The Direct Effect of TNF-α on Osteocyte RANKL Expression: Interactions in Osteoimmunology

論 文 内 容 要 旨

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Osteoimmunology peeks into the interaction of bone and the immune system, which has largely proved to be a multiplex reaction. Osteocytes have been shown to regulate bone resorption through the expression of RANKL in physiologic and pathologic conditions. TNF-*α*, a product of the immune system, is an important cytokine regulating bone resorption in inflammatory conditions either directly or by increasing RANKL and M-CSF expressions by osteoblasts and stromal cells. The effect of TNF-*α* on a wide range of cell types has been documented; however, the direct effect of TNF-*α* on osteocytes has not been established yet; partially due to the hard to access nature of the osteocyte in a mineralized bony matrix. In this work, an efficient method for isolating pure primary osteocytes was provided and the direct effect of TNF-*α* on primary isolated osteocytes was investigated. Primary osteocytes were isolated by cell sorting from neonatal calvaria of Dmp1-Topaz mice, which express the green fluorescent protein under the influence of dentin matrix protein 1 promoter. The results showed that osteocytes have a significantly higher RANKL mRNA expression when cultured with TNF-*α*. A co-culture system of osteocytes and TNF receptors I and II deficient osteoclast precursors with TNF-*α* showed a significant increase in the number of TRAP-positive cells while cultures without TNF-*α* failed to show TRAP-positive cells. Additionally, *in vivo* experiments of TNF-*α* injected to mouse calvaria showed an increase in TRAP-positive cell number in the suture mesenchyme and an increase in the percentage of RANKL-positive osteocytes compared to PBS-injected calvaria. Osteocytes cultured with TNF-*α* showed up-regulation of MAPKs phosphorylation which was measured by western blot. In addition to that, adding MAPKs inhibitors to osteocytes cultured with TNF-*α* significantly decreased RANKL mRNA expression compared to osteocytes cultured with TNF-*α* alone. TNF-*α* activated the NF-*k*B pathway in osteocytes which was measured as a function of p65 subunit nuclear translocation. TNF-*α* directly affected osteocyte RANKL expression and increased osteoclastogenesis. We also assessed whether TNF-*α* influenced RANKL expression in osteocytes during orthodontic tooth movement by using wild-type (WT) and TNF receptor I and II deficient (TNFRsKO) mice. A Nickel-titanium closed coil spring was attached to the maxillary alveolar bone near the incisors and the upper left first molar, and the first molars were moved mesially in WT and TNFRsKO mice. The number of RANKL-positive osteocyte in the alveolar bone significantly increased in WT mice than in TNFRsKO mice after OTM. These results demonstrated that osteocytes guard an important role in inflammatory bone resorption as well as mechanically driven bone resorption mediated by TNF-*α*.