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基础研究

hTERT 短发夹状RNA 抑制前列腺癌细胞生长的研究

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Study on Inhibition of Growth and Proliferation in Prostate Cancer Cell by the shRNA of hTERT

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摘要

目的 研究靶向端粒酶逆转录酶（hTERT）的短发夹状RNA（shRNA）基因转染对前列腺癌细胞体外生长的抑制效应及其促细胞凋亡作用。方法 在脂质体介导下将针对hTERT基因的shRNA表达载体psilencer-TRT转染前列腺癌细胞PC-3m，得到稳定细胞株PC-3m / shRNA-TRT。采用RT-PCR检测hTERT基因表达情况，western blot分析各组细胞hTERT及c-myc蛋白表达变化，细胞计数并绘制细胞生长曲线，Hoechst33258染色、透射电镜、流式细胞仪检测细胞凋亡情况。结果 重组质粒psilencer-TRT转录生成的shRNA使实验组细胞的hTERT基因表达显著下调，抑制率约为89．02%；同时实验组细胞hTERT及c-myc蛋白水平较对照组有明显下降，细胞的生长增殖能力也显著降低（P〈0．05），生长速率明显变慢，部分细胞呈现凋亡形态学改变，凋亡率为（19．69±4．75）%。结论 hTERT短发夹状RNA能有效抑制前列腺癌细胞中hTERT表达及癌细胞生长，诱导PC-3m细胞凋亡，可望为前列腺癌的基因治疗提供新方法。

关键词： 端粒酶逆转录酶 短发夹状RNA 前列腺癌 凋亡

Abstract: Objective To investigate the effect of the short hairpin RNA (shRNA) against human telomerase reverse t ranscriptase (hTERT) on the proliferation and apoptosis of prostate cancer cells PC-3m in vitro. Methods The recombinant plasmid psilencer-TRT was transfected into prostate cancer cell line PC-3m via liposome reagent . The level of hTERT mRNA was examined by reverse t ranscription polymerase chain reaction (RT-PCR) . The expressions of hTERT and c-myc protein were detected by western blot analysis. The effect of hTERT shRNA on the cellular proliferation capacity of PC-3m cells was assayed by the growth curve. The cell apoptosis was detected by Hoechst33258 staining , elect ron microscope , and flow cytomet ry analysis. Results The vector-mediated shRNA significantly reduced the level of hTERT mRNA by 89. 02 % af ter psilencer-TRT transduction in PC-3m cells. Meanwhile , the levels of hTERT and c-myc protein were also decreased in transfected cells. The cell proliferation was markedly inhibited compared with the cont rol cells. Partial cancer cells presented morphological changes of apoptosis , and the apoptosis rate was (19. 69 ±4. 75) %. Conclusion hTERT shRNA can suppress hTERT expression and cell proliferation , in addition to acceleration of apoptosis. This implied the possibility of RNA interfering to hTERT as the potential method for gene therapy of prostate cancer.

Key words: Human telomerase reverse t ranscriptase Short hairpin RNA Prostate cancer Apoptosis

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