Enhancement of osteoblastogenesis and suppression of osteoclastogenesis by inhibition of de-phosphorylation of eukaryotic translation initiation factor 2 alpha

Kazunori Hamamura¹, Andy Chen¹, Hiroki Yokota¹,²

¹Department of Biomedical Engineering, Indiana University Purdue University Indianapolis, Indianapolis, IN 46202, USA
²Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Correspondence: Kazunori Hamamura
E-mail: hamak@dpc.agu.ac.jp
Received: December 30, 2014
Published online: February 1, 2015

The phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) is activated in response to various stresses such as viral infection, nutrient deprivation, and stress to the endoplasmic reticulum. Severe stress to the endoplasmic reticulum, for instance, induces an apoptotic pathway, while mild stress, on the contrary, leads to a pro-survival pathway. Little has been known about the elaborate role of eIF2α phosphorylation in the development of bone-forming osteoblasts and bone-resorbing osteoclasts. Using salubrinal and guanabenz as inhibitors of the de-phosphorylation of eIF2α, we have recently reported that the phosphorylation of eIF2α significantly alters fates of both osteoblasts and osteoclasts. Based on our recent findings, we review in this research highlight the potential mechanisms of the enhancement of osteoblastogenesis and the suppression of osteoclastogenesis through the elevated level of phosphorylated eIF2α.

Keywords: osteoclast; eIF2α; Salubrinal; Guanabenz; principal component analysis


Mechanism of the enhancement of osteoblastogenesis by the inhibition of de-phosphorylation of eIF2α

Bone remodeling is a combined process of bone formation by osteoblasts and bone resorption by osteoclasts. We first examined the involvement of eIF2α in regulation of osteoblasts. Stress to the endoplasmic reticulum leads to the elevated phosphorylation level of eIF2α and suppresses general translation initiation except for some stress-responsive genes, including activating transcription factor 4 (ATF4) [¹, ²]. ATF4 is an important transcription factor for differentiation of mature osteoblasts [³], prompting a question: Does the inhibition of de-phosphorylation of eIF2α stimulate development of osteoblasts? Salubrinal and guanabenz are synthetic chemical agents known to specifically de-phosphorylate eIF2α by inhibiting protein phosphatase 1 (PP1) [⁴, ⁵]. They are also known as suppressors of stress to the endoplasmic reticulum.
Help identify axes that best explain the variance of the data [11]. PCA can be used to analyze genome-wide microarray data and identify principal axes and genes that highly contribute to those axes. PCA predicted a set of stimulatory and inhibitory transcription factor candidates underlying salubrinal- and guanabenz-driven suppression of osteoclastogenesis. Among these, two AP-1 transcription factors (c-Fos and JunB) were included.

As predicted, expression levels of c-Fos and JunB were upregulated by RANKL, and their upregulation was suppressed by salubrinal and guanabenz in mouse primary macrophage and RAW264.7 cells [9]. In RAW264.7 cells, a partial silencing of c-Fos by RNA interference attenuated RANKL-driven expression of NFATc1, TRAP, and cathepsin K. A partial silencing of JunB reduced NFATc1 and TRAP, but not cathepsin K. To further analyze regulatory linkages among NFATc1, c-Fos and JunB, a partial silencing of NFATc1 was conducted. Twelve hours after RANKL treatment in RAW264.7 cells, treatment with NFATc1 siRNA did not alter expression of c-Fos and JunB. In 24 h, however, the level of c-Fos was significantly reduced without affecting the level of JunB [9]. Collectively, the result suggests a potential feedback loop between NFATc1 and c-Fos.

Conclusions

Inhibition of de-phosphorylation of eIF2α promotes differentiation and mineralization of osteoblasts through the upregulation of ATF4. The same inhibition in osteoclasts, on the other hand, suppresses osteoclastogenesis by downregulating RANKL-driven NFATc1. A genome-wide expression analysis demonstrates that the regulation of NFATc1 is mediated by c-Fos and JunB (Fig. 1). In summary, the current study demonstrates that inhibitors of de-phosphorylation of eIF2α such as salubrinal and guanabenz may provide a novel therapeutic possibility for bone diseases including osteoporosis.

Acknowledgment

This work was supported by grant DOD W81XWH-11-1-0716.

Conflict of interests

All authors declare that they have no conflict of interests.

References


