Small molecule T63 suppresses osteoporosis by modulating osteoblast and osteoclast differentiation

Zhen Wang, Xiaoli Zhao, Shuyi Si

Biochemistry, Institute of Medicinal Biotechnology, Peking Union Medical College and Chinese Academy of Medical Sciences, China

Background: Osteoporosis results from the imbalance between bone resorption and bone formation, and restoring the normal balance of bone remodeling is highly desirable for identification of better treatment. In this work, we aim to identify novel small-molecule compounds to restore the balance of bone metabolism by promoting osteogenesis and/or inhibiting osteoclastogenesis. Methods: A cell-based high-throughput screening model was established that represents Runt-related transcription factor 2 (RUNX2) transcriptional activity, and high-throughput screening was performed with a library containing over 20,000 compounds. Alkaline phosphatase (ALPL) activity and mineralization, as well as gene expression of Alpl and other osteogenic marker genes, upon induction in osteogenic medium in mouse osteoblasts MC3T3-E1 cells and mesenchymal stem cell-like cells C3H10T1/2 were analyzed after drug treatment. Western blots were performed to study the BMPs and WNT signaling pathways involved in osteogenesis regulation. Ovariectomized and dexamethasone treated rat osteoporosis model was used to observe the in vivo effect of the compound. Also, the effect of the compound on osteoclastogenesis was observed in RANKL-induced RAW264.7 cells by TRAP staining and expression analysis of osteoclastogenesis-related genes expression. Results: A small molecule compound T63 was identified from the screening to be an efficient up-regulator of RUNX2 activity. T63 increased osteoblast differentiation in both dose- and time-dependent manner in MC3T3-E1 cells and C3H10T1/2 cells upon induction. T63 up-regulated RUNX2 mRNA and protein levels, and knockdown of RUNX2 reduced the osteogenic role of T63. Mechanistically, T63 activated both BMPs and WNT signaling pathways. Inhibition of either signaling pathway with specific inhibitor suppressed T63-induced RUNX2 expression as well as the osteogenic phenotypes. Notably, T63 was also found to directly suppress RANKL-induced osteoclast differentiation, as evidenced by decreased TRAP-staining and osteoclastogenesis-related genes expression in RAW264.7 cells upon induction. Moreover, T63 markedly protected against bone mass loss in the ovariectomized and dexamethasone treated rat osteoporosis model. Conclusion: Our data demonstrate that T63 protects against bone loss by exerting dual effects on osteogenesis and osteoclastogenesis. The compound could be a promising drug candidate and deserves further development for potential therapeutics in bone metabolism disorders such as osteoporosis.