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ESTABLISH AN OSTEOLASTIC MODEL REFLECTING CBFA1 ACTIVITY BY FLUORESCENCE

Abstract

Objective: To obtain MG63 cell lines which were stably transfected with EGFP reporter gene driven by 6OSE2 promoter and select the cell strain which can reflect Cbfa1 activity by EGFP fluorescence. **Method:** 6OSE2 promoter was synthesized and cloned into pUC57-T vector. The 140bp promoter fragment was obtained from amplified vector by double digestion with restriction enzyme and inserted into CMV-promoter deleted pEGFP-N1 vector to construct eukaryotic expression vector. MG63 cell was transfected with this vector by Lipofectamine2000 and stably selected by G418. Analysis the EGFP fluorescence intensity of the stably transfected MG63 cell stain and detection of ALP activity after treatment with different concentration of IGF-I or VD3 to selected one which can reflecting Cbfa1 activity. **Result:** The pUC-6OSE2 amplified vector and p6OSE2-EGFP expression vector were constructed. Through stably transfection and selection, we gained one OSE-MG63 cell strain which fluorescence intensity can reflect the treatment of IGF-I or VD3. **Conclusion:** The osteoblastic model which can reflect Cbfa1 activity was establishment successfully and verified by microgravity experiment. This cell line will be useful for studying the effects of microgravity on the activity of Cbfa1 and the signal pathways related with bone form.

Key words:Osteoblast; 6OSE2; MG63; Gene expression

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