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Radixin定点突变过表达对HepG2细胞膜转运蛋白B

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Title: Effect of radixin phosphorylation on bile salt export pump expression on HepG2 cell membrane

作者: 封欣婵; 柴进; 程英; 陈文生
第三军医大学西南医院全军消化病研究所

Author(s): Feng Xinchan; Chai Jin; Cheng Ying; Chen Wensheng
Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing, 400038, China

关键词: RDX定点突变; 载体构建; pcDNA3.1载体; 胆盐输出泵

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摘要: 目的 构建pcDNA3.1-RDX定点突变真核过表达质粒，研究其在胆汁淤积时对HepG2细胞膜转运蛋白胆盐输出泵(bile salt export pump, Bsep)定位表达的影响。 方法 从含有RDX野生型质粒中，利用PCR方法钓取RDX野生型基因片段并以野生型为基础进行定点突变，PCR扩增后转入pcDNA3.1载体，其产物转化DH-5α感受态细胞。对长出的单克隆进行菌落PCR鉴定，再对PCR鉴定阳性的克隆进行测序和比对分析，比对正确即为构建成功的目的质粒。将目的质粒转染HepG2细胞，经G418筛选构建稳转细胞株。提取各株细胞的总蛋白，检测磷酸化RDX是否影响HepG2细胞膜上转运蛋白Bsep的表达。 结果 PCR和测序结果均证实pcDNA3.1-RDX WT、pcDNA3.1-RDX T564D、pcDNA3.1-RDX T564A过表达载体构建成功。蛋白免疫印迹表明，与转染pcDNA-3.1-RDX-WT的HepG2相比，转染pcDNA-3.1-T564D的HepG2的Bsep膜蛋白表达量显著增加(P<0.05)，而转染pcDNA-3.1-T564A的HepG2的Bsep膜蛋白表达量有所下降（P>0.05）。 结论 成功构建了pcDNA3.1-RDX WT、pcDNA3.1-RDX T564D、pcDNA3.1-RDX T564A过表达载体，并证实RDX的磷酸化能增强HepG2细胞膜上Bsep的表达。

Abstract: Objective To construct radixin (RDX) wild type and mutant over-expression vectors, pcDNA3.1-RDX WT, pcDNA3.1-RDX T564D, and pcDNA3.1-RDX T564A, and to study the effects of RDX phosphorylation on bile salt export pump (Bsep) expression of HepG2 cell membrane. Methods RDX wild type gene was

used as template to carry out point mutation, T564D and T564A. All target DNAs were transferred to pcDNA3.1 to construct 3 over-expression vectors, pcDNA3.1-RDX WT, pcDNA3.1-RDX T564D, and pcDNA3.1-RDX T564A. DH-5α competent cells were transformed with the recombinant plasmids for positive clone identification. Then target clones were used to transfect HepG2 cells and stable cell lines were established. Western blotting was used to confirm the expression of RDX, p-Thr564-RDX and Bsep. Results All the recombinant plasmids were correctly constructed. Western blot results demonstrated that the cell membrane Bsep expression was slightly higher in HepG2 cells transfected with pcDNA3.1-RDX WT than in the cells transfected with pcDNA-3.1-RDX T564A (P>0.05) and significantly lower than in the cells transfected with pcDNA3.1-RDX T564D (P<0.05). Conclusion RDX wild type and mutant over-expression vectors are successfully constructed. RDX phosphorylation improves Bsep expression on HepG2 cell membrane.

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