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论文

慢病毒载体介导的siRNA抑制Jiyoye细胞c-myc基因表达的实验研究

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摘要：

目的 探讨RNAi技术经慢病毒载体介导的siRNA对Jiyoye细胞c-myc基因表达的抑制作用。方法 设计并合成RNA干扰序列,退火后连接到pLVX干扰载体上，构建PLVX-c-myc表达载体,经慢病毒介导转染人Jiyoye细胞株培养72h。实验分为空白对照（未转染）、c-myc-neg、c-myc-1、c-myc-2、c-myc-3组。采用流式细胞术检测各组细胞转染率，采用real-time PCR和Western blot法检测各组细胞 c-myc mRNA及蛋白表达水平的变化。结果 构建pLVX-c-myc-neg、pLVX-c-myc-1、pLVX c-myc-2、pLVX-c-myc-3重组表达载体。与c-myc-neg组相比， c-myc-1、c-myc-2和c-myc-3组c-myc mRNA及蛋白表达水平均明显下调（P<0.05），以c-myc-3组下降最显著（P<0.05）。结论 成功构建c-myc shRNA表达载体，利用慢病毒介导转染Jiyoye细胞的c-myc基因表达下调，为进一步探讨沉默c-myc基因在白血病和淋巴瘤靶向治疗中的作用奠定了实验基础。

关键词： 基因，myc；siRNA；shRNA；Jiyoye细胞；RNA干扰

Suppression of c-myc expression by lentiviral vector-mediated- small interfering RNA in Jiyoye cells

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Abstract:

Objective To explore the effect of lentiviral vector-mediated siRNA on c-myc gene expression in Jiyoye cells by using the RNAi technique in vitro. Methods Three interference sequences c-myc-1, c-myc-2 and c-myc-3 and the negative control c-myc-neg that targeted human c-myc mRNA were designed and synthesized. After annealing, all the fragments were cloned into the pLVX vector, which were transfected into human leukemia Jiyoye cells by lentivirus and were cultured for 72 hours. The cells were divided into five groups: the blank control group(untransfected), the c-myc-neg group, the c-myc-1 group, the c-myc-2 group and the c-myc-3 group. After 72 hours, the transfection rate in each group was determined by flow cytometry. Expressions of the c-myc mRNA and c-Myc protein were detected by Real-time PCR and Western blot. Results PLVX-c-myc-neg, PLVX-c-myc-1, PLVX-c-myc-2 and PLVX-c-myc-3 were constructed. C-myc mRNA and protein expression levels in the three groups respectively transfected with c-myc-1, c-myc-2 and c-myc-3 were significantly down-regulated -compared with the negative control group transfected with c-myc neg(P<0.05). The c-myc-3 group decreased most significantly compared with the c-myc-1 group and the c-myc-2 group (P<0.05). There was no significant difference between the untransfected group and the negative control group (P>0.05). Conclusion The successfully constructed shRNA expression vector for the c myc gene suppresses expression of c myc in Jiyoye cells, which might provide an experimental basis for further study of the role of c myc gene silencing in targeting treatment of leukemia and lymphoma.

Keywords: Genes, myc; Small interfering RNA; Small hairpin RNA; Jiyoye cells; RNA interference

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