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Author: Mrs. Feima Guo
China Astronaut Research and Training Center, China

Dr. Zhongquan Dai
China Astronaut Research and Training Center, China
Mrs. Feifei Tong
China
Ms. Lina Qu
China
Mr. Peng Shang
Northwestern Polytechnical University, China
Prof. Yinghui Li
China Astronaut Research and Training Center, China

SIMULATED MICROGRAVITY INHIBITS THE RESPONSE OF RUNX2 TO VD3

Abstract

Introduction: Bone loss in humans and experiments animals resulted from spaceflight mainly caused by decreased bone formation, osteoblast proliferation and differentiation. Runx2 plays important roles in osteoblast differentiation and function. Previous studies have showed 1, 25-dihydroxyvitamin D3 (VD3) regulates osteoblast differentiation through Runx2 transcript factor. In present, we examined the effect of VD3 on Runx2 activity under simulated microgravity conditions. Methods: To this study destination6OSE2 promoter, which consists with 6 repeats of osteoblast specific element 2 (OSE2) upstream of a minimal 34bp mOG2 promoter, was synthesized and cloned into pUC57-T vector. This 140bp promoter was digested by restriction enzyme KpnI and BamH I and inserted into pGL4.14 vector (Promega USA) digested by Kpn I and Bgl II, so that the reporter gene firefly luciferase was driven by 6OSE2 promoter and regulated by Runx2. Osteoblast MC3T3-E1 and myogenic cells C2C12 were transfected with this vector by Lipofectamine2000 respectively and stably selected by Hygromycin B. The stable cell lines were treated with BMP2 and VD3 for 48h and detect the activity of firefly luciferase to test if reporter gene can respond to osteogenic factor. Selected cell line was cultured on clinostat to simulated microgravity with or without VD3. Firefly luciferase activity was assayed by using the Single Luciferase Reporter Assay System (Promega). Result: p6OSE2-LUC expression vector was constructed. Through stable transfection and selection, we gained OSE-MC3T3E1 and OSE-C2C12 cell strains in which luciferase activity can reflect the effects of osteogenic factors treatment. In OSE-MC3T3E1 and OSE-C2C12 cells, luciferase activity increased after 48h of VD3 treatment in normal condition. In simulated microgravity, alkaline phosphatase and luciferase activity reduced after 48h clinorotation culture. The degree of luciferase activity increase in simulated microgravity was lower than in 1g condition when simultaneously treated with VD3 for 48h. Conclusion: The cell model of OSE-MC3T3E1 and OSE-C2C12 can response the osteogenic induction by the activity of reporter luciferase and can be used for further investigation about bone loss mechanism. Simulated microgravity inhibited the osteogenesis and attenuated the induction of VD3 for osteoblast mature. Keywords: Runx2; microgravity; 1,25-dihydroxyvitamin D3; luciferase; osteoblast

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