

Dissection the Osteogenic and Angiogenic Signal Pathways in Bone Development and Regeneration with Biochips

Jeremy M, Schon LC and Zhang Z*

Orthobiologic Laboratory, MedStar Union Memorial Hospital, Baltimore, MD, USA

Abstract

Osteogenesis is the cellular and molecular foundation of skeletal development and bone regeneration related to fracture healing, revitalization of bone graft and therapies for osteoporosis. A hallmark of osteogenesis is the mineralization of extracellular matrix. Because of its potential impact on developmental biology and human health, understanding and regulation of osteogenesis are subjects of intensive study. Although significant advancements have been made over the past decades, there are still unsolved puzzles in regulations of osteogenesis. Angiogenesis generally refers to new blood vessels branching out from established vasculature. Besides of fundamental physiology, angiogenesis involves in pathology, such as growth of cancer, and is essential for the repair of virtually all types of tissues. It has long been recognized that osteogenesis and angiogenesis are coupling events during bone formation. The classic osteogenic and angiogenic pathways intertwine and cross-talk during bone formation. To better understand the signal pathways and coupling factors of osteogenesis and angiogenesis is critically important for enhancing bone regeneration and tissue engineering of bone. The conventional biological models, however, have very limited capacity of isolating angiogenic and osteogenic events from the cascade of bone regeneration, and precisely quantifying the effects of angiogenic and osteogenic factors on bone formation at a molecular level. Biochips and tissue chips provide a powerful tool to simulate and quantify angiogenic and osteogenic events on the chips and effectively untangle these biologically important and clinically relevant molecular events during bone formation.

Keywords: Osteogenesis; Angiogenesis; Extracellular matrix; Blood vessels; Regeneration

Bone Development and Regeneration

Bone development occurs through either endochondral ossification or intramembranous ossification. Intramembranous ossification, which does not require a cartilaginous intermediate, occurs mainly in the development of flat cranium and facial bones. Endochondral ossification is essential for fetal development of the musculoskeletal system, growth of long bones, and fracture healing. The process begins with a cartilage template formed from the differentiation of mesenchymal condensations. Chondrocytes in the core of the anlagen produce specialized extracellular matrix composed of types II, IX, and XI collagen as well as aggrecan, chondromodulin-1, and matrilin-3 [1]. As the core chondrocytes rapidly proliferate, peripheral chondrocytes become hypertrophic and synthesize type X collagen. The hypertrophic chondrocytes direct peripheral perichondrial cells to differentiate into osteoblasts to form bone collar, which further develops into cortical bone. As the hypertrophic chondrocytes further mature, they mineralize the surrounding matrix and produce specific growth factors and enzymes to promote vascular invasion-an invitation of angiogenesis to bone development. These molecules that are involved in angiogenesis include osteopontin, Matrix Metalloproteinase-9 (MMP-9), MMP-13, and Vascular Endothelial Growth Factor (VEGF) [2]. Vascular invasion is, at least, responsible for resorption of the mineralized cartilage matrix to form the bone marrow cavity and deposition of trabecular bone.

During endochondral ossification, chondrocyte maturation and osteoblasts differentiation are key events of osteogenesis and regulated by several signal pathways, such as Indian Hedgehog (IHH), parathyroid Hormone-Related Peptide (PTHrP), Fibroblast Growth Factor (FGF), Bone Morphogenetic Proteins (BMP), WNT and Notch [3].

Angiogenesis in Bone Development and Regeneration

Both endochondral and intramembranous ossifications require vascular support that comes in the form of angiogenesis. Angiogenesis, as opposed to vasculogenesis, is the formation of new blood vessels from preexisting vasculature. Sprouting angiogenesis requires external angiogenic signals, among which VEGF is the most notable one. Upon binding to the receptors on endothelial cells, VEGF activates endothelial cells to produce proteases that allow endothelial cell migration out of the parent vessel and toward the angiogenic signals. The migrating endothelial cells eventually adhere to one another forming the lumen of a neo-vessel.

VEGF is a family of growth factors that include Placenta Growth Factor (PGF), VEGF-A, -B, -C, -D, -E, and -F. The different forms of VEGF have functional consequences for angiogenesis, heparin-binding affinity, bioavailability, and VEGFR activation [4,5]. The VEGF-A splice variant is the most abundant and commonly studied isoform of VEGF. VEGF binds to its corresponding receptor tyrosine kinase (RTK), of which two forms have been identified: Flt-1 (Fms-Like-Tyrosine Kinase) and KDR (Kinase Domain Region). The VEGF receptors consist of seven immunoglobulin-like extracellular domains, a single transmembrane region, and an intracellular tyrosine-kinase domain.

***Corresponding author:** Zijun Zhang, Orthobiologic Laboratory, Medstar Union Memorial Hospital, 201 E. University Parkway, Bauernschmidt Building, Room 763, Baltimore, MD 21218, USA, Tel : 410-554-2830; Fax: 410-554-2289; E-mail: zijun.zhang@medstar.net

Received March 17, 2014; **Accepted** March 29, 2014; **Published** April 03, 2014

Citation: Jeremy M, Schon LC, Zhang Z (2014) Dissection the Osteogenic and Angiogenic Signal Pathways in Bone Development and Regeneration with Biochips. J Biochips Tiss Chips 4: 109. doi:[10.4172/2153-0777.1000109](https://doi.org/10.4172/2153-0777.1000109)

Copyright: © 2014 Jeremy M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Tangled Signal Pathways in Osteogenesis and Angiogenesis

Although VEGF is most commonly recognized as a potent endothelial cell mitogen and pro-angiogenic factor, it has a wide range of biologic activities. VEGF promotes the expression of plasminogen activators, MMPs, and urokinase receptors that aid in endothelial cell invasion and migration [5]. It has been shown to regulate microvascular permeability in tumors and wounds while inducing vasodilation *in vivo* [6]. VEGF can influence metabolic processes through manipulating the rate of hexose transport and influence integrin expression during immunologic events [7]. Clearly, VEGF's biologic functions are broad with activity spanning numerous cell types and organ systems.

VEGF is a well-known signaling protein that shares common pathways in both osteogenesis and angiogenesis. VEGF exerted dose-dependent stimulatory effects on the proliferation, maturation and matrix mineralization of osteoblasts [8] and strong osteogenic induction for mesenchymal progenitor cells [9]. Maximum VEGF expression was noted during matrix mineralization. VEGF stimulated the expression of Flt-1 and KDR on osteoblasts as well as increased kinase activity of Flt-1. It is evident that VEGF has a significant role in manipulating the function of the bone-forming osteoblasts.

Furthermore, VEGF has also been shown to influence the bone formation and remodeling process through interaction with the osteoclasts. The bone-resorbing osteoclasts enzymatically catabolize mineralized matrix through a specialized 'ruffled-border' on their surface. Osteoclast activity is tightly coupled with bone formation through the receptor activator of nuclear factor kappa beta (RANK). During bone development and remodeling, osteoblasts express RANK Ligand (RANKL) which binds to and activates receptors on osteoclasts. A decoy receptor, osteoprotegerin modulates these events such that equilibrium exists between anabolic and catabolic activities in the bone according to the environmental demands. VEGF has been shown to increase the transcription and protein expression of RANK in vascular endothelial cells [10]. Thus, there is a feedback loop among bone formation, bone resorption, and vascularization in bone remodeling. VEGF also plays an important role in bone development through the RANK/RANKL pathway. During endochondral ossification, high levels of VEGF are expressed by hypertrophic chondrocytes [11]. This is noteworthy because vascular invasion is crucial for proper bone growth and repair, and a strong temporal correlation has been demonstrated between matrix mineralization and vascular invasion through VEGF. Furthermore, Gerber et al. found impaired trabecular bone formation when VEGF was inactivated through a soluble receptor chimeric protein in a murine model of endochondral ossification [12].

Similar conclusions implicating VEGF signal as an important mediator of bone formation and remodeling have also been observed in fracture healing. Street et al. impaired the healing of femoral fracture by using an inhibiting antibody to Flt-1 [13]. Conversely, overexpression of VEGF, either through gene transfection or other means, improved fracture healing and soft-callus conversion [14,15]. VEGF also enhances BMP signals, which are largely responsible for bone formation, during fracture healing. Peng et al. found a synergistic response to mesenchymal cell recruitment and soft-callus formation when VEGF and BMP-4 were administered concurrently [16].

Vascular invasion and VEGF expression are also regulated by the local microenvironment of new bone development whether it occurs as part of a repair process or in skeletal maturation. The cartilage anlagen of endochondral ossification are hypoxic in comparison to most tissues

in the body [17]. It has been shown that a hypoxic microenvironment can regulate VEGF expression through stabilization of the Hypoxia Inducible Factor (HIF). Under hypoxic conditions, Prolyl-Hydroxylase Domain Proteins (PHDs) are unable to hydroxylate specific regions of the Oxygen Dependent Degradation Domain (ODDD) of HIF-1 α . This allows HIF-1 α to accumulate and sets off a cascade of events resulting in transcription of hypoxia response elements [18].

Recent studies have shown that HIF is a coupling agent between the process of angiogenesis and osteogenesis through VEGF. Hypoxia is a strong inducer of VEGF-An expression in osteoblasts and a lack of HIF-1 α decreases overall bone volume [19,20]. Factors other than hypoxia have also been implicated in increased HIF expression, including insulin growth factor-1 (IGF-1). IGF-1 increases VEGF-An expression in osteoblasts by maintaining HIF-2 α protein levels and thus catalyzing the cascade of hypoxia response element activation [21].

There are also sheer evidences that signals, which classically involve in osteogenesis such as Notch and BMP, are critical for angiogenesis [22]. Angiogenesis and osteogenesis during bone formation/regeneration are complicated, because of the overlapping and interactions of signal pathways. It remains a challenge to dissect the molecular and cellular events and refine the role of each signal pathway in angiogenesis and osteogenesis.

Biochips and Tissue Chips

The advent of biochips and tissue chips has launched an entirely new paradigm of studying biological processes at the molecular scale. These chips turn biologic processes into quantifiable electrical signals. The ability to detect specific molecules can be tailored by using various advanced surface materials such as microporous gold, graphite, glassy carbon, and indium tin oxide [23,24]. Recent developments in nanotechnology have greatly expanded the range and specificity of the biological recognition elements in biochips. Different forms of nanomaterials including nanotubes, nanowires, nanoparticles, graphene, and quantum dots allow researchers create sophisticated molecular sensing devices that are capable of discerning events at the single molecule level [25].

The biochips and tissue chips could be instrumental in the future direction of research for osteogenesis-angiogenesis coupling in bone development and regeneration. Protein signaling events could be recorded for the osteoblasts, osteoclasts, vascular endothelial cells, and chondrocytes for different permutations of interactions. Using biochips, the influence of VEGF on the osteoblasts and corresponding feedback loops with vascular endothelial cells could be better understood.

Rather than focusing on a single protein, transcription factor or gene, biochips and tissue chips would allow arrays of targets to be evaluated simultaneously providing high-throughput data more efficiently. Gene microarrays have long been used for studying gene expression changes at the global scale for osteogenesis and angiogenesis [26]. Because biochips are capable of recording molecular events in real-time, temporal relationships between osteogenesis and angiogenesis could also be unveiled in a precise and quantitative fashion.

Using biochips and tissue chips to study the signal pathways involving angiogenesis and osteogenesis would not only provide insight of molecular biology of bone development but also advance the field of bone tissue engineering. The technologies involving in biochips and tissue engineering share a common feature that is interacting with cells *in vitro*. The similar conditions and environment allow discoveries

of biochip and tissue chips on the signal pathways of angiogenesis and osteogenesis to be applied directly to tissue engineering of bone.

Acknowledgement

This review was support in part by Dr. Lew Schon Innovation Fund.

References

1. Hojo H, Ohba S, Yano F, Chung UI (2010) Coordination of chondrogenesis and osteogenesis by hypertrophic chondrocytes in endochondral bone development. *J Bone Miner Metab* 28: 489-502.
2. Kronenberg HM (2003) Developmental regulation of the growth plate. *Nature* 423: 332-336.
3. Long F, Ornitz DM (2013) Development of the endochondral skeleton. *Cold Spring Harb Perspect Biol* 5: a008334.
4. Arondéguy T, Lacazette E, Millevoi S, Prats H, Touriol C (2013) VEGF-A mRNA processing, stability and translation: a paradigm for intricate regulation of gene expression at the post-transcriptional level. *Nucleic Acids Res* 41: 7997-8010.
5. Ferrara N (2009) VEGF-A: a critical regulator of blood vessel growth. *Eur Cytokine Netw* 20: 158-163.
6. Ylä-Herttua S, Rissanen TT, Vajanto I, Hartikainen J (2007) Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. *J Am Coll Cardiol* 49: 1015-1026.
7. Patil AS, Sable RB, Kothari RM (2012) Occurrence, biochemical profile of vascular endothelial growth factor (VEGF) isoforms and their functions in endochondral ossification. *J Cell Physiol* 227: 1298-1308.
8. Li P, Bai Y, Yin G, Pu X, Huang Z, et al. (2013) Synergistic and sequential effects of BMP-2, bFGF and VEGF on osteogenic differentiation of rat osteoblasts. *J Bone Miner Metab* [Epub ahead of print].
9. Fiedler J, Leucht F, Waltenberger J, Dehio C, Brenner RE (2005) VEGF-A and PlGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. *Biochem Biophys Res Commun* 334: 561-568.
10. Min JK, Kim YM, Kim YM, Kim EC, Gho YS, et al. (2003) Vascular endothelial growth factor up-regulates expression of receptor activator of NF-kappa B (RANK) in endothelial cells. Concomitant increase of angiogenic responses to RANK ligand. *J Biol Chem* 278: 39548-39557.
11. Patil AS, Sable RB, Kothari RM (2012) Occurrence, biochemical profile of vascular endothelial growth factor (VEGF) isoforms and their functions in endochondral ossification. *J Cell Physiol* 227: 1298-1308.
12. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, et al. (1999) VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 5: 623-628.
13. Street J, Bao M, de Guzman L, Bunting S, Peale FV Jr, et al. (2002) Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci U S A* 99: 9656-9661.
14. Geiger F, Bertram H, Berger I, Lorenz H, Wall O, et al. (2005) Vascular endothelial growth factor gene-activated matrix (VEGF165-GAM) enhances osteogenesis and angiogenesis in large segmental bone defects. *J Bone Miner Res* 20: 2028-2035.
15. Li R, Stewart DJ, von Schroeder HP, Mackinnon ES, Schemitsch EH (2009) Effect of cell-based VEGF gene therapy on healing of a segmental bone defect. *J Orthop Res* 27: 8-14.
16. Peng H, Wright V, Usas A, Gearhart B, Shen HC, et al. (2002) Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. *J Clin Invest* 110: 751-759.
17. Simon MC, Keith B (2008) The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol* 9: 285-296.
18. Mahon PC, Hirota K, Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* 15: 2675-2686.
19. Trebec-Reynolds DP, Voronov I, Heersche JN, Manolson MF (2010) VEGF-A expression in osteoclasts is regulated by NF-kappaB induction of HIF-1alpha. *J Cell Biochem* 110: 343-351.
20. Wang Y, Wan C, Deng L, Liu X, Cao X, et al. (2007) The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *J Clin Invest* 117: 1616-1626.
21. Akeno N, Robins J, Zhang M, Czyzyk-Krzeska MF, Clemens TL (2002) Induction of vascular endothelial growth factor by IGF-I in osteoblast-like cells is mediated by the PI3K signaling pathway through the hypoxia-inducible factor-2alpha. *Endocrinology* 143: 420-425.
22. Beets K, Huylebroeck D, Moya IM, Umans L, Zwijsen A (2013) Robustness in angiogenesis: notch and BMP shaping waves. *Trends Genet* 29: 140-149.
23. Grieshaber D, MacKenzie R, Vörös J, Reimhult E (2008) Electrochemical biosensors-Sensor principles and architectures. *Sensors* 8: 1400-1458.
24. Pavlickova P, Schneider EM, Hug H (2004) Advances in recombinant antibody microarrays. *Clin Chim Acta* 343: 17-35.
25. Gu LQ, Shim JW (2010) Single molecule sensing by nanopores and nanopore devices. *Analyst* 135: 441-451.
26. Xue Y, Xing Z, Bolstad AI, Van Dyke TE, Mustafa K (2013) Co-culture of human bone marrow stromal cells with endothelial cells alters gene expression profiles. *Int J Artif Organs* 36: 650-662.

Citation: Jeremy M, Schon LC, Zhang Z (2014) Dissection the Osteogenic and Angiogenic Signal Pathways in Bone Development and Regeneration with Biochips. *J Biochips Tiss Chips* 4: 109. doi:[10.4172/2153-0777.1000109](https://doi.org/10.4172/2153-0777.1000109)

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 350 Open Access Journals
- 30,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: www.omicsonline.org/submit