成纤维生长因子(FGF) 23基因沉默对甲状旁腺激素(PTH)促成骨细胞分化作用的影响

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Influence of FGF23 silence on differentiation of rat calvarial osteoblastic cells by parathyroid hormone in vitro

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摘要

目的 研究内源性成纤维生长因子23(fibroblast growth factor 23,FGF23)对甲状旁腺激素(parathyroid hormone,PTH)促成骨细胞分化作用的影响。方法 采用酶消化法分离培养新生SD大鼠头盖骨成骨细胞,应用小发夹RNA(short hairpin RNA,shRNA)干扰技术(RNAi)沉默成骨细胞FGF23表达,应用MTT法和PNPP法检测细胞增殖和碱性磷酸酶(alkaline phosphates,ALP)活性,应用real-time RT-PCR法检测其FGF23、ALP和骨钙素(osteocalcin,OCN)等基因mRNA水平,研究PTH对培养成骨细胞和FGF23基因沉默细胞的作用。结果 rhPTH1-34对培养成骨细胞增殖促进作用明显,分化促进作用弱。1×10-10~1×10-8 mol/L rhPTH1-34 作用3天,细胞增殖率增加31.6%~50.5% (P<0.05),而细胞比活性未见明显改变。同时其ALP和OCN转录水平轻度上调,分别较对照组增加35% (P<0.05)和16%(P>0.05)。rhPTH1-34上调成骨细胞FGF23 mRNA水平(4倍),该作用可被针对FGF23特异的shRNA转染所抑制。FGF23基因沉默后PTH促分化作用明显增强,其ALP和OCN mRNA水平分别较对照组增加1.8倍(P<0.05)和5.8倍(P<0.05),显示内源性FGF23对PTH促分化的干扰作用。结论 内源性FGF23可能参与PTH促分化作用的调节,FGF23上调可干扰PTH的促成骨细胞分化作用。

关键词 ： 甲状旁腺激素(PTH)，成纤维细胞生长因子(FGF) 23 ， RNA干扰(RNAi)，成骨细胞，大鼠

Abstract :

Objective To investigate the roles of fibroblast growth factor 23 (FGF23) in osteoblast by RNA interference (RNAi) and its influence on cell differentiation by parathyroid hormone (PTH) in vitro.Methods The primary rat calvarial osteoblasts were cultured in MEM medium containing 10% charcoal stripped fetal bovine serum(CSFBS) and treated with rhPTH1-34 for 3 days.The changes of proliferation and alkaline phosphates (ALP) activity were measured by MTT and PNPP methods respectively,and the ALP and OCN mRNA levels were determined by real-time RT-PCR.Further FGF23 was transiently silenced by shRNA method in osteoblasts and the expression levels of FGF23,ALP and OCN were determined in transcriptional levels after rhPTH1-34 treatment. Results rhPTH1-34 prompted osteoblasts proliferation while less effect on its differentiation.The cells proliferations were increased 31.6%~50.5% (P<0.05) by rhPTH1-34 in range of 1×10-10-1×10-8 mol/L,while the cell ALP activities (calculated by D405/D570) were slightly but no significantly changed.Meanwhile,the mRNA levels of ALP and OCN were slightly increased 35% (P<0.05) and 16% (P>0.05) by 1×10-9 mol/L rhPTH1-34 in 2 hours treatment.The FGF23 expression was up regulated (about 4 folds) in osteoblasts by 1×10-9 mol/L rhPTH1-34 in transcriptional level and which was turned down by the transfection of shRNA of FGF23.Further,the marked stimulating effects of rhPTH1-34 on ALP and OCN expression (about 1.8 and 5.8 folds respectively) were showed in the transfected osteoblasts.Conclusions The expression of FGF23 might involve in osteogenesis regulation by PTH.The up regulation of FGF23 expression by PTH could suppress its stimulating effect on osteoblasts differentiation,and the underlying mechanisms remain to be clarified.

Key words : parathyroid hormone(PTH) fibroblast growth factor (FGF) 23 RNA interference(RNAi) osteoblasts rats

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