Effects of deficiency of Kelch-like ECH-associated protein 1 on osteoclastogenesis by constitutive activation of nuclear factor E2 p45-related factor 2

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[Background] Kelch-like ECH-associated protein 1 (Keap1) binds to nuclear factor E2 p45-related factor 2 (Nrf2), a transcription factor of antioxidant enzymes. Previous studies indicated that oxidative stresses stimulate osteoclastogenesis, which is responsible for bone loss, by activating receptor activator of NF-kappaB ligand (RANKL)-dependent pathways. In addition, recent studies reported that Nrf2 deficiency stimulates osteoclastogenesis. However, the effects of Keap1 deficiency remain unclear.

[Methods] Keap1-deficient-, Nrf2-deficient-, Keap1-Nrf2-double deficient mice were used for osteoclast formation.

[Results] By using Keap1-deficient newborn mice, we observed that osteoclast number was reduced and that talus and calcaneus bone formation was partially retarded without severe gross abnormalities in vivo. Moreover, Keap1-deficient macrophages were unable to differentiate into osteoclasts in vitro via attenuation of RANKL-regulated signaling and expression of nuclear factor of activated T cells-1 (NFATc1), a key transcription factor for osteoclastogenesis. During osteoclastogenesis, RANKL-induced gene expression of mitochondrial enzyme is required for down-regulation of IFN regulatory factor (IRF8), a negative transcriptional regulator of NFATc1. Our results indicate that Keap1 deficiency down-regulated peroxisome proliferator-activated receptor-gamma coactivator 1beta and mitochondrial gene expression and up-regulated Irf8 expression, as well as up-regulation of MafB, a negative regulator of NFATc1. Furthermore, Keap1-dependent phenotypes, such as attenuation of NFATc1 expression and the up-regulation of Irf8 as well as MafB in Keap1-deficient cells were reversed in Keap1-Nrf2-double deficient cells, suggesting that constitutive Nrf2 activation is responsible for the IRF8/MafB-mediated NFATc1 down-regulation and impaired osteoclastogenesis. These results also support the possibility that Nrf2 activators might have potential activity to inhibit osteoclast differentiation via NFATc1-dependent pathways. To elucidate this possibility, we examined effects of tert-butylhydroquinone (tBHQ) and sulforaphane (SR), both are widely used as Nrf2 activators, on the differentiation of macrophages into osteoclasts. Both compounds inhibited the formation of osteoclasts in a dose-dependent manner, accompanied by increased levels of Irf8 and MafB expression. Moreover, our observations with tBHQ and SR were reversed in Nrf2-deficient cells.

[Conclusion] These results suggest that the Keap1/Nrf2 axis plays important roles in NFATc1 expression and osteoclast differentiation.