogenic, chondrogenic, or adipogenic lineages depending on the signals they receive. Lineage tracing studies illustrate that Nestin-expressing cells found on arteries and type H vessels represent early mesenchymal stem and progenitor cells (MSPCs), with the potential to generate a wide range of cell types in the bone marrow stroma and bone lineages.[23] LepR-expressing cells at type L vessels contribute to the bone lineage during early development, but to the adipocyte lineage later in adults. CAR cells support hematopoietic stem cells (HSCs) and the HSC niche.[10,23–25] In addition, quiescent $CXCL12^+$ bone marrow stromal cells in perisinusoidal space can transform into osteoblast precursor cells in a manner mediated by canonical Wnt signaling during bone regeneration.[26] Another mesenchymal cell type that preferentially associates with type H vessels is Osterix and Runx2 expressing.[2,3] These cells are rarely detected around the type L sinusoidal vessels. Osterix and Runx2 expressing cells are associated with a wide range of lineages during neonatal bone development, including the bone marrow stroma, bone, chondrocyte, and adipocyte lineages.[23] However, they show more limited potential in adults, as osteoblast precursors, which play an essential role in active bone repair and remodeling.[12,23] Further, a recent study shows that a new type of adipogenic lineage cells containing no lipid droplets, named as marrow adipogenic lineage precursors (MALPs), play critical roles in maintaining marrow vasculature and suppressing bone formation.[27]

Functions of Bone Vasculature During Bone Development

After mesenchymal condensation, hypoxia and hypoxia-inducible factor-1α (HIF-1α), regulate chondrogenesis, osteogenesis, and angiogenesis in bone analgen. HIF-1α plays a pleiotropic role in regulating chondrogenesis process, supporting the growth of chondrocytes by utilizing an anaerobic metabolism during bone development.[28,29] Moreover, hypoxia is a crucial regulator of vascular endothelial growth factor (VEGF) expression, and HIF-1α can trigger downstream VEGF signaling pathways.[30,31] Accordingly, the conditional deletion of HIF-1α in osteoblasts results in reduced bone volume and bone vascularity,[32] and the overexpression of HIF-1α leads to improved osteogenesis and angiogenesis.[33]

During early mouse development, around embryonic day 15 (E15), hypertrophic chondrocytes at the primary ossification centre (POC) secrete pro-angiogenic factors to stimulate blood vessel invasion.[10,34] This leads to blood vessel invasion into the cartilage and further extends toward the epiphysis of growing bone accompanied by ossification processes.[35] Because of the coupling of bone formation and blood vessel growth, VEGF derived from cartilage promotes the vascular invasion and enhance osteogenesis.[36,37] Conversely, ECs release angiocrine factors such as transforming growth factor-beta 1 (TGFβ1), which upregulates connective tissue growth factor (CTGF) in the perichondrium, regulating surrounding cells during early bone formation.[38]

In actively growing bones, type H vessels couple angiogenesis and osteogenesis and regulate bone development.[2] The proximity and dense arrangement of $Osterix^+$ osteoprogenitors to type H ECs provide a resource of osteoblasts for bone formation.[2] Importantly, the type H blood vessels secrete osteogenic factors such as platelet-derived growth factors (PDGFs), fibroblast growth factor1 (FGF1), and transforming growth factors (TGFs) to support osteoprogenitor cell survival and proliferation.[2] Further, type H vessels support vessel-associated osteoclasts (VAOs) through a RANKL–RANK signaling pathway to promote cartilage resorption and directional bone formation.[39] Matrix metalloproteinases (Mmps), including Mmp2, Mmp9, and Mmp14, derived from type H ECs are crucial for cartilage resorption to promote longitudinal bone growth.[39] The abundance of the type H vessels declines with age leading to the decline in osteoprogenitor numbers and thereby bone mass.[4] Genetic or pharmacological reactivation of type H vessels in aged mice leads to an increase in osteoprogenitor numbers and bone volume.[4]

Moreover, molecular and mechanistic investigations indicated that numbers of angiocrine or angiogenic factors such as HIF-1α, PDGF-BB, and slit guidance ligand 3 (SLIT3) can couple angiogenesis and osteogenesis.[2,40,41] Wnt5a secreted from ECs mediates the β-catenin signaling pathway, which regulates osteogenesis.[42,43] Semaphorin-3a derived from ECs actively target on both osteoclast and osteoblast function during bone formation.[44–46] Interleukin-33 (IL-33) produced by a subset of CD105 expressing ECs in bone marrow facilitate osteogenesis and hematopoiesis.[47]

Blow Flow and Oxygenation Patterns in Bone

The vascular organization in bone dictates both blood flow and oxygenation patterns in the bone. Young adult long bones exhibit a largely centrifugal blood flow pattern.[1,12,16,48] Thus

blood enters the bone tissue centrally, through high-pressure central arteries, and flows outward toward the lower-pressure periosteal system. However, centripetal flow patterns can also be observed in some situations. These include cases of injury, such as destruction of the nutrient artery or bone fracture. In this case, blood flow from the periosteum can increase to compensate for the loss of central blood flow.[1,18] Centripetal patterns are also observed in aged individuals due to progressive ossification of blood vessels in the bone marrow.[16] The overall level of blood flow also has significant functional consequences. For instance, there is a positive effect of skeletal muscle use or stimulation on bone mass and vice versa.[1,13] Muscle contractions directly lead to an increase in bone blood flow due to increased interstitial fluid flow and intramedullary pressure, which then stimulates bone growth.[13,16,49]

The mean pO2 in the bone marrow of healthy human volunteers is around 6.6%, whereas in other healthy tissues the median interstitial pO2 is 3% to 9%.[13,50] Because arteries feed into the capillary network at the metaphysis and endosteum, those regions exhibit a higher pO2 than the central sinusoidal regions of the bone marrow.[3,10,14,21] Mathematical models predict that pO2 may fall to as low as 1% in distant capillary regions of the bone marrow.[13] Interestingly, this oxygenation profile is significantly altered in response to stresses such as irradiation and chemotherapy.[21] The major consumption of oxygen in the bone is by the hematopoietic cells. In line with this, ablation of hematopoiesis significantly increases oxygen levels in the bone marrow space.[25] Furthermore, pO2 varies depending on the vicinity of either arteriolar or venular vessels, with pO2 being higher in the former case.[15] The pO2 heterogeneity has critical functional consequences for different cell types and is an important defining feature of multiple and distinct vascular niches in bone.[3,13,50]

Bone Marrow Vascular Niches for Hematopoietic Stem Cells

Bone vasculature has an established function in providing specialized niches to support HSCs. Further recent studies show that HSCs identified by novel markers such as α-catulin and Hoxb5 reside mainly in perivascular niches.[51,52] Under complex niche microenvironments, HSCs interact with different types of vascular cells,[25,53,54] and angiocrine factors regulate the functions of HSCs.[55,56] For example, multiple types of ECs secrete stem cell factor (SCF), which is essential for HSC maintenance and erythropoiesis.[57] CXCL12 from perivascular stromal cells, ECs and osteoblasts is an important chemokine for HSC maintenance and homeostasis.[24,58] Accordingly, EC-specific deletion of CXCl12 or SCF leads to decline in HSC numbers and reduced HSC repopulating activity.[24,59] Further, type H blood vessels play an essential role in HSC maintenance. Activation of Notch signaling in ECs results in the expansion of type H vessels, increase in PDGFRβ-positive, Nestin-positive, and NG2-positive perivascular cells and enhanced SCF levels.[8] Notably, EC-specific activation of HIF signaling solely promotes type H capillaries but fail to enhance HSC numbers, suggesting that ECs, mainly, arterial cell and type H EC Notch signaling, are a critical regulator of HSCs frequency.[4] Such interactions between HSC and their complex vascular niches have been extensively reviewed.

Interleukins (ILs) are produced by multiple cell types, including ECs and regulate HSCs. For instance, EC-derived IL-33 regulates the HSC fate.[47] Perivascular stromal cell–derived IL-7 maintain a pro-B cell niche associated with the HSC niche, which plays a critical role in early B lymphocyte development.[60,61] Further, ECs express a range of toll-like receptors (TLRs), which sense pathogens and contribute to the hematopoietic response. Upregulation of TLR4 and MyD88 proteins by ECs in response to lipopolysaccharide are required to recruit neutrophils and to trigger emergency granulopoiesis.[62,63] The response of vascular niches to different stress conditions and their impact on HSCs has been reviewed elsewhere.[9]

Coupling of Angiogenesis and Osteogenesis in Bone

Coupling of angiogenesis with osteogenesis suggests the existence of molecular crosstalk between ECs and cells of the bone lineage (Fig. 2, Table 1). Mainly, this coupling is mediated by ECs of type H blood vessels through their osteogenesis promoting signals. Conversely, bone lineage cells also have been reported to release multiple factors that enhance angiogenesis.

Diverse roles of hypoxia inducible factor (HIF-1)

HIF is an important transcriptional regulator that mediates hypoxia-related intracellular signaling pathways and supports neo-angiogenesis.[64,65] HIF1-α is one of the subunits of HIF heterodimers and is essential for physiologic and pathological vascularisation as well as bone formation and regeneration.[10,66] HIF1-α plays a vital role in regulating chondrogenesis and supporting chondrocytes by utilizing anaerobic metabolism during collagen production and bone development.[28,29] The age-dependent reduction of type H ECs and bone loss is associated with a decrease in expression of HIF1-α. Genetic and pharmacological experiments show that endothelial HIF1-α promotes the formation of type H vessels and osteogenesis. Accordingly, EC-specific inactivation of HIF1-α leads to a decrease in the number of type H vessels and decline in osteogenesis.[2] EC-specific deletion of Von Hippel–Lindau (*Vhl*), which stabilizes endothelial HIF1-α, increases type H vessel angiogenesis and osteogenesis.[2] Administration of iron chelator deferoxamine mesylate, which stabilizes HIF-1α by inactivating prolyl hydroxylase domain-containing protein (PHD) enzymes,[67] significantly increases type H vessels, osteoprogenitors, and osteoblasts in aged mice.[2] Further, HIF1-α and HIF2-α can also transcriptionally regulate the expression of extracellular matrix genes including collagen type II, and the absence of HIF1-α affects chondrogenesis and osteogenesis due to impaired extracellular matrix secretion.[28,68,69] Taken together, HIF1-α is one of the key players involved in the coupling of angiogenesis and osteogenesis during bone formation under hypoxia.[33]

Role of endothelial Notch in bone formation

Notch signaling is an important pathway to modulate cell–cell interactions. In most soft tissues, Notch signaling restricts EC proliferation and sprouting and negatively controls angiogenesis.[70,71] Surprisingly, activation of Notch signaling in the bone leads to enhanced angiogenesis and osteogenesis.[8] Genetic manipulation of a Notch receptor inactivator (Fbxw7) led to increased type H vessels, osteoprogenitors, metaphyseal distal arches, and secretion of Noggin from ECs, which is an antagonist of BMPs.[8] Conversely, EC-specific Notch loss-of-function mice showed loss of type H vessels, defective vascular endothelial growth factor (VEGFA) expression, irregular growth plates, and shortened bones.[8] A disintegrin and metalloproteinase domain

(ADAM) family metalloprotease ADAM10 mediates Notch signaling activation as it triggers proteolytic processing, thus allowing the Notch intracellular domain to enter the nucleus and activate Notch-dependent genes, such as *Hes1*, *Hes5*, *Hey1*, and *Hey2*.[72] EC-specific deletion of ADAM10 results in impaired bone formation, growth plate defects, and increased pathological neovascularisation.[72] Interestingly, blood flow positively regulates Notch signaling. Hence type H vessels with smaller diameter, which possess higher blood flow, leading to Notch activation and promotion of angiogenesis and osteogenesis.[3] In contrast, decreased blood flow can downregulate Notch signaling.[3] Therefore. Notch signaling is a crucial component in the crosstalk between vascular cells and cells of the bone lineage during bone formation and remodeling.

Role of VEGFA during bone development

VEGFA is a critical regulator of physiological and pathological angiogenesis in bone and most other tissues.[73] Bone lineage cells such as hypertrophic chondrocytes, osteoblasts, and osteoclasts can secrete VEGFA, thereby controlling migration and proliferation of ECs, hypertrophic cartilage remodeling, ossification, and angiogenesis.[10,36,74] VEGFR2 (Flk-1) and VEGFR1 are the primary tyrosine kinase receptors for VEGFA. EC-specific deletion of VEGFR2 results in reduction and disorganization of blood vessels in the metaphyseal region closed to the growth plate.[75]

VEGFA exists in multiple isoforms—$VEGF^{120}$, $VEGF^{164}$, and $VEGF^{188}$ in mouse and $VEGF^{121}$, $VEGF^{145}$, $VEGF^{165}$, $VEGF^{183}$, $VEGF^{186}$, $VEGF^{189}$, and $VEGF^{206}$ in human, which elicit diverse biological processes.[76,77] Nevertheless, reduced endochondral angiogenesis and mineralization occurs in mice expressing only $VEGF^{120}$ isoform.[78] Mice with a combined loss of $VEGF^{164}$ and $VEGF^{188}$ demonstrate reduced angiogenesis, perturbed vessel invasion into hypertrophic chondrocytes, impaired bone formation and shortened limbs.[77,79] The temporal–spatial effect and appropriate concentration of VEGFA are crucial for blood vessel growth and bone formation. High concentrations of VEGFA can elevate osteoclast recruitment, increasing bone resorption and bone loss.[80–82]

PDGF-B in the bone marrow microenvironment

PDGF-BB secreted from ECs and preosteoclasts is critical in supporting migration, proliferation, and differentiation of various bone marrow–derived mesenchymal cell types to promote angiogenesis and osteogenesis.[41,83–86] During the processes of both bone modeling and remodeling, PDGF-BB can bind to PDGFRβ and then trigger mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling cascade, promoting the formation of type H blood vessels and bone.[41,85] Monocytes differentiate into periosteal tartrate-resistant acid phosphatase (TRAP)-positive mononuclear cells during bone formation and release PDGF-BB, which induces periostin expression and recruitment of periosteum-derived cells (PDCs) to the periosteum. These cells, in turn, differentiate into osteoblasts leading to enhanced osteogenesis and type H vessel formation.[87] Further, $PDGFR\beta^{+}$ skeletal stem and progenitor cells (SSPCs) mainly reside in perivascular niches, and PDGF-PDGFRβ signaling induces SSPC proliferation, trafficking, and angiotropism during bone injury.[88]

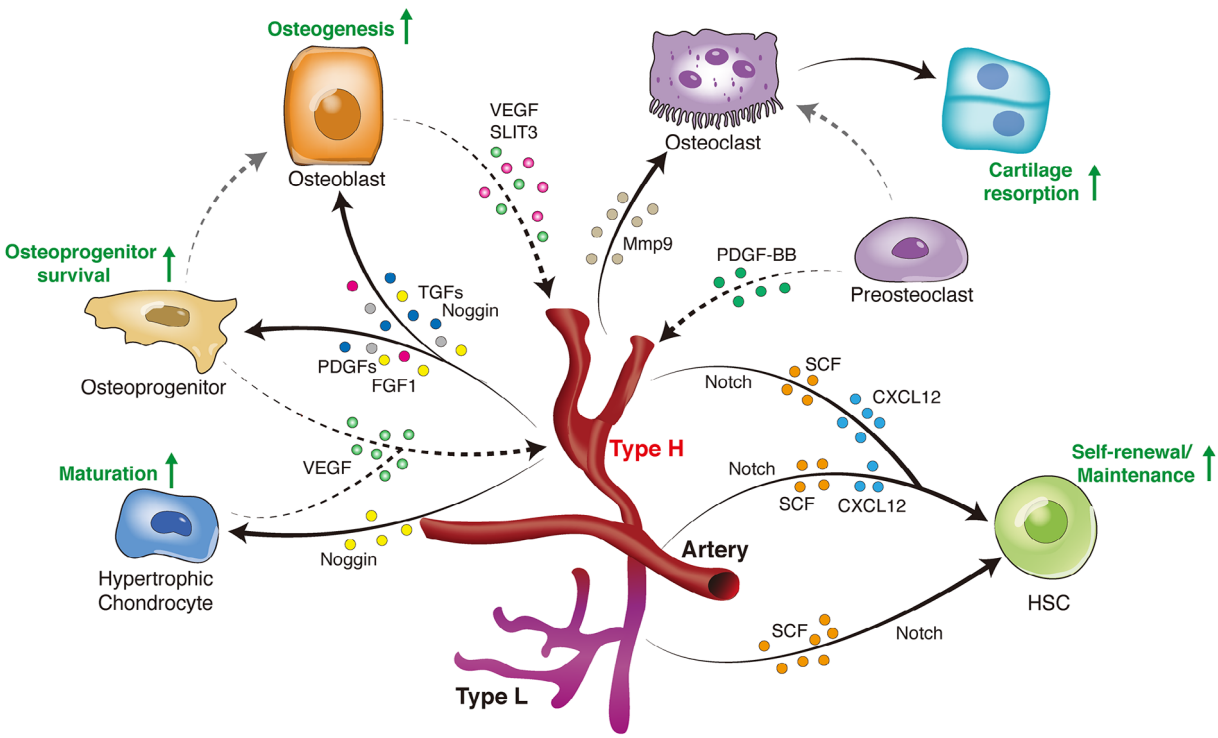

Fig 2. Blood vessel–derived angiocrine factors and their target cell-types in bones. Type H vessels release various angiocrine factors to promote survival, proliferation, and differentiation of bone lineage cells and thereby drive osteogenesis. CXCL12 = C-X-C motif chemokine 12; FGF = fibroblast-derived growth factor; HSC = hematopoietic stem cell; Mmp = matrix metalloproteinase; PDGF = platelet-derived growth factor; SCF = stem cell factor; SLIT3 = slit guidance ligand 3; TGF = transforming growth factor; VEGF = vascular endothelial growth factor.

Table 1. Blood Vessel–Derived Signals in Physiological and Pathological Conditions

Factor	Source cell	Target cell	Condition	Function	Reference
Ang-1	Perivascular mesenchymal cells	HSCs	Homeostasis	HSC quiescence and maintenance	Arai and colleagues[124]
BMP-2	ECs	Osteoblasts; chondrocytes	Bone remodeling; inflammation	Osteogenesis: endochondral bone formation	Bouletreau and colleagues[106]; Basic-Jukic and colleagues[147]
BMP-4	ECs	HSPCs	Homeostasis	HSPC expansion and maintenance	Kobayashi and colleagues[125]
CXCL12	Perivascular mesenchymal cells; ECs	HSCs	Development; normal	HSC quiescence and maintenance	Ding and colleagues[24]
DHH	ECs	HSPCs	Homeostasis	HSPC expansion and maintenance	Kobayashi and colleagues[125]
EGFL7	ECs	ECs	Normal; injury; tumor	Physiological and pathological angiogenesis	Parker and colleagues[177]
E-selectin	ECs	Leucocytes; fibroblasts	Rheumatoid arthritis	Leucocyte and fibroblast migration	Kriegsmann and colleagues[141]; Klimiuk and colleagues[142]; Zimmermann-Geller and colleagues[143]
FGF1	ECs	Osteoprogenitor	Normal	Osteoprogenitor survival and proliferation	Kusumbe and colleagues[2]
FGF2	ECs	HSPCs	Normal; bone repairing	HSC self-renewal	Kigami and colleagues[117]
ICAM-1	ECs	Leucocytes; fibroblasts	Rheumatoid arthritis	Leucocyte and fibroblast migration	Klimiuk and colleagues[142]
IGFBP2	ECs	HSPCs	Normal	HSPC expansion and maintenance	Kobayashi and colleagues[125]
IL-6	ECs	HSPCs	Inflammation; myelosuppressive injury	HSPC proliferation and differentiation	Poulos and colleagues[149]
IL-7	Perivascular mesenchymal cells; ECs	Pro-B cells	Development; normal	Early B lymphocyte development	Pillai and colleagues[60]; Dias and colleagues[61]
IL-33	$CD105^{+}$ ECs	HSCs; osteoblasts	Development; bone remodeling	Hematopoiesis; osteogenic differentiation	Kenswil and colleagues[47]
Jag-1	ECs	HSCs	Homeostasis	HSC self-renewal and regeneration	Poulos and colleagues[118]
Jag-2	ECs	HSCs	Aging; myelosuppressive injury	HSC protection; hematopoietic recovery	Saçma and colleagues[103]
Thrombospondin-1	ECs	DTCs	Tumor	DTC quiescence	Ghajar and colleagues[76]
Mmps	type H ECs	Chondrocytes	Normal; bone repairing	Cartilage resorption; bone elongation; bone remodeling;	Romeo and colleagues[39]
Nidogen-1	Perivascular stromal cells	Pro-B cells	Homeostasis	Pro-B cell retention; hematopoiesis	Balzano and colleagues[217]

(Continues)

Table 1. Continued

Factor	Source cell	Target cell	Condition	Function	Reference
Noggin	ECs	Osteoblasts; osteoprogenitor; chondrocytes	Development; normal	Osteogenic differentiation	Ramasamy and colleagues[8]
NOS2	ECs	Osteoblasts	Homeostasis	Negative regulation of osteoblast differentiation	Veeriah and colleagues[218]
OPG	ECs	Osteoclasts	Diabetic conditions	Anti-osteoclastogenesis	De Ciriza and colleagues[148]
PDGFs	ECs	Osteoprogenitor	Homeostasis	Osteoprogenitor survival and proliferation	Kusumbe and colleagues[2]
SCF	ECs; Perivascular cells	HSCs	Development; normal	HSC maintenance and homeostasis	Comazzetto and colleagues[57]
Sema-III	ECs	Osteoblasts; Osteoclasts	Normal; bone remodeling; osteoporosis	Anti-osteoclastogenesis	Serini and colleagues[45]; Kang and Kumanogoh[46]
TGFβ	ECs	Osteoprogenitor	Development; normal; bone repairing	Osteoprogenitor survival and proliferation; mesenchymal condensation	Kusumbe and colleagues[2]; Song and colleagues[38]
Tenascin-C	ECs	HSCs	Normal; inflammation	Hematopoietic regeneration	Nakamura-Ishizu and colleagues[56]
Timps	type H ECs	Chondrocytes	Normal; bone repairing	Cartilage resorption; bone elongation; bone remodeling;	Romeo and colleagues[39]
TNF-α	ECs	HSPCs	Inflammation; myelosuppressive injury	HSPC proliferation and differentiation	Poulos and colleagues[149]
VCAM-1	ECs	Leucocytes; fibroblasts; DTCs	Rheumatoid arthritis; tumor	Leucocyte and fibroblast migration: DTC protection	Klimiuk and colleagues[142]
von Willebrand factor	ECs	DTCs	Tumor	DTC protection	Carlson and colleagues[182]

Vascular roles of fibroblast growth factor

Fibroblast growth factors (FGFs) are mainly secreted by bone lineage cells, including chondrocytes and osteoblasts,[89,90] and the receptors FGFR1 and FGFR2 expressed in vascular cells.[91] FGFs induces the expression of multiple angiogenic molecules, including VEGFA and VEGFR2, stimulating the growth of blood vessels and contributing to chondrocyte proliferation and the initiation of chondrocyte hypertrophy.[92,93] Mice lacking FGF2 show reduced trabecular bone volume,[94] and mutations in FGF signaling molecules cause skeletal malformations in humans.[90] The absence of FGF9 and FGF18 leads to a reduction in the numbers of hypertrophic chondrocytes and chondrocyte proliferation, delayed skeletal vascularization, and reduced osteoblast/osteoclast recruitment to the growth plate.[95,96] Moreover, EC-specific deletion of FGFR1 and FGFR2 induce loss of pericytes and increases vessel permeability in the bone.[97]

Aging of Bone Vasculature and Bone Marrow Vascular Niches

Skeletal aging is not only associated with the loss of bone mass but also involves striking changes in vasculature and vascular niches in bone (Fig. 1). Blood circulation to the bone is severely reduced upon aging.[98] The decrease in the number of arterioles upon aging leads to reduced blood flow to the bone.[3] Because blood flow is critical for inducing and maintaining the Notch signaling, a drop of blood flow leads to a decline in endothelial Notch, which is a critical regulator of type H blood vessels.[3] This loss of Notch signaling leads to reduced type H vessels, osteoprogenitor cell numbers, and bone formation.[3] In line with this, EC-specific activation of Notch signaling in aged mice increases not only new type H vessel formation but also promotes perivascular niche function and the abundance of HSCs.[4] However, the functionality of HSCs is not improved in these niche-activated aged mice, due to the cell-autonomous aspects of HSC aging, such as the accumulation of DNA damage.[7,99] Further, androgens via the androgen receptor in neurons can slow the age-related cortical thinning in mice.[100]

Age-associated decrease in HSC functionality in congruence with the changes in the cellular composition of the bone marrow is indicative of the critical role played by the microenvironment in regulating HSC aging. In addition to changes in blood vessels, another apparent age-dependent change in the bone marrow microenvironment is the dramatic alterations in the mesenchymal cells in bone. Along with the reduction in osteoprogenitors, PDGFRβ-positive, NG2-positive, and Nestin-positive perivascular cells decline, while the adipocytes increase with reduced functionality of HSCs. Increases in the BM fat content with age is indicative of the alteration in lipid metabolism with age.[101,102] A subpopulation of HSCs with high regenerative capacity, active Notch signaling, and cellular polarity resides predominantly in perisinusoidal niches in the aged mice.[103]

Role of Bone Vasculature in Bone Repair and Regeneration

The invasion and reorganization of blood vessels are essential processes in bone repair and regeneration. After bone damage, inflammatory exudation and hematoma formation occur due to the disruption of local blood vessels, which leads to chronic hypoxia with enhanced expressions of HIF-1α, VEGF, and bone morphogenetic protein 2 (BMP-2).[104–106] Following this, new vessel invasion occurs from the bone marrow, compact bone, and the periosteum to the fracture site to reestablish the local blood supply and recruit osteoblast precursors, forming a soft callus containing fibroblasts and chondroblasts.[10,48] Next, vascularisation and ossification occur in order to convert the soft callus into rigid calcified tissue, followed by further mineralization and normalization of the vasculature at the fracture site.[107,108] Interestingly, the obstruction of vascular invasion during bone healing favors chondrogenic over osteogenic differentiation of skeletal progenitor cells, which is driven by SOX9 acting as a regulator of cellular metabolism by suppressing oxidation of fatty acids.[109]

During bone repair, several pro-angiogenic and angiocrine factors participate in inducing vascularisation and new bone growth. For instance, VEGFA is an essential proangiogenic factor that promotes bone repair. VEGFR1 negatively regulates blood vessel growth and inhibit fracture repair,[110,111] whereas placental growth factor (PIGF), a ligand for VEGFR1, can facilitate bone healing.[112,113] FGF signaling is another crucial signaling pathway for bone repair,[114] and increased expressions of FGFs and FGF receptors are found during bone healing[115]; specifically, FGF2 and FGF9 stimulate angiogenesis and osteogenesis during bone repair and regeneration.[116–118] TGFβ, BMP-2, BMP-7, and growth differentiation factor (GDF) also lead to stimulation of angiogenesis and osteogenesis during the healing process.[119–121] Further, angiocrine crosstalk via Notch signaling has been known to promote fracture repair.[122] EC-specific deletion of the Notch ligand Jag1 demonstrates a decrease of HSC regeneration after bone marrow ablation.[123] Besides, ECs upregulate *Fgf2*, *Bmp4*, *Igfbp2*, and *Angiopoietin1*, leading to the expansion of haemopoietic stem progenitor cells (HSPCs), which contribute to hematopoietic recovery and bone repair after acute injury to the bone marrow microenvironment such as chemotherapy and irradiation.[124,125]

Type H vessels could play a crucial role in cartilage resorption to enhance bone formation in repair and regeneration.[39] SLIT3 can promote EC migration via roundabout homologue signaling.[126] Conditional deletion of SLIT3 leads to a decline in type H vessels and impaired bone repair, whereas overexpression of SLIT3 increases the abundance of type H vessels and accelerates fracture healing.[40] Low-intensity pulsed ultrasound increases spinal fusion by enhancing type H vessels during spinal fusion.[127] Moreover, expansion of type H vessels after irradiation and chemotherapy mediates regenerative angiogenesis and promotes pericyte expansion via blood flow-mediated secretion of PDGF-B.[128]

Importance of blood flow in the bone healing process has been widely recognized.[128] Aged mice with decreased blood flow demonstrate impaired bone repairing ability.[129] This is in line with a study in elderly individuals with reduced blood flow to bone, demonstrating reduced bone healing capacity.[130] Changes in blood supply to bone is related to disuse-induced osteopenia conditions such as bed rest and hindlimb unloading.[131,132] Recently, manipulation of blood flow has been found to improve responsiveness to both irradiation and chemotherapy in mouse models of metastatic breast cancer.[128]

Maladaptation of Bone Vasculature in Diseases and Stress

Blood vessels and their angiocrine factors are critical for bone formation, stem cell functions, bone repair, and bone

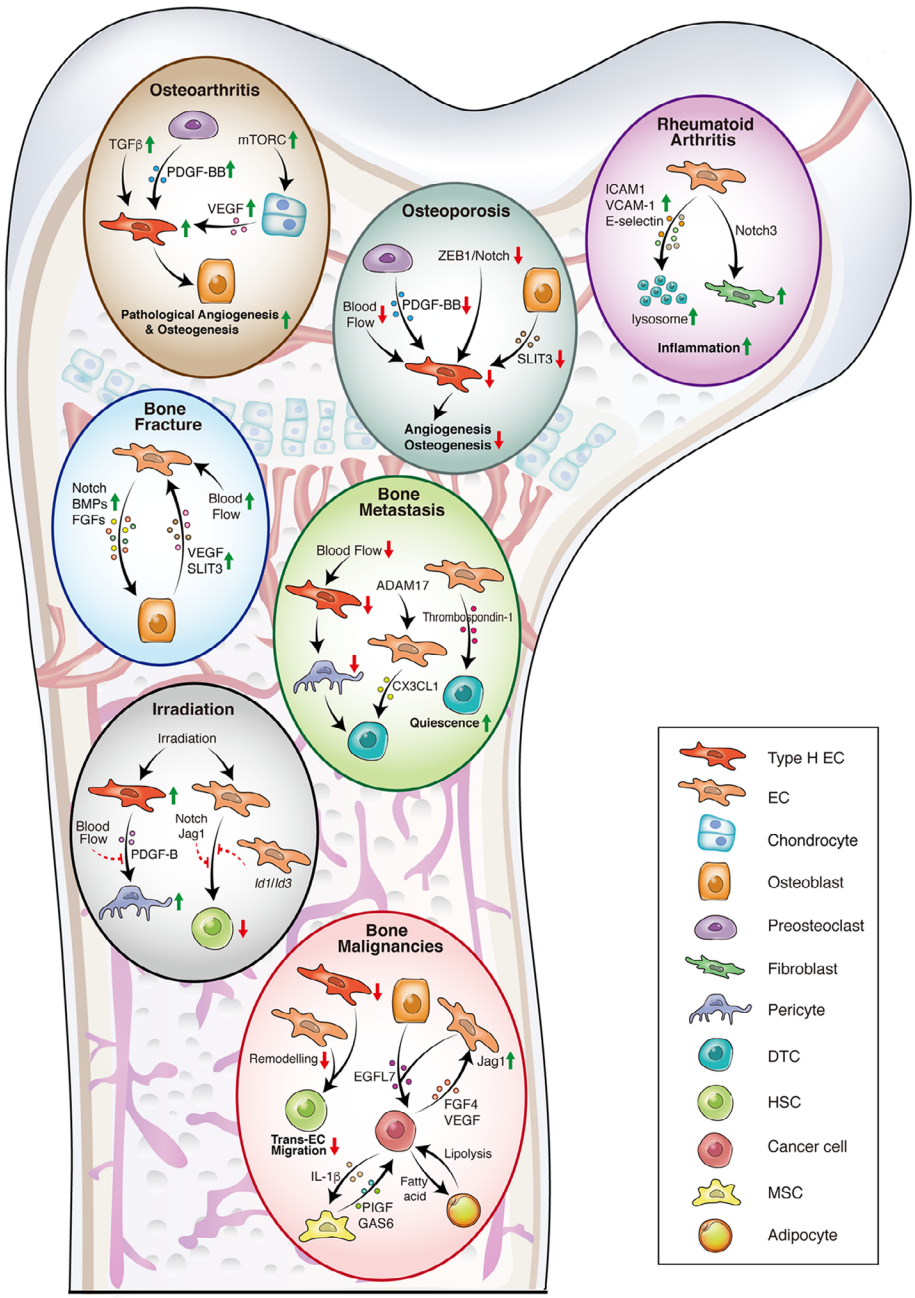

Fig 3. Blood vessel–driven signaling during various pathological conditions in the bone, including osteoarthritis, rheumatoid arthritis, osteoporosis, bone fracture, bone malignancies, bone metastasis, and irradiation. The angiocrine signals from ECs play crucial roles during these pathological conditions. ADAM = a disintegrin and metalloproteinase domain; BMP = bone morphogenetic protein; CX3CL1 = C-X3-C motif chemokine ligand 1; DTC = disseminated tumor cell; EC = endothelial cell; EGFL7 = epidermal growth factor-like domain 7; FGF = fibroblast-derived growth factor; GAS6 = growth arrest-specific 6; HSC = hematopoietic stem cell; ICAM = intercellular adhesion molecule; IL-1β = interleukin-1β; MSC = mesenchymal stem cell; mTORC = mechanistic target of rapamycin complex; PDGF = platelet-derived growth factor; PlGF = placental growth factor; SLIT3 = slit guidance ligand 3; TGF = transforming growth factor; VCAM = vascular cell adhesion protein; VEGF = vascular endothelial growth factor; ZEB1 = Zinc-finger E-box-binding homeobox 1.

regeneration. Recently, maladaptations of blood vessels, including the type H vessels, have been identified underlying bone diseases (Fig. 3). Understanding the dysregulation of blood vessels and their angiocrine factors in bone pathologies will aid to explore potential therapeutic targets for multiple bone diseases such as osteoarthritis, osteoporosis, and osteonecrosis.

Osteoarthritis

Osteoarthritis (OA) is a type of joint disease that results in progressive cartilage breakdown, subchondral bone vascular invasion, and abnormal bone formation.[133,134] Disruption of blood flow and ischemia in the subchondral bone reduce nutrient diffusion to the articular cartilage and lead to osteocyte death and articular

damage in OA. Further, vasculature, especially the type H vessels, play essential roles in OA progression. For example, Mmp2, Mmp9, and Mmp14 derived from type H blood vessels digest cartilage matrix and promote cartilage degeneration.[39] Mechanistic target of rapamycin complex (mTORC) in the articular chondrocytes increases VEGFA production into the subchondral bone and promotes the formation of type H vessels and OA progression.[135] Suppression of mTORC inhibits pathological angiogenesis in subchondral bone and decreases OA progression.[135] Moreover, abnormally increased PDGF-BB secretion by preosteoclasts increases type H vessel formation and induces pathological subchondral bone angiogenesis, contributing to OA development.[136] Likewise, TGFβ mediates signaling cascade to promote recruitment of mesenchymal stem cells and type H vessel formation.[137] At the same time, Halofuginone attenuates OA by inhibition of TGFβ activity and type H vessel formation in the subchondral bone.[138]

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a long-term autoimmune disorder and inflammatory disease, causing degradation of underlying bone and cartilage.[139] Increase in osteochondral angiogenesis and vessel density in non-calcified articular cartilage regions occur in RA.[140] RA is characterized by lymphocyte infiltration, and active ECs are essential for trafficking of leucocytes into the joint during the progression of RA.[140] Further, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion protein 1 (VCAM-1), and E-selectin expressed by ECs promote leucocyte and fibroblast migration into RA joints.[141–143] Specifically, endothelial Notch3 signaling drives the differentiation of synovial fibroblasts, which acquire invasive phenotype during the disease.[144]

Inflammation

Under inflammatory stress, vasculature in the bone marrow is essential to support bone remodeling. ECs express BMP-2 to promote bone formation.[145–147] Similarly, ECs can release osteoprotegerin (OPG) to minimize osteoclastogenesis during diabetes.[148] Moreover, multiple cytokines such as IL6, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) can be produced by ECs under inflammatory conditions, inducing nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) signaling to regulate HSPC functions.[149] Inhibition of the endothelial NF-κB pathway improves HSPC proliferation and hematopoietic recovery in mice following myelosuppressive injury.[149] IL-33, a pro-inflammatory cytokine produced by CD105-expressing ECs, promotes the differentiation of bone marrow–derived stromal cells to osteoblasts and enhance calcium deposition.[47]

Osteoporosis

Osteoporosis is a progressive bone loss condition that results in reduced bone mass and density. Postmenopausal osteoporosis, a predominant type of osteoporosis, is a leading cause for high incidences of bone fracture in older women,[150] which is tightly associated with the reduction of type H blood vessels. Ovariectomized mouse models of postmenopausal osteoporosis demonstrate a decrease in type H vessels.[41] Cathepsin K, expressed by active osteoclasts in osteoporosis mouse models leads to a decrease in PDGF-BB secreted from preosteoclasts, reducing type H vessels and thereby angiogenesis and osteogenesis.[41] In line with this, a specific SHP-2 inhibitor, NSC-87877, inhibits the fusion of preosteoclasts into mature osteoclasts and increases the concentrations of PDGF-BB in the postmenopausal osteoporosis model, thereby enhancing type H vessel formation and reversing the bone loss.[151] Moreover, EC-specific deletion of Zinc-finger E-box-binding homeobox 1 (ZEB1) impairs type H vessel formation and reduces osteogenesis, whereas administration of *Zeb1*-packaged liposomes reverses osteoporosis.[152] These findings are translational from mice to humans, because the loss of abundance of type H vessels is also a crucial indicator for osteoporosis and bone loss in humans.[153,154]

Because glucocorticoids decrease blood vessels and blood flow to the bone,[155–157] blood vessels are important targets of glucocorticoid-induced osteoporosis (GIO). Tetramethylpyrazine was found to prevent steroid-induced osteonecrosis of the femoral head by enhancing femoral head vascularization through the inhibition of the negative steroid effect on the VEGF/FLK1 signaling pathway.[156] Preosteoclast-derived PDGF-BB inhibited by glucocorticoids, leads to a decline in type H ECs and osteogenesis.[158] Further, L-235, a cathepsin K inhibitor, restored bone loss by maintaining PDGF-BB secretion and preserving type H vessels in GIO mouse models, and consequently reduced the effect of GIO on the growing mice.[158] The age-dependent decline of type H vessels and osteoprogenitors is associated with bone loss with age in both mice and humans. Tetramethylpyrazine induces the formation of type H vessel through upregulation of the AMPK-mTORC-HIF-1α-VEGF signaling pathway and promotes bone homeostasis and maintenance of HSCs niche during aging.[159] Also, microRNA-497~195 promotes the formation of type H vessel and thereby bone mass in aged mice due to its positive effect on endothelial Notch activity and HIF-1α stability.[160]

Osteonecrosis

Interruption of blood supply leads to the death of bone tissue and induces the development of osteonecrosis. In the glucocorticoid treatment, glucocorticoid-induced osteonecrosis is a severe complication, characterized by impaired intraosseous circulation, reduced blood flow, and vascular dysfunction.[161,162] Type H ECs decline in glucocorticoid-treated mice compared with the vehicle group.[163] Further, a combination treatment of core decompression and parathyroid hormone (PTH) has been used to promote neo-angiogenesis and bone repair, decreasing the prevalence of osteonecrosis in mouse models. Nevertheless, further research is required to investigate the benefit of blood vessel targeted therapy under clinical settings of osteonecrosis in humans.[164]

Bone Malignancies

Blood vessel microenvironments also support the proliferation and survival of cancer cells. Moreover, EC-derived angiocrine factors have the potential to trigger tumor growth and aggressiveness.[165] Disorganized vasculature and reduced type H vessels are identified in bones of acute myeloid leukemia (AML) mice.[166,167] Further, AML cells localized in proximity to ECs display resistance to chemotherapy, and inhibition of vascular niche remodeling rescues HSCs in AML.[168,169] Enhanced perivascular hypoxia in AML xenografts promotes the remodeling of ECs

and increases the oxidative stress, ROS, and NO production.[167] Moreover, Pazopanib, a receptor tyrosine kinase inhibitor of VEGFRs, PDGFRs, and c-Kit, removes EC protection of AML cells and promotes AML cell sensitivity to cytarabine.[170] Further, the bone marrow vascular niche remodeling contributes to bone malignancy progression. For instance, enhanced growth arrest–specific 6 (GAS6) and PlGF expression by mesenchymal stem cells promotes leukemia cell survival, proliferation, and therapy resistance, whereas IL-1β produced by leukemia cells drives apoptosis of Nestin$^+$ mesenchymal cells.[171] Malignant hematopoietic cells induce lipolysis from adipocytes and, in turn, adipocytes release fatty acids, which are used as an energy source by leukemic cells.[171]

The crosstalk between cancer cells and vascular cells are complex in bone, which may help to explore novel approaches to treat leukemia and other bone malignancies. For example, VEGFA facilitates the proliferation of leukemic cells, whereas blocking VEGFR2 enhances the sensitivity of leukemic cells to chemotherapy.[172] Lymphoma cells secrete FGF4 and activate FGFR1, upregulating the Notch ligand Jag1 on neighboring ECs.[173] Moreover, in multiple myeloma, interfering RNA knockdown of several genes such as *Bnip3*, *Ier3*, and *Sepw1* affects EC functions, decreasing the overangiogenic phenotype and tumor progression.[174] Epidermal growth factor-like domain 7 (EGFL7) secreted by bone lineage cells and ECs is associated with multiple types of cancers,[175,176] and inactivation of EGFL7 may lead to the reduction of blood vessel formation and regulate cancer development in the bone.[177]

Bone Metastasis

Bone is one of the most common sites for metastasis for several primary tumor types.[178,179] Once within the bone, disseminated tumor cells (DTCs) can survive within the bone in the dormant state up to decades before reactivation and relapse. Reactivation and metastasis of the DTCs are closely related to the ECs in the bone marrow.[180] ECs can produce thrombospondin-1 and induce DTC quiescence.[181] Endothelial Von Willebrand factor (VWF) and vascular cell adhesion molecule 1 (VCAM-1) signaling pathway inhibit the interaction between DTCs and the perivascular niche, sensitizing DTCs to chemotherapy.[182] A recent study shows that ADAM17-regulated C-X3-C motif chemokine ligand 1 (CX3CL1) expression produced by bone marrow ECs promotes spinal metastasis from hepatocellular carcinoma.[183] Although sinusoids and low blood flow facilitate more significant interactions between tumor cells and ECs in bone,[180] type H vessels with higher speed of blood flow and more abundant oxygen, cytokine, and growth factors may promote tumor cell survival. Reduced blood flow leads to a reduction of type H vessels and inhibition of pericyte expansion, which regulate EC-derived PDGF-B signaling, rendering DTCs susceptible to irradiation and chemotherapy.[128]

Irradiation

Irradiation is commonly used to treat various types of malignancies. However, the whole-body radiation often leads to bone injury, adversely affecting the microenvironment for stem cells and destroying the vasculature and sinusoidal ECs in the bone marrow. Vascular niches harboring HSC population play an essential role in enhancing bone recovery after irradiation.[184] For instance, irradiated mice transplanted with the bone marrow ECs demonstrate enhanced hematopoiesis and survival.[185] EC-specific deletion of the Notch ligand *Jag1* resulted in a decrease in HSC regeneration and increased lethality after irradiation.[123] Apart from the Notch signaling, ECs upregulate factor such as *Fgf2*, *Bmp4*, *Igfbp2*, and *Angiopoietin1*,[124,125] contributing to bone and hematopoietic recovery after irradiation. A recent study shows that endothelial *Id1* and *Id3* genes are required for the survival of bone marrow sinusoidal ECs to maintain HSC development and function during both homeostasis and acute stress such as irradiation.[186] Further, deletion of E-selectin during irradiation, which is mainly expressed by bone marrow ECs, promotes HSC quiescence and self-renewal.[187] Moreover, type H vessels expand after irradiation, which can regulate the regenerative angiogenesis through blood flow–mediated secretion of PDGF-B.[128]

Angiogenesis and Vascularization in Bone Tissue Engineering

Angiogenesis or blood vessel growth is necessary to establish blood supply in engineered bone tissues. Several strategies have been reported to enhance vascularization in bone tissue engineering, including the delivery of angiogenic growth factor, incorporation of optimal seed cells, and in vivo prevascularization approaches for bone regeneration (Table 2).[188,189]

Because of the coupling of angiogenesis and osteogenesis, angiogenic growth factors have gained great attention as a target for improving the success rate of bone tissue engineering. Most notably, the incorporation of VEGF from bone scaffolds can promote neovascularization and endochondral ossification as observed in a rat defect model in the presence of a bioactive glass coating with an additive VEGF release effect.[190] Likewise, the implantation of a VEGF-incorporated scaffold into a rat calvarial defect model led to advanced bone regeneration.[191] Moreover, Angiogenin (ANG), a normal constituent of circulating blood and a stimulator of angiogenesis, can be used to fabricate a porous scaffold, and the sustained release of ANG increases vascularized bone regeneration.[192] Additionally, PDGF can spatially coordinate with VEGF in the bone scaffold, promoting the formation of mature vascular structures.[193] It should be noted that delivery systems are superior to bolus injection, which can permit a prolonged and sustained release of growth factors at the appropriate dose.[193] Based on the proper delivery system, various other angiogenic growth factors such as Sphingosine 1-phosphate (S1P),[194] Mmp2,[195] and FGF2[196] can be applied in bone tissue engineering due to their capabilities to improve bone healing outcomes.

Incorporation of the optimal seed cells is another important strategy to enhance angiogenesis in bone tissue engineering. Because of the critical role of ECs, transplanted ECs can interact with host ECs to facilitate a vascular supply and promote osteogenesis in different types of the substrate such as polycaprolactone (PCL),[197] polylactide-glycolic acid (PLGA),[198] collagen,[199] and polycaprolactone-starch (SPCL) fiber-mesh scaffolds.[200] It has been reported that co-implantation of ECs and perivascular cells could contribute to engineering a microvascular network in vivo and therefore successfully overcome the high oncogenic risk of genetic manipulations.[201,202] However, mature ECs have low availability and proliferation activity and exhibit distinct phenotypic and genotypic heterogeneity in different organs.[203] Thus, endothelial progenitor cells (EPCs)

Table 2. Design, Strategy, and Material Properties for Vascularized Tissue–Engineered Bone Regeneration

Material	Strategy	Vascularization mechanism	Animal model	Reference
FTY/MBG-PLGA scaffolds	Supercritical CO_2 foaming technique	FTY720 and therapeutic ions released from the scaffolds synergistically induce type H vessel formation	Rat critical-sized calvarial defect model	Li and colleagues[211]
Ca-P-coated Mg-Zn-Gd scaffolds	Alloying, extrusion and surface modification	Mg^{2+} increases angiogenesis including increased ratio of type H ECs	Rat orbital bone defect model	Zhang and colleagues[212]
PCL/DFO scaffolds	3D printing	Deferoxamine promotes vascular growth, enhanced HIF1-α and type H ECs	Rat femur defect model	Yan and colleagues[213]
Poly-GLP-1 molecule	Polymeric pro-drug strategy	poly-GLP-1 promote transduction of Smad2 pathway, with increased type H ECs	Mouse femur defect model	Wang and colleagues[216]
PVA-PCL-HAB	Electrospinning	Degradation of HBA stimulate VEGF production to promote vascularization	Mouse subcutaneous implantation	Prabna and colleagues[220]
PPCN-gelatin	Sequential polycondensation and radical polymerization	A superior scaffolding environment for angiogenic factors such as VEGF	Mouse model of ectopic bone formation	Ye and colleagues[219]
HA-collagen-chitosan fibers	Lyophilization	HA/collagen induced growth of endothelial cells, facilitating pre-vascularization in vitro	Rabbit radius bone defect	Liu and colleagues[221]
ANG loaded Fibrin/bone powder scaffold	Fibrin glue mediated bone powder and ANG mixture	Fibrin glue serves as a binding reservoir for ANG	Rabbit calvarial defect model	Kim and colleagues[192]
PCL-BCP scaffold	3D printing	PCL provides a biomimetic nanofiber surface for EC attachment and formation of endothelialized lumens	In vitro	Temple and colleagues[222]
Cylindrical porous PCL-HA scaffolds	Particulate leaching technique	Pre-seeding scaffold with EPCs promote neovascularization	Mouse femur defect model	Yu and colleagues[204]
VEGF loaded HA-collage scaffold	Spray-drying	Sustained delivery of VEGF promotes angiogenesis	Rat critical-sized cranial defect model	Quinlan and colleagues[191]
VEGF and PDGF loaded PLG scaffold	Standard double emulsion	Sequential delivery of VEGF and PDGF promote vascularization	Mouse model of hindlimb ischemia	Chen and colleagues[193]
SIP loaded PLAGA scaffold	Encapsulation of S1P in polymeric thin films	Sustained release of S1P increase numbers of blood vessels in the defect site	Rat critical-sized calvarial defect model	Sefcik and colleagues[194]
BMP-2/VEGF biomimetic PLA scaffold	3D printing	Angiogenesis induced by MMP-2 regulative mechanism deliv erdual growth factors	In vitro	Cui and colleagues[195]
FGF2/VEGF loaded collagen-heparin scaffold	Lyophilization	FGF2 and VEGF promote vascularization	Rat subcutaneous implantation	Nillesen and colleagues[196]
Porous PCL scaffold	Hydrolysis, hydroxide and immersion	Co-culture of bone marrow fibroblasts and EC promote angiogenesis	In vitro	Choong and colleagues[197]

have been reported to support proangiogenic therapies in tissue engineering.[204,205]

Prevascularization strategy by generating vascular bundles in vivo using graphene oxide-copper nanocomposites (GO-Cu) coatings, in vivo bioreactors, or stem cell-materials interactions are proving to be effective treatment for the repair of the large bone defect.[206–208] in vivo prevascularization offers the transient perfusion after implantation and promotes the formation of a rapid capillary in growth. Additionally, in vivo bioreactors such as morselized autologous bone[209,210] combined with tissue engineering scaffolds show good vascular circulation and better capillary networks in a large mandibular defect and segmental defect model, respectively.

A number of strategies have been investigated in the field of bone tissue engineering on target to increase type H vessel during bone regeneration. CO_2 foamed composite scaffold incorporating bioactive lipids promotes vascularization and bone regeneration via HIF1-α upregulation and enhanced type H vessel formation.[211] Mechanobiologically optimized magnesium (Mg) scaffolds (CaP-coated Mg-Zn-Gd scaffolds) enhance the ratio of type H ECs and osteogenic differentiation of bone mesenchymal stem cells (BMSCs) during repair of orbital bone defects.[212] Moreover, deferoxamine-loaded 3D-printed biodegradable scaffold fabricated via surface aminolysis and layer-by-layer assembly technique shows enhanced HIF1-α, CD31 high expression ECs, and osteogenesis.[213]

Moreover, specific small molecules accelerate bone formation by enhancing type H vessels formation. Administration of Harmine, a widely distributed tricyclic β-carboline alkaloid in plants, increases the number of type H vessels and promotes preosteoclast PDGF-BB–induced angiogenesis and osteogenesis in ovariectomy (OVX)-induced osteoporotic mice.[214] Treatment with ophiopogonin D, a Krüppel-like factor-3 (KLF3) inhibitor, enhances the abundance of type H vessels and accelerates bone healing.[215] A novel poly-GLP-1 molecule using a polymeric prodrug strategy was found to increase the number of type H ECs and promote bone formation in a mouse femoral defect model.[216]

Conclusion

Blood vessels in bone are critical to normal osteogenesis and hematopoiesis. Loss or dysfunction of these blood vessels underlies skeletal aging and bone diseases. Thus, the in-depth analysis of vascular heterogeneity and dissection of molecular signals regulating the skeletal tissue and blood vessel interactions in the bone will provide opportunities for harnessing the full therapeutic potential of the vasculature in bone regeneration and in management of bone diseases and aging.

Disclosures

The authors have declared that no conflict of interest exists.

Acknowledgments

A.P.K is supported by Medical Research Council (CDA: MR/P02209X/1), European Research Council (StG: metaNiche, 805201), Leuka (2017/JGF/001), The Royal Society (RG170326), Kennedy Trust for Rheumatology Research (KENN 15 16 09) and John Fell Fund OUP Research Fund (161/061). S.K.R is a Sir Henry Dale Fellow of the Wellcome Trust and the Royal Society (202300/Z/16/Z) and American Bone and Mineral Research Society.

Authors' roles: JC, MH, SKR, and APK wrote the original draft. JC prepared figures. APK, JC, and AC edited the manuscript. APK, SKR, and JC designed the review structure and figures.

Author contributions: JC: Writing-original draft; writing-review and editing. MH: Writing-original draft; writing-review and editing. AC: Writing-review and editing. SKR: Conceptualization; funding acquisition; supervision; writing-review and editing. APK: Conceptualization; funding acquisition; project administration; supervision; writing-original draft; writing-review and editing.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1002/jbmr.4171.

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