The Osteogenic–Angiogenic Interface: Novel Insights into the Biology of Bone Formation and Fracture Repair

Dwight A. Towler, MD, PhD

Corresponding author
Dwight A. Towler, MD, PhD
Washington University School of Medicine, Internal Medicine–BMD, Barnes-Jewish North Campus Box 8301, 660 South Euclid Avenue, St. Louis, MO 63110, USA.
E-mail: dtowler@im.wustl.edu

Current Osteoporosis Reports 2008, **6:**67–71
Current Medicine Group LLC ISSN 1544-1873

Bone never forms without vascular interactions. Although this is a very simple and obvious statement, the biological, clinical, and pharmacologic implications are incompletely appreciated. The vasculature is not only the conduit for nutrient–metabolite exchange and the rate-limiting "point-of-reference" for Haversian bone formation, but also provides the sustentacular niche for the self-renewing osteoprogenitor. This past year, significant advances have been made in our understanding of the osteogenic–angiogenic interface that are immediately germane to osteoporosis disease biology and fracture management. The critical contributions of the osteoblast oxygen-sensing machinery, paracrine vascular endothelial growth factor and placental growth factor signaling, fracture-mobilized circulating osteoprogenitors, and the osteogenic CD146(+) marrow sinusoid stem cell have been recently discovered. This brief review recounts these revelations, highlighting the potential impact to human bone health and fracture repair.

Introduction

Tremendous unmet needs exist in musculoskeletal medicine. The World Health Organization estimates that the annual costs associated with musculoskeletal disease exceed $100 billion annually in the United States alone—a fraction of the worldwide burden [1]. Age- and disease-specific anabolic therapies are required to rapidly—but safely—restore bone mass and quality, and mechanical and metabolic functions, in the multitude of clinical scenarios faced by physicians. During endochondral bone development, vascular invasion of initially avascular cartilage templates is recognized as a rate-limiting step for bone formation [2]. Throughout postnatal bone growth, maturation, and repair, vascular formation by angiogenesis and arteriogenesis is a critical component of musculoskeletal physiology [3–5]. However, in the past 2 years, skeletal angiogenesis and osteogenic–angiogenic interactions have emerged as rate-limiting biological processes that can be pharmacologically, genetically, or mechanically manipulated to promote bone formation and matrix mineralization [6,7••]. This brief review recounts some of the most enlightening discoveries into the osteogenic–angiogenic interface—recent insights that could be translated to improve bone regeneration and fracture repair in the near-term.

Angiogenesis, Bone Formation, and the Oxygen-Sensing Machinery

Bone never forms without vascular interactions, whether it be in skeletal anlage, at the growth plate, or during fracture repair. A diverse array of angiogenic peptides and growth factors are elaborated by mineralizing hypertrophic chondrocytes and osteoblasts; however, vascular endothelial growth factor (VEGF) is the prototypic coupling factor of the osteogenic–angiogenic interface. Seminal data by Zelzer et al. [2,8] first demonstrated that VEGF was necessary for multiple aspects of normal endochondral bone development. This was not a trivial accomplishment; VEGF is necessary for prenatal life, and mice even haploinsufficient for the VEGF gene exhibit hemorrhagic embryonic lethality before the onset of bone formation. The VEGF gene normally expresses three major VEGF products generated by alternative splicing [9]; in the mouse, these are called VEGF120, VEGF164, and VEGF188. By characterizing skeletal maturation and mineralization in the VEGF120/120 mouse—a VEGF hypomorph homozygote that survives to later embryonic stages—it was shown that skeletal mineralization was tightly coupled with vascular invasion [2]. Earlier studies had demonstrated that Runx2/Cbfa1—the master osteo/chondrogenic transcriptional regulator of skeletal development—upregulated VEGF expression in hypertrophic

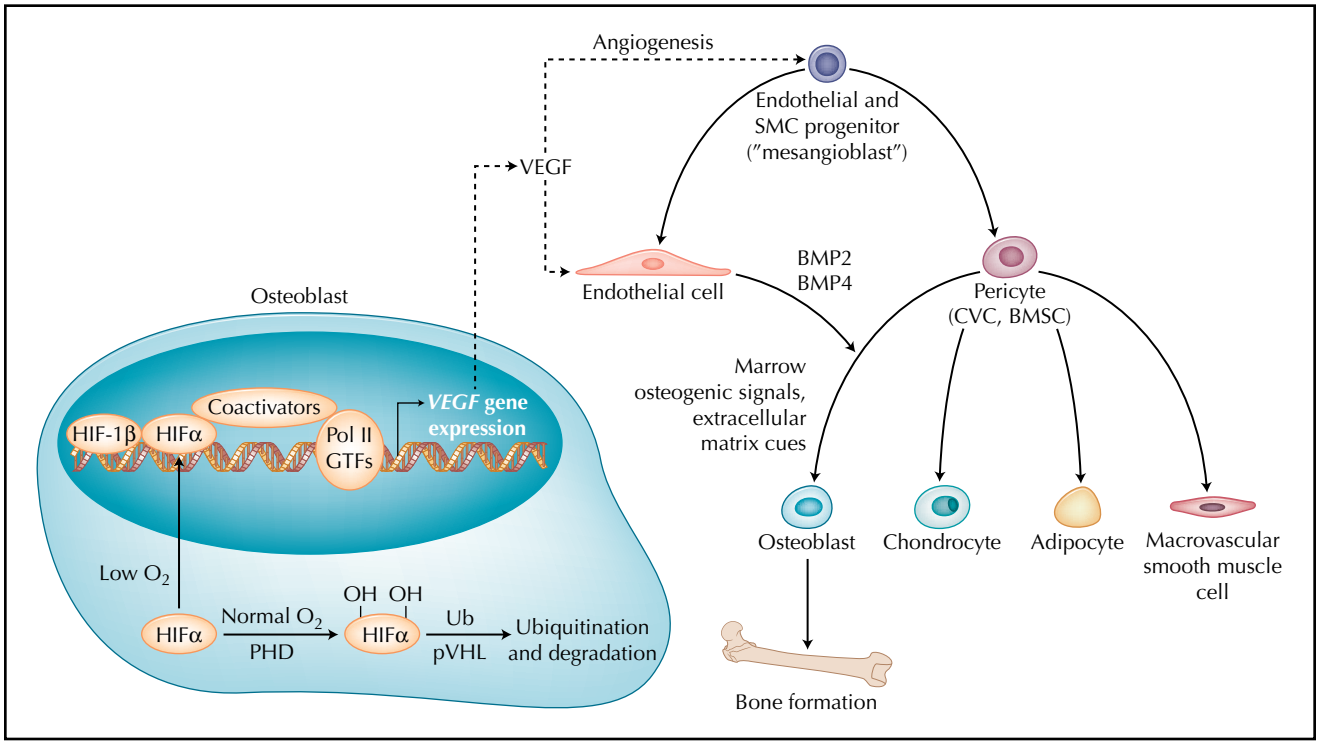

Figure 1. Working model of osteogenic–angiogenic coupling in trabecular bone. BMP—bone morphogenetic protein; BMSC—bone marrow stromal cell; CVC—calcifying vascular cell; GTF—general transcription factor; HIF—hypoxia-induced factor; PHD—prolyl hydroxylase; pVHL—von Hippel Lindau protein; SMC—smooth muscle cell; VEGF—vascular endothelial growth factor. (*From* Towler [15], with permission.)

chondrocytes and in mineralizing osteoblasts [10]. This indicates that VEGF expression is entrained to osteo/chondrogenic maturation, and that Runx2/Cbfa1-dependent programs are most critical to VEGF control during endochondral bone development. However, exciting new data indicate that the hypoxia-induced factors (HIFs) represent physiologically and pharmacologically relevant regulators of VEGF expression and osteogenic–angiogenic coupling in postnatal bone physiology.

HIF was initially identified by Semenza and colleagues in the early 1990s as a nuclear DNA binding activity that recognized and regulated hypoxia-activated response elements in the erythropoietin [11] and VEGF [12] genes. The core of the HIF transcription factor complex is a heterodimer of an α (HIF-1α, HIF-2α) and a β (HIF-1β) subunit [13]. Once considered functionally redundant, recent data indicate overlapping yet distinct functions of the HIF-α subunits (vide infra). Via actions on angiogenesis and erythrocyte mass, HIF activity elicits physiologic homeostatic responses that restore tissue oxygenation. The mechanisms of HIF regulation by hypoxia are conveyed by oxygen-dependent enzymes—prolyl hydroxylases (PHDs)—that tag HIF for recognition by the von Hippel Lindau protein, pVHL [13,14]. pVHL serves as an E3 ligase for the ubiquitination and subsequent proteasomal degradation of HIFs [14,15]. The role of HIF in bone has been intensely studied [6,15]. The critical components of oxygen sensing in the osteoblast were first identified; HIF-α subunits, pVHL, and PHDs were all functionally elaborated. By implementing Cre-Lox transgenic technology, it was demonstrated that genetic augmentation or inhibition of HIF-α subunits osteoblasts results in increased or decreased bone mass. The increases in bone mass achieved by augmenting osteoblast HIF-α activity were astounding; trabecular bone volume/tissue volume increased by 70% [6]. Importantly, the bone anabolic effects of augmenting osteoblast HIF-α activity were not osteoblast cell autonomous. Rather, it was demonstrated that activation of angiogenesis—mediated via the VEGF secreted by the HIF-augmented osteoblast—was driving increases in bone mass [6]. Signaling via VEGF receptors 1 and 2 was required for osteogenic–angiogenic coupling [7••]. Because the process of angiogenesis increases the pool of multipotent mesenchymal progenitors and endothelial progenitors necessary for bone formation, the prevailing environment that couples osteogenesis with angiogenesis in healthy bone marrow increases bone mass (Fig. 1) [15].

It was then demonstrated that pharmacologic activation of the HIF-α pathway in vivo was not only possible but also accelerated bone wound repair [7••]. Desferrioxamine is a semi-selective chelator that inhibits PHD activity by sequestering the non-heme iron (II) necessary for oxygen- and oxoglutarate-dependent hydroxylation of HIF-α subunits. Ex vivo, desferrioxamine upregulates osteoblast HIF-α protein accumulation, induces VEGF expression, and promotes neoangiogenesis in embryonic bone organ culture. Using distraction osteogenesis—an orthopedic model of bone healing mediated via mechanically induced membranous ossification—local administration of desferrioxamine at

the repairing osteotomy gap profoundly accelerated osseous repair [7••]. Depending on the ossicle and fracture site, fracture nonunions and necrosis occur in 5% to 10% of patients and are much more likely with underlying vascular disease associated with diabetes, uremia, and advanced age, or vascular insult induced by radiation and pharmacotherapy for cancer [16–21]. Thus, strategies that improve bone regeneration after injury hold promise not only to more quickly restore skeletal integrity and mobility, but also to reduce rates of fracture nonunion. It is unknown whether mechanical loading or distraction is requisite for optimal repair via HIF-dependent osteogenic–angiogenic coupling [7••].

In principle, targeted inhibition of either HIF PHDs or the pVHL E3 ubiquitin ligase could be used to locally augment skeletal HIF-α, and thus the secreted factors such as VEGF that mediate osteogenic–angiogenic coupling (Fig. 1). Three HIF PHDs have been identified to date. The specific contributions of each PHD to HIF cellular economy and osteoblast oxygen sensing have yet to be explored. Moreover, very recent data from Aragones and colleagues [22] indicate that specific PHD-HIF subunit combinatorial interactions convey unique metabolic roles in the musculoskeletal system and that PHD-HIF signals control musculoskeletal tolerance to hypoxic stress independent of VEGF production. This is an additional biological consideration because chronic hypoxia is a common setting for many patients with osteoporosis and lung disease [23]. Moreover, Fei et al. [24] have shown that chronic hypoxia uncouples angiogenesis and osteogenesis in vivo—an extremely important observation and fundamental biological principle. Thus, targeting specific PHDs for inhibition may have desirable effects in certain skeletal repair settings beyond activation of anabolic osteogenic–angiogenic coupling via paracrine VEGF signaling [6,22].

Vasculature as Both Niche and Conduit: The Comings and Goings of Osteoprogenitors

Seven years ago, Hauge and colleagues [25,26] discovered a novel histoanatomic feature structure of bone, the bone remodeling compartment (BRC). This difficult-to-visualize compartment consists of a cancellous bone resorption lacunae covered by a thin cellular membrane contiguous not only with the bone-lining cell layer, but also the vascular sinusoids of hematopoietic marrow [26]. The latter contiguity is apparent from the visualization of erythrocytes within this sinus, underneath the osteocalcin(+) alkaline phosphatase(+) membranous canopy of the BRC [25,27]. The full significance of this novel anatomic osteogenic–angiogenic interface is only now becoming apparent. In 2007, Sacchetti and colleagues [28••] identified the marrow residence of the human osteogenic stem cell. This CD146(+) self-renewing osteoprogenitor is a mural resident of the bone marrow vascular sinusoid—a subendothelial cell intimately juxtaposed to the sinusoidal vascular endothelium. The topological relationship of this CD146(+) bone stem cell with the vascular endothelium [28••] is highly reminiscent to that of the pericyte—a microvascular smooth muscle cell that is also a multipotent mesenchymal osteoprogenitor [29,30]. However, the CD146(+) cell of the bone marrow sinusoid is not only self-renewing but also is sufficient to organize bone replete with marrow elements when implanted ectopically [28••]. Whether these properties are exhibited by pericytes has not been carefully established [31].

Why is the contiguity of the BRC of the resorption envelope with the vascular sinusoid space of such interest? Recently, Eghbali-Fatourechi and colleagues [32] identified that an osteocalcin(+) alkaline phosphatase(+) osteoprogenitor circulates at remarkably high levels—about 1% of blood mononuclear cells. The numbers of these circulating osteoprogenitors were significantly increased during periods of intense skeletal growth (eg, puberty) and intense osteogenic repair (eg, fracture healing) [32]. Because the absolute number of BRCs increases with bone remodeling [25], a working model emerges in which the contiguity between vascular sinusoids containing mural CD146(+) osteoprogenitors and the BRC locally provides osteogenic cells for growth, remodeling, and repair. A certain fraction of these cells escape bone to enter the general circulation [32] and may contribute to the global augmentation in bone formation observed with localized skeletal injury [33]. Very recent characterization of the circulating osteoprogenitor population reveals heterogeneity in the expression of CD34 [34]—a cell surface glycoprotein characteristic of hematopoietic and endothelial progenitor cells [35] but also expressed by pericytes [35]. Matsumoto and colleagues [36••] first demonstrated that circulating osteocalcin(+) CD34(+) progenitors—donated by healthy human volunteers—could promote vascularization and mechanically sound bone healing when infused as a xenograft in a femoral fracture nonunion model. Very recently, by using an elegant transgenic mouse model to tag murine endothelial progenitor cells, Matsumoto et al. [37] established that these fracture-mobilized circulating progenitors are indeed recruited to sites of skeletal repair as first posited by Eghbali-Fatourechi et al. [32]. Thus, the vasculature also provides a conduit for progenitors that promote formation and fracture repair [32,37]. The upside potential of human CD34(+) cell-based therapies for treatment of fracture nonunion is evident from early preclinical studies [36••].

Bone Morphogenetic Protein 2: A Critical Permissive Factor for Postnatal Bone Osteogenic–Angiogenic Coupling?

Bone morphogenetic protein 2 (BMP2) plays an indispensable role in cardiovascular development; global loss of BMP2 results in very early embryonic lethality [38]. To better understand the role of BMP2 in skeletal physiology, Tsuji

and colleagues [39] implemented Cre-Lox mouse transgenic technology to selectively ablate BMP2 expression in mesenchymal cell lineages. They demonstrated that although mesenchymal BMP2 expression was not required for bone formation during development, it was critical for postnatal skeletal integrity. Spontaneous fractures occurred throughout adulthood in Prx1-Cre:BMP2(CKO;CKO) mice—a highly unusual event in the life of a mouse—and fracture repair was blocked [39]. How can this be explained? Recently, Maes and colleagues [40•] found that placental growth factor (PlGF)—a VEGF receptor 1 angiogenic ligand—was necessary for mesenchymal osteoprogenitor recruitment during fracture repair. BMP2 signals mesenchymal cells to produce PlGF [41,42], and thus recruits and sustains viability of the endothelial progenitor cell discussed earlier [42]. Moreover, BMP2 synergizes with VEGF to enhance bone formation and repair [43]. Thus, it is tempting to speculate that loss of mesenchymal BMP2 tone impairs skeletal PlGF and VEGF cues required postnatally to maintain or restore skeletal integrity via coupled osteogenic–angiogenic signaling. This notion has yet to be tested in vivo.

Conclusions

This abbreviated overview presents a small fraction of the available data demonstrating the importance of osteogenic–angiogenic interactions to bone formation, skeletal integrity, and fracture repair. A better understanding of the osteogenic–angiogenic interface will significantly improve our ability to successfully manage patients with bone health disorders in multiple disease settings [16–21].

Disclosure

Dr. Towler receives research salary support from the National Institutes of Health (NIH) and Barnes-Jewish Hospital Foundation. He serves as a paid scientific advisory board consultant for Wyeth, Eli Lilly, and GlaxoSmithKline. He serves as a paid grant review consultant for the NIH Center for Scientific Review, SBDD and AICS study sections. He serves as a paid scientific advisory board consultant for the UCLA Geffen School of Medicine, Department of Medicine.

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