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基础研究

RNA干扰技术沉默STAT3 对人肺癌细胞生长抑制的作用

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Knockdown of STAT3 Gene Expression Using siRNA Inhibits Growth of Lung Cancer Cell Lines

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摘要 目的 探讨RNA干扰技术沉默STAT3基因表达对肺腺癌A549细胞的生长抑制作用。方法针对STAT3mRNA序列设计合成2对编码si RNA的DNA模板,构建pSUPER-si RNA-STAT3重组质粒,转染A549细胞;采用RT-PCR法检测重组质粒对STAT3基因表达的影响;在荧光显微镜下观察细胞的凋亡情况。结果 成功构建pSUPER-si RNA-STAT3重组质粒,并成功转染A549细胞。特异性si RNA片段能有效降低STAT3mRNA水平,最大干扰效率达85.32%,明显高于空质粒对照组;干扰作用于转染后24h即可出现,48h达高峰,72h降低。对应不同位点的两个si RNA片段对STAT3均可产生干扰作用,彼此间差别不大。在荧光显微镜下,与未转染细胞相比,转染si RNA的A549细胞中凋亡细胞所占比例明显增加。结论 pSUPER-si RNA-STAT3可抑制STAT3在人肺腺癌A549细胞中的表达,并抑制肿瘤细胞的生长,促进其凋亡。以STAT3为靶点的RNA干扰技术可望成为肺癌基因治疗的新方法。

关键词: 肺癌 RNA 干扰 STAT3 凋亡

Abstract: Objective To study the effect s of pSUPER-siRNA-STAT3 gene on the growth of A549 cells.Methods Two pairs of DNA template coding siRNA were synthesized against STAT3 to reconstruct pSUPER-siRNA- STAT3. Which was transfected into A549 cells. The STAT3 expression in A549 were transfected with pSUPER-siRNA-STAT3 ,and it was detected by RT-PCR and cells viability by fluorescence microscope. Results The siRNA eukaryotic expression vector against STAT3 mRNA was successfully conct ructed , which was transfected into A549 cells. The level of STAT3 mRNA in A549 cells was inhibited by the specific siRNAs. The decrease of STAT3 mRNA expression began to appear 24 hours after transfection. And the most apparent interfering efficiency was 85. 32 % ,48 hours after transfection ,which was markedly higher than that in the cells transfected with the control siRNAs. Both siRNAs from different loci had interfering effect on STAT3 mRNA expression ,but there was no significant difference between them. Compared with those control cells ,the apoptotic rates were significantly higher in siRNA transfected cells by fluorescence microscope. Conclusion pSUPER-siRNA-STAT3 could significantly inhibit STAT3 expression ,suppress the growth of A549 cells. The RNA interfering technique targeted on STAT3 may provide a new method in the gene therapy of lung cancer.

Key words: Lung cancer RNAi STAT3 Apoptosis

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