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siRNA沉默 RhoC 表达诱导人肝癌BEL7402细胞凋亡

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摘要：

目的：研究siRNA沉默 RhoC 基因表达对人肝癌细胞BEL7402凋亡的影响及其机制，为肝癌的基因治疗提供实验依据。方法: 构建 RhoC -siRNA真核表达载体pU6mRFP RhoC -siRNA，转染BEL7402细胞，激光共聚焦显微镜检测转染效率，RT-PCR和Western blotting鉴定 RhoC 基因沉默效果；流式细胞术、琼脂糖凝胶电泳和瑞氏染色检测BEL7402细胞凋亡，RT-PCR检测细胞凋亡相关基因 Bcl-2和Bax 的表达。结果: 成功构建pU6mRFP RhoC -siRNA重组载体，转染BEL7402细胞的效率为70%，RT-PCR和Western blotting检测 RhoC 基因沉默效率分别为85%和82%。pU6mRFP RhoC -siRNA转染组 BEL7402细胞凋亡显著高于未转染BEL7402细胞和pU6mRFP Scramble-siRNA转染组BEL7402细胞\[（21.00±2.23）% vs （ 6.47±1 64）%、（4.63±0.47）%， P <0.01）\]，pU6mRFP RhoC -siRNA转染组BEL7402细胞DNA呈典型的“梯状”条带，瑞氏染色见转染组BEL7402细胞中有大量凋亡细胞。pU6mRFP RhoC -siRNA转染组BEL7402细胞 Bcl-2 基因水平显著低于、而 Bax 基因水平显著高于未转染BEL7402细胞和pU6mRFP Scramble-siRNA转染组BEL7402细胞（0.28±0.15 vs 0.96±0.21，1.03±0 24， P <0.05；1.09±0.21 vs 0.26±0.10，0.25±0.07， P <0.01）。结论：siRNA沉默 RhoC 基因可诱导人肝癌BEL7402细胞凋亡，其机制与下调 Bcl-2 基因、上调 Bax 基因表达有关。

关键词：RhoC 基因 siRNA 肝肿瘤 凋亡

siRNA silencing RhoC expression induces apoptosis of human hepatocellular carcinoma BEL7402 cells

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Abstract:

Objective:To study the effect of siRNA silencing RhoC expression on apoptosis in human hepatocellular carcinoma BEL7402 cells and the related mechanism, so as to provide an experimental evidence for liver cancer gene therapy. Methods: RhoC-siRNA eukaryotic vector pU6mRFP RhoC-siRNA was constructed and transfected into BEL7402 cells, and the transfection efficiency was examined by confocal microscope. RT-PCR and Western blotting analysis were conducted to identify the effect of RhoC gene silencing; flow cytometry, agarose gel electrophoresis and Wright staining were applied to detect apoptosis of BEL7402 cells; and the expression levels of apoptosis related genes were determined by RT-PCR. Results: The pU6mRFP RhoC-siRNA recombinant plasmid was successfully constructed, and its transfection efficiency in BEL7402 cells was 70%. RT-PCR and Western blotting analysis results showed that the silencing rate of RhoC were 85% and 82%, respectively. The apoptosis rate of BEL7402 cells in pU6mRFP RhoC-siRNA transfected group was significantly higher than those in untransfected and pU6mRFP Scramble-siRNA transfected groups (\[21.00±2.23\]% vs \[6.47±1 64\]%,\[4.63±0.47\]%, P <0.01). Typical apoptotic DNA “ladder” appeared in pU6mRFP RhoC-siRNA transfected BEL7402 cells in gel electrophoresis analysis, and Wright staining showed a lot of apoptotic BEL7402 cells in pU6mRFP RhoC-siRNA group. Compared with untransfected and pU6mRFP Scramble-siRNA transfected BEL7402 cells, Bcl-2 gene expression in pU6mRFP RhoC-siRNA group was significantly decreased and Bax gene expression was significantly increased (0.28±0.15 vs 0.96±0.21, 1.03±0.24, P <0.05; 1.09±0.21 vs 0.26±0.10, 0.25±0 07, P <0.01). Conclusion: siRNA silencing RhoC gene expression can induce apoptosis in human hepatocellular carcinoma BEL7402 cells, which may be related to the down-regulated expression of Bcl-2 gene and up-regulated expression of Bax .

Keywords:RhoC gene siRNA hepatocellular carcinoma apoptosis

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