Glutaredoxin2 isoform b (Glrx2b) promotes RANKL-induced osteoclastogenesis through activation of the p38-MAPK signaling pathway

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Receptor activator of NF-κB ligand (RANKL) triggers the differentiation of bone marrow-derived monocyte/macrophage precursor cells (BMMs) of hematopoietic origin into osteoclasts through the activation of mitogen-activated protein (MAP) kinases and transcription factors. Recently, reactive oxygen species (ROS) and antioxidant enzymes were shown to be closely associated with RANKL-mediated osteoclast differentiation. Although glutaredoxin2 (Glrx2) plays a role in cellular redox homeostasis, its role in RANKL-mediated osteoclastogenesis is unclear. We found that Glrx2 isoform b (Glrx2b) expression is induced during RANKL-mediated osteoclastogenesis. Over-expression of Glrx2b strongly enhanced RANKL-mediated osteoclastogenesis. In addition, Glrx2b-transduced BMMs enhanced the expression of key transcription factors c-Fos and NFATc1, but treatment with SB203580, a p38-specific inhibitor, completely blocked this enhancement. Conversely, down-regulation of Glrx2b decreased RANKL-mediated osteoclastogenesis and the expression of c-Fos and NFATc1 proteins. Also, Glrx2b down-regulation attenuated the RANKL-induced activation of p38. Taken together, these results suggest that Glrx2b enhances RANKL-induced osteoclastogenesis via p38 activation. [BMB reports 2012; 45(3): 171-176]

INTRODUCTION

Bone homeostasis is maintained through regulation of the balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation; an imbalance can lead to the development of diseases such as osteoporosis, periodontal disease, rheumatoid arthritis, multiple myeloma, and metastatic cancers (1). Osteoclasts are multinucleated giant cells that resorb mineralized tissues and are differentiated from hematopoietic stem cells by key factors, such as macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). In particular, the binding of RANKL to its receptor, RANK, triggers osteoclast differentiation via the activation of mitogen-activated protein (MAP) kinases and transcription factor NF-κB (2). In addition to NF-κB, AP-1 transcription factor member c-Fos and nuclear factor of activated T cells (NFAT) c1 are strongly induced during RANKL-induced osteoclast differentiation and play critical roles in the regulation of genes required for osteoclast differentiation and function. c-Fos is essential for RANKL-mediated induction of NFATc1 (3, 4), and NFATc1 regulates a number of osteoclast-specific genes, including DC-STAMP, tartrate-resistant acid phosphatase (TRAP), Atp6v0d2, and osteoclast-associated receptor (OSCAR) (5).

Reactive oxygen species (ROS), including free radicals and radical-derived/non-radical reactive species, are involved in the control of many cellular functions and play important roles as regulatory mediators in signaling processes (6). Interestingly, the involvement of ROS in osteoclastogenesis has been reported in several studies: an increase in intracellular ROS caused by RANKL was observed in bone marrow-derived monocyte/macrophage precursor cells (BMMs), and ROS (e.g., hydrogen peroxide) have been shown to be associated with enhanced bone resorption (7, 8).

Increased levels of the antioxidant glutathione (GSH), which plays a major role in oxidant defense via an enzymatic coupling reaction, enhance bone resorption activity (9). In contrast, GSH depletion by L-buthionine-(S,R)-sulfoximine (BSO), a specific inhibitor of GSH synthesis, inhibits osteoclastogenesis and bone pit formation by blocking nuclear importation of NF-kB and AP-1 in RANKL-mediated signaling in RAW 264.7 cells (10). Furthermore, antioxidant enzymes regulating ROS have been shown to

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be closely associated with RANKL-mediated osteoclast differentiation; thioredoxin-1 (Trx1) increases osteoclast formation through the AP-1 and NF-κB signaling pathways in RAW 264.7 cells, but other antioxidant enzymes, specifically glutathione peroxidase-1 (Gpx) and peroxiredoxin-1 (Prx), inhibit RANKL-mediated osteoclastogenesis (11). These results indicate the involvement of ROS and antioxidant enzymes in osteoclastogenesis.

The antioxidant protein glutaredoxin (Glrx) catalyzes deglutathionylation of protein-glutathione mixed disulfides (protein-SSG) and plays important roles in redox homeostasis and signal transduction (12). Among Glrx family members, Glrx2 has been shown to play a central role in the regulation of apoptosis. In a functional study, Glrx2 decreased apoptosis induced by doxorubicin and the antimitabolite 2-deoxy-D-glucose by sustaining mitochondrial redox homeostasis (13), but its down-regulation induced sensitivity to phenylarsine oxide and the anticancer agent doxorubicin in HeLa cells (14). However, to date, no investigation has addressed the potential role of Glrx2 in osteoclastogenesis. Therefore, we investigated the functional involvement of Glrx2 in RANKL-induced osteoclastogenesis in BMMs. Mouse Glrx2 is localized in the mitochondria and has mRNA variants encoding two distinct proteins: mitochondrial isofrom a, Glrx2a (UniProt ID: A2A5W4), and non-mitochondrial isofrom b, Glrx2b (UniProt ID: Q3UQ95) (15). Because we found that Glrx2b, but not Glrx2a, is strongly induced during RANKL-mediated osteoclast differentiation, we focused on evaluating the functional role of Glrx2b in osteoclastogenesis.

RESULTS

Glrx2b is time-dependently induced during RANKL-mediated osteoclastogenesis

To investigate the expression pattern of Glrx2b in RANKL-mediated osteoclastogenesis, Western blot analysis was performed using BMMs that were differentiated into osteoclasts by treatment with RANKL in the presence of M-CSF. Interestingly, Glrx2b protein was time-dependently induced by RANKL (Fig. 1). RANKL-mediated osteoclastogenesis was confirmed by evaluating the expression of transcription factors c-Fos and NFATc1, which play major roles in the regulation of osteoclastogenesis-related genes. RANKL-induced expression of Glrx2b was also confirmed in a RAW 264.7 cell-based model of osteoclast differentiation (data not shown).

Over-expression of Glrx2b enhances RANKL-induced osteoclastogenesis

When BMMs were transduced with Glrx2b retrovirus, selected with puromycin, and cultured with M-CSF and RANKL, more mature TRAP-positive multinucleated osteoclasts (MNCs) were formed compared to the control (Fig. 2A). The number of TRAP-positive MNCs and RANKL-mediated TRAP activity were increased by over-expression of Glrx2b (Fig. 2B-D).

Because RANKL is expressed mainly as a membrane-bound form on osteoblasts and triggers the signaling essential for osteoclast differentiation (4, 16), the effect of endogenous RANKL generated by osteoblasts on Glrx2b-induced osteoclastogenesis