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慢病毒载体介导RNA干扰体外抑制人胰腺癌细胞增殖诱导配体的表达

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摘要：

目的:观察慢病毒表达载体介导的RNA干扰（RNAi）对人胰腺癌细胞株CFPAC-1增殖诱导配体(a proliferation-inducing ligand, APRIL)表达的影响，为后续的以APRIL基因为靶点的胰腺癌基因治疗研究奠定基础。方法:应用基因工程技术，筛选出3条针对APRIL基因的RNAi靶序列，分别与pGCL-GFP载体连接，构建3个重组慢病毒表达载体LV-APRIL shRNA1、LV-APRIL shRNA2、LV-APRIL shRNA3；将连接产物转化到DH5α感受态细胞，经PCR筛选阳性克隆、测序鉴定。将LV-APRIL shRNA、pHelper 1.0、pHelper 2.0共转染293T细胞，包装产生慢病毒颗粒并测定病毒滴度。将包装产生的3种重组慢病毒分别感染CFPAC-1细胞，实时定量PCR和Western印迹检测CFPAC-1细胞APRIL mRNA和蛋白的表达，并与未转染及空转染细胞进行比较。结果:3个慢病毒载体PCR和测序结果与预期结果一致，经包装产生的病毒滴度分别为5×107、6×107、4×107 TU/ml。感染CFPAC-1细胞后，APRIL基因mRNA和蛋白的表达量与未感染慢病毒的细胞组及空载体感染组相比均明显下降（P<0.05），其中LV-APRIL shRNA1、LV-APRIL shRNA2作用较明显，使mRNA表达分别下降73%和68%，蛋白表达分别下降66%和59%（P<0.05）；而未感染慢病毒的细胞组与空载体组相比无统计学差异。结论:成功构建针对APRIL基因的3个慢病毒载体LV-APRIL shRNA，体外感染CFPAC-1细胞后可有效抑制APRIL基因和蛋白的表达。

关键词：增殖诱导配体 RNA干扰 慢病毒 胰腺癌

Inhibitory effects of lentiviral vector-mediated RNA interference on proliferation-inducing ligand expression in human pancreatic cancer in vitro

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Abstract:

Objective: To observe the influence of lentiviral vector-mediated RNA interference on expression of human APRIL (a proliferation-inducing ligand) gene in human pancreatic cancer cell line CFPAC-1, so as to pave a way for APRIL gene-targeted gene therapy of pancreatic cancer. Methods: Gene engineering technique was used to screen 3 RNA interference sequences targeting APRIL gene; the sequences were separately cloned into the pGCL-GFP vector to construct LV-APRIL shRNA1, LV-APRIL shRNA2 and LV-APRIL shRNA3, which were subsequently confirmed by PCR and DNA sequencing analysis. The titer of lentivirus was determined after 293T cells were cotransfected with LV-APRIL shRNA, pHelper 1.0 and pHelper 2.0. The 3 kinds of recombinant lentiviruses were injected into CFPAC-1 cells and the APRIL mRNA and protein expression were examined by real-time RT-PCR and Western blotting, respectively,and the result was compared with those of the non-transfected and blank vector transfected CFPAC-1 cells. Results: PCR analysis and DNA sequencing confirmed that the 3 APRILshRNA sequences were successfully inserted into the lentiviral vectors. The titers of concentrated virus were 5×107 TU/ml,6×107 TU/ml and 4×107 TU/ml, respectively. APRIL expression in CFPAC-1 cells was significantly inhibited at both mRNA and protein levels compared with the non-transfected and empty vector transfected CFPAC-1 cells (P<0.05). After transfection with LV-APRIL shRNA1 and LV-APRIL shRNA2, APRIL mRNA expression decreased by 73% and 68%, APRIL protein expression decreased by 66% and 59% (P<0.05), respectively; there was no significantly difference between the non-transfected and empty vector transfected CFPAC-1 cells.Conclusion: Three lentiviral RNAi vectors of APRIL gene have been successfully constructed, and they can effectively inhibit the expression of APRIL gene in CFPAC-1 cells in vitro.

Keywords:a proliferation-inducing ligand RNA interference lentivirus pancreatic cancer

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