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SIMULATED MICROGRAVITY ATTENUATE THE RESPONSIVENESS OF CBFA1 TO CYTOKINES

Abstract

Bone loss resulted from spaceflight mainly caused by decreased bone formation, osteoblast proliferation and differentiation. Transcript factor Cbfa1 plays important roles in osteoblast differentiation and function through responding to microenvironment change including cytokines and mechanical factor. In present, we examined the activity and responsiveness of Cbfa1 to cytokines under simulated microgravity condition and their possible mechanism. First we established cell models (6OSE2-Luc-MC3T3E1/6OSE2-EGFP-MG63) which was stably transfected with p6OSE2-Luc/p6OSE2-EGFP expression vector into MC3T3E1/MG63. Using the models, Cbfa1 activity can be reflected by the activity of reporter luciferase or EGFP. The selected cell line was cultured on clinostat to simulated microgravity with or without cytokine (IGF-I, VD3, BMP2), then the reporter acitvity was assayed using the Single Luciferase Reporter Assay System (Promega) or established semi-quantitative fluorescence intensity analysis methods. The expression of Cbfa1 was examined by Western blot and the interaction between Cbfa1 and VDR (receptor of VD3), IRS (signal molecular of IGF-I) and integrin 3 were detected by Co-immunoprecipitation methods. In normal condition, the reporter activity increase after 48h of cytokines treatment. In simulated microgravity, the ALP activity and reporter activity reduced after 48h clinostation, so does Cbfa1 expression. The degree of reporter acitivity increase in simulated microgravity was lower than in normal condition. Using CO-IP, we demonstrated that simulated microgravity attenuated the interaction between Runx2 and VD3 or integrin 3 and IRS. Microgravity coused a thinning and dispersed distribution of microfilament. In regard as BMP2, microfilament disruptor cytochalasin B significantly attenuated BMP2 induction to Cbfa1 as well as DNA binding activity of Cbfa1 to OSE2. The addition of F-actin stabilizer Jasplakinolide reversed the inhibitory effect of microgravity on the responsiveness of Cbfa1 to BMP2. Taken together, the cell models can be used for the investigation about bone loss mechanism. Our reults demonstrated that microgravity inhibited the osteogenesis by decreaseing the activity and responsiveness of Cbfa1 to cytokines for osteoblast mature. Microgravity decrease the interaction between Cbfa1 with cofactor and the integrin-cytoskeleton system participated these inhibitory effects of microgravity. The detail mechanism need to be further studied.

Keywords: Runx2; simulated microgravity; VD3; luciferase; osteoblast

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