

Osteocyte-induced angiogenesis via VEGF–MAPK-dependent pathways in endothelial cells

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Received: 4 June 2013 / Accepted: 26 September 2013
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Abstract Recently, it has been suggested osteocytes control the activities of bone formation (osteoblasts) and resorption (osteoclast), indicating their important regulatory role in bone remodelling. However, to date, the role of osteocytes in controlling bone vascularisation remains unknown. Our aim was to investigate the interaction between endothelial cells and osteocytes and to explore the possible molecular mechanisms during angiogenesis. To model osteocyte/endothelial cell interactions, we co-cultured osteocyte cell line (MLOY4) with endothelial cell line (HUVECs). Co-cultures were performed in 1:1 mixture of osteocytes and endothelial cells or by using the conditioned media (CM) transfer method. Real-time cell migration of HUVECs was measured with the transwell migration assay and xCELLigence system. Expression levels of angiogenesis-related genes were measured by quantitative real-time polymerase chain reaction (qRT-PCR). The effect of vascular endothelial growth factor (VEGF) and mitogen-activated phosphorylated kinase (MAPK) signaling were monitored by western blotting using relevant antibodies and inhibitors. During the bone formation, it was noted that osteocyte dendritic processes were closely connected to the blood vessels. The CM generated from MLOY4 cells-activated proliferation, migration, tube-like structure formation, and upregulation of angiogenic genes in endothelial cells suggesting that secretory factor(s) from osteocytes

could be responsible for angiogenesis. Furthermore, we identified that VEGF secreted from MLOY4-activated VEGFR2–MAPK–ERK-signaling pathways in HUVECs. Inhibiting VEGF and/or MAPK–ERK pathways abrogated osteocyte-mediated angiogenesis in HUVEC cells. Our data suggest an important role of osteocytes in regulating angiogenesis.

Keywords Osteocyte · Endothelial cells · Bone · Angiogenesis · Co-culture

Introduction

Bone remodelling and angiogenesis are two processes that are intimately linked. Given that vascular status is important for proper bone homeostasis, defining the roles of osteoblasts, osteoclasts, osteocytes, endothelial cells, and angiogenic factors and their interactions in bone is critical to understand bone pathology and for the development of new strategies for bone regeneration and fracture healing.

Several lines of evidence suggest that bone angiogenesis is regulated by different cell types present in the bone tissue [1]. Close interaction between the bone-forming osteoblasts and the vessel-forming endothelial cells is crucial for bone vascularisation and osteogenesis [1, 2]. For example, interaction between osteoblasts and endothelial cells formed microvessel-like structures and gap junction communications [3]. Paracrine factors, such as vascular endothelial growth factor (VEGF), are implicated as mediators during these communications [4]. Furthermore, the conditioned medium from human umbilical endothelial cells (HUVEC) enhances the proliferation of human bone marrow stromal cells when co-cultured in vitro [5]. On the other hand, various studies support the idea that multi-nucleated osteoclasts play an

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