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RBM5 基因表达载体构建、稳定转染A549 细胞系的建立及功能的初步研究

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Construction of RBM5 vector, establishment of stably transfected A549 cell line and preliminary research on the function of RBM5 gene

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摘要

目的：通过构建表达载体、建立稳定转染RNA结合基序5(RNA binding motif 5，RBM5)的A549细胞系，初步研究RBM5基因过表达对A549 细胞增殖以及天冬-谷-丙-组氨酸盒多肽15[DEAH box polypeptide 15，DHX15]表达的影响。方法：应用分段克隆法构建pcDNA3.1(+)/RBM5真核表达载体；将测序验证后的重组质粒pcDNA3.1(+)/RBM5转染肺腺癌A549细胞并以G418进行筛选，采用Western印迹鉴定RBM5基因过表达阳性细胞，流式细胞仪分别检测经pcDNA3.1(+)/RBM5稳定转染的A549细胞[pcDNA3.1(+)/RBM5-A549]及pcDNA3.1(+)空白质粒转染的A549细胞[pcDNA3.1(+)-A549]的周期分布；运用RT-PCR技术分别检测pcDNA3.1(+)/RBM5-A549和pcDNA3.1(+)-A549细胞中剪接相关因子DHX15的表达情况。结果：成功构建出pcDNA3.1(+)/RBM5真核表达载体，筛选出RBM5基因稳定转染过表达阳性细胞株；pcDNA3.1(+)/RBM5-A549较pcDNA3.1(+)-A549细胞处于G1期细胞比例增大、S期细胞比例减小(均$P<0.01$)；cDNA3.1(+)/RBM5-A549较pcDNA3.1(+)-A549细胞的DHX15表达上调($P<0.01$)。结论：成功构建重组质粒pcDNA3.1(+)/RBM5，并建立了RBM5稳定转染的A549细胞系；初步证实RBM5基因过表达可抑制肺腺癌A549细胞的细胞周期，并使DHX15表达上调。

关键词 ： RNA结合基序5，A549细胞系，天冬-谷-丙-组氨酸盒多肽15，基因克隆，质粒构建，肿瘤抑制，细胞周期

Abstract :

Objective: To establish a stable A549 cell line transfected by RNA binding motif 5 (RBM5) expression vector, and to investigate the effect of RBM5 gene on proliferation of A549 cell line and the expression of DEAH box polypeptide 15 (DHX15).
Methods: The eukaryotic expression vector pcDNA3.1 (+)/RBM5 was constructed by a twostep PCR technique. Then, the recombinant plasmid pcDNA3.1 (+)/RBM5 was verified by DNA sequencing and transfected into the lung adenocarcinoma cell A549. The positive cells with overexpression of RBM5 gene were identified by Western blotting. Flow cytometry was used to analyze the cell cycles of the positive A549 cells [pcDNA3.1 (+)/RBM5-A549] and the negative controls [pcDNA3 .1 (+)- A549]. Finally, RT-PCR was used to detect the expression of DHX15, a splicing-related factor, in the positively transfected A549 cells and the negative controls.
Results: A pcDNA3.1 (+)/RBM5 eukaryotic expression vector has been constructed successfully, and the A549 cell line that stably transfected with RBM5 gene has been established. Compared with negative control cells, the percentage of G1 phase cells in the positive cells was increased, while the percentage of S phase was decreased (both $P<0.01$), and the expression of DHX15 is upregulated ($P<0.01$).
Conclusion: RBM5 gene can inhibit the cell cycle and upregulate the expression of DHX15 in A549 cells.

Key words : RNA binding motif 5 A549 cell line DEAH box polypeptide 15 gene cloning plasmid construction tumor suppression cell cycle

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