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技术与方法

FAK siRNA质粒表达载体的构建及其对肺巨细胞癌细胞FAK基因表达的抑制

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摘 要 目的 应用RNA干扰技术，构建针对FAK的siRNA表达载体，抑制肺巨细胞癌细胞BE-1中FAK的表达。方法 依据设计siRNA的原则，针对人FAK的mRNA序列，设计并合成编码siRNA的两条寡核苷酸序列，经退火成互补双链，再克隆到pSilencer™ 2.1-U6真核表达载体中构建重组体pSilencer-FAK，进行测序鉴定。然后转染重组体至BE-1细胞中，经G418 筛选，以空质粒转染为对照，获得稳定转染克隆，运用Western 印迹检测FAK基因的表达。结果 测序证实目的寡核苷酸片段已被克隆到pSilencer™ 2.1-U6载体中 ，pSilencer-FAK转染细胞后，FAK基因在蛋白水平的表达量受到明显抑制。结论 成功构建了针对人FAK的siRNA表达载体，通过转染BE-1细胞，可有效抑制细胞中FAK的表达，为后续研究以及肺癌的基因治疗奠定了基础。

关键词 SiRNA RNAi FAK 基因表达抑制

分类号

Construction of FAK siRNA expressing vector and the inhibitory effects on the expression of FAK in lung cancer cell

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Abstract Objective To construct the siRNA expression vector of FAK and inhibit the expression of FAK through RNA interference in lung cancer cell line BE-1. Methods According to the encoding sequence of mRNA of FAK , two pieces of oligonucleotide sequences were designed and synthesized. The annealed oligonucleotide fragments were subcloned into pSilencer™ 2.1-U6 siRNA expression vector. After being identified by sequencing, the recombinant plasmids pSilencer-FAK were transfected into BE-1 cells. The treated cells were selected by G418. FAK expression in the stable transfected cells was assayed by western blot. Results DNA sequencing showed that the oligonucleotide fragments were correctly inserted into pSilencer™ 2.1-U6 vector,and FAK expression in the transfected cells was down-regulated significantly by pSilencer-FAK at the protein level. Conclusion The siRNA expression vector of FAK was successfully constructed , and could inhibit FAK expression in BE-1 cells , which will facilitate further studies of gene therapy for tumors such as lung cancer.

Key words SiRNA RNAi FAK Inhibition of gene expression

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