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论文

shRNA-FHIT对胃癌细胞株BGC-823增殖和凋亡的影响

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摘要：

目的 构建靶向抑制脆性组氨酸三联体（FHIT）基因的短发夹双链RNA（shRNA）真核表达质粒,观察FHIT抑制后对胃癌细胞株BGC-823增殖和凋亡的影响。方法 构建FHIT基因特异性的小RNA干扰质粒PGPU6/GFP/Neo-shRNA1、PGPU6/GFP/Neo-shRNA2,利用脂质体LipofactamineTM2000转染胃癌细胞BGC-823,实验分为未转染组、阴性对照组（转染PGPU6/GFP/Neo-shNC组）及PGPU6/GFP/Neo-shRNA1转染组和PGPU6/GFP/Neo-shRNA2转染组,G418筛选得到稳定表达株,Real-time PCR检测在mRNA水平干扰质粒对FHIT的抑制效应,MTT法、流式细胞术观察转染前后细胞生长特性的变化。结果 与阴性对照组相比,转染shRNA-FHIT重组质粒的BGC-823细胞FHITmRNA表达明显下降(P＜0.05)。与未转染组和阴性对照组相比,转染干扰质粒组细胞增殖活性增强,凋亡率减低、生长周期出现S期和G2/M期的比例上调（均P＜0.05）。结论 成功将重组shRNA-FHIT表达载体转染BGC-823细胞,并筛选出稳定低表达FHIT的细胞。shRNA-FHIT可以促进胃癌细胞增殖、降低凋亡率,并削弱G0/G1期阻滞,为进一步研究FHIT基因在肿瘤中的作用机制奠定了实验基础

关键词： 三联脆性组氨酸基因；胃肿瘤；RNA干扰；增殖；凋亡

Effect of the short hairpin RNA of fragile histidine triad on proliferation and apoptosis of the gastric cancer cell line BGC-823

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Abstract:

Objective To construct two eukaryotic plasmids expressing short hairpin RNA (shRNA) of fragile histidine triad (FHIT) and to study their effects on proliferation and apoptosis of the gastric cancer cell line BGC-823. Methods Specific shRNA plasmids to FHIT were constructed, and then transfected into the BGC-823 cells with lipofectamine methods. Cells were divided into four groups：the control group，the PGPU6/GFP/Neo-shNC transfected group as the negative group and the PGPU6/GFP/Neo-shRNA transfected groups .After selection with G418, the stable cell clones were attained. Expression of FHIT mRNA was determined by quantitive real-time PCR. The effect of FHIT on the growth characteristics of gastric cancer cells was observed by methyl thiazolyl tetrazolium (MTT) and flow cytomery (FCM). Results Stable clones with shRNA-FHIT plasmids were obtained. Compared with the negative control cells, expression of FHIT mRNA was down-regulated in the shRNA-FHIT plasmid transfected cells(P＜0.05). Proliferation was promoted, whereas the cell apoptosis rate was decreased. Cells at the G0/G1 cell stage decreased, while the cells at S and G2 cell stages increased. All these differences between shRNA-FHIT transfected cells and the two control groups of gastric cancer cells had statistical significances (P＜0.05). Conclusions shRNA-FHIT plasmids were successfully transfected into BGC-823 cells, and the cells which express FHIT in a stable lower level were obtained. FHIT-tageted shRNA could obviously decrease the FHIT expression in BGC-823 cells at a stable lower level, promote the proliferation of BGC-823 cells, suppress their apoptosis and weaken the G0/G1 phase block of cell cycle.

Keywords: Fragile histidine traid gene；Stomach neoplasm；RNA interference；Proliferation；Apoptosis

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