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基础研究

miR-132在食管癌细胞系KYSE150 中对靶基因FOXA1的调控作用

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Regulation of miR-132 on Target Gene FOXA1 in Esophageal Carcinoma Cell Line KYSE150

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- 摘要
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全文: PDF (877 KB) HTML (0 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 目的构建miR-132真核表达载体和融合靶基因FOXA1表达载体，在食管癌细胞KYSE150中验证miR-132对靶基因FOXA1的调控作用。方法根据miR-132序列在基因组中的位置及其上下游序列，以EC9706细胞基因组DNA为模板，扩增包含miR-132前体序列，克隆到pMD18-T Simple中，经BamHⅠ和EcoRⅠ酶切后亚克隆到质粒pcDNA3.1(+)上；通过Real-time PCR检测转染重组表达载体pcDNA3.1-miR-132的KYSE150成熟miR-132的表达水平。用生物信息学软件对miR-132的靶基因进行预测，将候选靶基因FOXA1的3′ UTR区融合到pMIR荧光素酶基因下游，通过双荧光素酶报告基因检测分析miR-132对靶基因FOXA1的调控作用。将pCDNA3.1-miR-132表达质粒转染人食管癌细胞KYSE150，通过Western blot检测miR-132对FOXA1蛋白表达的影响。结果成功构建了miR-132真核表达载体，Real-time PCR验证表明pCDNA3.1-miR-132在KYSE150细胞中能够显著地过表达成熟miR-132。生物信息学预测FOXA1可能是miR-132的靶基因。双荧光素酶报告基因分析表明miR-132能够作用于FOXA1的3′ UTR。Western blot进一步证实miR-132能够抑制内源性FOXA1蛋白的表达。结论FOXA1是miR-132直接调控的靶基因。

关键词： KYSE150 miR-132 FOXA1

Abstract： ObjectiveTo construct an eukaryotic expression vector of miR-132 and FOXA1，to verify the regulation effect of miR-132 on the target gene FOXA1 in human esophageal carcinoma cell KYSE150. MethodsAccording to sequence of the mature miR-132 with its flank sequences，PCR primers were designed and miR-132 precursor sequence was amplified from genomic DNA of EC9706 cell. PCR product was cloned into pMD18-T Simple vector and then subcloned into pCDNA3.1(+) between BamHⅠand EcoRⅠ. Human esophageal carcinoma cell line KYSE150 was transfected by pCDNA3.1(+) and pCDNA3.1-miR-132,miR-132 were detected by real-time PCR. FOXA1 was predicted by using bioinformatics. The 3′ UTR of candidate target gene FOXA1 was cloned into the downstream of pMIR vector,mutation expression vector pMIR-FOXA1-Mut was also constructed. Co-transfection of both vectors were performed in KYSE150 cells and dual-luciferase reporter assay was analyzed. Western blot was used to detect the expression of FOXA1 protein in KYSE150 cell line transfected with pCDNA3.1(+)-miR-132 and pCDNA3.1(+) respectively. ResultsThe miR-132 expression vector has been constructed successfully and it can effectively express mature miR-132 in esophageal carcinoma cell KYSE150. FOXA1 was selected as a candidate of target genes which has 7 base pairs completely matched to miR-132 in the 3′ UTR seed region. Recombined vector pMIR-FOXA1 fused FOXA1 3′ UTR and mutation expression vector pMIR-FOXA1-Mut were constructed. Dual-luciferase reporter assay showed that the luciferase activity decreased in the group co-transfected with pCDNA3.1(+)-miR-132 and pMIR-FOXA1. Overexpression of exogenous miR-132 in KYSE150 cell line can significantly suppress the expression of FOXA1 protein. ConclusionmiR-132 can suppress FOXA1 gene expression at the post-transcriptional level by targeting to the specific sequence of FOXA1 gene 3′ UTR,FOXA1 is a target gene of miR-132.

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