Mechanism Underlying Post-menopausal Osteoporosis: HIF1α is Required for Osteoclast Activation by Estrogen Deficiency

Takeshi Miyamoto

Department of Orthopedic Surgery and Department of Integrated Bone Metabolism and Immunology, Keio University School of Medicine, Tokyo, Japan

(Received for publication on February 18, 2015)
(Revised for publication on April 16, 2015)
(Accepted for publication on May 7, 2015)
(Published online in advance on August 8, 2015)

The aging of the population worldwide has sharply increased the number of post-menopausal osteoporosis patients. Bone fragility caused by osteoporosis often results in fractures; therefore, controlling osteoporosis is crucial to prevent such injuries. To date, various drugs to treat osteoporosis have been developed and launched; however, the molecular mechanisms underlying post-menopausal osteoporosis have not been fully elucidated, and additional factors that could be targeted to treat patients remain to be characterized. Recently, hypoxia inducible factor 1 alpha (HIF1α) was identified as essential for osteoclast activation, an activity that promotes bone loss following menopausal estrogen deficiency. Although osteoclasts, which are located in hypoxic regions of the bone surface, express HIF1α mRNA, in pre-menopausal conditions the presence of estrogen decreases HIF1α protein levels in these cells. In menopausal conditions, however, estrogen deficiency allows HIF1α protein to accumulate in osteoclasts, leading to osteoclast activation and bone loss. Osteoclast-specific conditional HIF1α inactivation protects mice from estrogen deficiency-induced osteoclast activation and bone loss, as does systemic administration of a HIF1α inhibitor. Therefore, HIF1α represents a potential therapeutic target to prevent osteoclast activation and bone loss in post-menopausal patients. (doi: 10.2302/kjm.2015-0003-RE; Keio J Med 64 (3) : 44–47, September 2015)

Keywords: osteoclasts, postmenopausal osteoporosis, HIF1α, hypoxia, estrogen

Activation of Osteoclasts in Postmenopausal Osteoporosis Patients

Bone homeostasis requires a delicate balance of activity between bone-resorbing osteoclasts and bone-forming osteoblasts (Fig. 1). In women from age 20 years to pre-menopause, these activities are mutually regulated, keeping bone volume stable. However, estrogen deficiency occurring at menopause activates both osteoclasts and osteoblasts, with activation of the former being dominant, resulting in decrease of bone mass (Fig. 1). Therefore, osteoclasts are considered targets when devising treatment strategies for post-menopausal osteoporosis patients. Indeed, various osteoclast-inhibiting agents reportedly increase bone mass and prevent fragility fractures to a significantly greater extent than placebos.1–5 However, the mechanisms underlying osteoclast activation induced by estrogen deficiency remain undefined.

Osteoclast Regulation as a Means of Treating Post-menopausal Patients

Osteoclast cell–cell fusion

Because osteoclasts are activated in estrogen-deficient conditions, administration of estrogen itself could block their activation and subsequent bone loss.6 However, prolonged estrogen administration to post-menopausal pa-

Reprint requests to: Takeshi Miyamoto, MD, PhD, Department of Orthopedic Surgery and Department of Integrated Bone Metabolism and Immunology, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan, E-mail: miyamoto@z5.keio.jp

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Patients could stimulate mammary gland or uterine tumor development or promote venous thrombosis. Thus, safer osteoclast-specific inhibitors have been sought.

Manipulation of osteoclast fusion has been considered one way to inhibit osteoclast activity, because fusion of mono-nuclear osteoclasts reportedly promotes the multi-nucleation required to remodel the osteoclast cytoskeleton via the creation of ruffled borders or sealing zones, leading to bone resorption. Among the factors that promote osteoclast cell–cell fusion, we identified two: the dendritic cell-specific transmembrane protein (DC-STAMP) and the osteoclast stimulatory transmembrane protein (OC-STAMP). We found that, both in vivo and in vitro, deficiency in either protein completely abrogated osteoclast cell–cell fusion, although formation of tartrate-resistant acid phosphatase-positive mono-nuclear osteoclasts remained unchanged. These observations indicate that both DC-STAMP and OC-STAMP are essential for osteoclast fusion but not for osteoclast differentiation (Fig. 2). We then observed that osteoclast bone-resorbing activity was indeed significantly down-regulated in DC-STAMP knockout mice and in OC-STAMP knockout mice, both of which exhibited mono-nuclear osteoclasts rather than their multi-nuclear counterparts typically seen in wild-type mice. However, levels of bone volume increase were limited in both DC-STAMP knockout mice and OC-STAMP knockout mice. Consequently, we sought other targets that might function to increase bone mass more robustly.

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**Fig. 1** Bone homeostasis is regulated by osteoclast and osteoblast activities. In pre-menopausal conditions, osteoclasts and osteoblasts work in concert to keep bone volume stable (left panel). However, post-menopausally, osteoclast activities outweigh those of osteoblasts, decreasing bone volume (right panel).

**Fig. 2** Generation of multi-nuclear osteoclasts. Osteoclasts and macrophages are derived from common precursor cells in the presence of macrophage colony-stimulating factor (M-CSF), a cytokine for macrophage differentiation, and receptor activator of nuclear factor kappa B ligand (RANKL) and M-CSF alone, respectively. Mono-nuclear osteoclasts are generated first, and then multi-nuclear osteoclasts form by fusion of mono-nuclear osteoclasts via DC-STAMP and OC-STAMP. Although mono-nuclear osteoclasts can resorb bone, resorption activity is significantly elevated in multi-nucleated cells.
Regulation of osteoclasts by hypoxia and estrogen

Osteoclasts localize to bone surfaces that are very hypoxic.\(^{11}\) We found that osteoclasts express mRNA encoding hypoxia inducible factor 1 alpha (HIF1\(\alpha\)), a transcription factor regulated by hypoxia.\(^{12}\) Interestingly, HIF1\(\alpha\) protein is detected in osteoclasts in ovarioctomized (OVX) but not in sham-operated mice,\(^{12}\) suggesting that osteoclast-specific HIF1\(\alpha\) protein is suppressed by estrogen.

In vitro, HIF1\(\alpha\) protein is found in osteoclasts cultured under hypoxia but not in cells cultured in normoxic conditions.\(^{12}\) Interestingly, HIF1\(\alpha\) protein is suppressed in osteoclasts by estrogen derivative estradiol (E2), even under hypoxia, without affecting HIF1\(\alpha\) mRNA levels, supporting the idea that HIF1\(\alpha\) protein in osteoclasts is normally suppressed by estrogen (Fig. 3). Based on this model, estrogen deficiency would promote HIF1\(\alpha\) protein accumulation in osteoclasts, leading to their activation and subsequent bone loss (Fig. 3).

To determine the consequences of HIF1\(\alpha\) protein accumulation in osteoclasts in these conditions, we generated osteoclast-specific HIF1\(\alpha\) conditional knockout mice\(^{12}\) and found that they were resistant to OVX-induced bone loss. Similarly, in wild-type mice, systemic administration of a HIF1\(\alpha\) inhibitor completely abrogated OVX-induced osteoclast activation and bone loss.\(^{12}\) These results suggest that HIF1\(\alpha\) could serve as a therapeutic target to prevent osteoclast activation and bone loss in this group of patients. At present, interactions between cell–cell fusion and HIF1\(\alpha\) expression are unclear, and the regulation by HIF1\(\alpha\) of DC-STEM or OC-STEM has not been reported. Because expression of both DC-STEM and OC-STEM is regulated by nuclear factor of activated T cells 1,\(^{10,13}\) a transcription factor essential for osteoclastogenesis, cell–cell fusion and HIF1\(\alpha\) activity are likely independent.

Not only does aberrant osteoclast activity cause osteoporosis, but osteoclasts in cooperation with osteoblasts are required for normal bone turnover. Thus, inhibition of osteoclasts beyond physiological levels could severely suppress bone turnover. However, our evidence suggests that targeting the osteoclast factors pathologically activated by estrogen deficiency in post-menopausal women is not likely to interfere with physiological bone turnover: we found that systemic administration of a HIF1\(\alpha\) inhibitor to sham-operated mice did not inhibit osteoclast activity, likely because HIF1\(\alpha\) protein is already sufficiently suppressed by estrogen in normal conditions. HIF1\(\alpha\) is reportedly essential for angiogenesis coupled to osteoblastogenesis in bone\(^{14,15}\); however, we found that systemic HIF1\(\alpha\) inhibition did not result in decreased bone mass in sham-operated animals in estrogen-sufficient conditions.\(^{12}\) Taken together, we propose that HIF1\(\alpha\) is a suitable therapeutic target to inhibit pathological but not physiological osteoclast activities and to maintain bone turnover at levels similar to those in pre-menopausal conditions in post-menopausal osteoporosis patients.

Conflict of Interest

The author has no conflicts of interest to report.

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