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RNAi沉默hTERT基因诱导大肠癌SW480细胞凋亡

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摘要：

目的：探讨RNA干扰人端粒酶逆转录酶（human telomerase reverse transcriptase，hTERT）的表达对大肠癌细胞SW480凋亡的影响。方法：构建携带hTERT小发夹干扰RNA（small hairpin RNA，shRNA）的重组表达载体pGPU6/GFP/Neo-hTERT-shRNA（简称hTERT-shRNA质粒），脂质体法转染SW480细胞，RT-PCR法检测不同转染时间点SW480细胞中 hTERT mRNA的表达。TRAP-PCR-ELISA法检测转染后48 h SW480细胞的端粒酶活性，透射电镜观察转染后48 h SW480细胞超微结构。结果：hTERT-shRNA质粒转染48 h时，hTERT-shRNA组SW480细胞 hTERT mRNA表达的抑制率显著高于空白组、脂质体组、NC-shRNA组（75.0% vs 39.2%、33.3%、28.0%， P <0.05）。hTERT-shRNA转染组SW480细胞端粒酶活性显著低于空白组、脂质体组、NC-shRNA组（2.242±0.285 vs 2.756±0.089、 2.693±0.225、2.691±0.120， P <0.05）。hTERT-shRNA质粒转染后的SW480细胞体积明显缩小、细胞核固缩、染色质不均匀地沿核膜下聚集、空泡形成增多，出现典型的凋亡形态。结论：RNAi可有效沉默SW480细胞中hTERT的表达，降低SW480细胞端粒酶活性，诱导SW480细胞凋亡。

关键词：RNA干扰 大肠癌 hTERT 端粒酶 凋亡

RNA interference-based hTERT gene silence induces apoptosis of colorectal cancer SW480 cells

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Abstract:

Objective:To investigate the effect of RNA-mediated interference of hTERT (human telomerase reverse transcriptase) expression on the apoptosis of colorectal cancer cell line SW480. Methods: Small hairpin RNA (shRNA) targeting hTERT was synthesized and recombinant plasmid pGPU6/GFP/Neo-hTERT-shRNA (named hTERT-shRNA plasmid) containing hTERT-shRNA was constructed. SW480 cells were transfected with hTERT-shRNA plasmid by liposome method, and the expression of hTERT mRNA in SW480 cells was detected by RT-PCR analysis at different time points. The telomerase activity of SW480 cells was examined by TRAP-PCR-ELISA analysis. The ultrastructure of SW480 cells was examined by TEM (transparent electron microscope) 48 h after hTERT-shRNA transfection. Results: The inhibitory rate of hTERT mRNA expression in SW480 cells of hTERT-shRNA group was significantly higher than those of blank group, liposome group, and NC-shRNA group (75.0% vs 39.2%, 33.3%, 28.0%, P <0.05). Telomerase activity in SW480 cells of hTERT-shRNA group was significantly decreased compared with those of blank group, liposome group, and NC-shRNA group (2.242±0.285 vs 2.756±0.089, 2.693±0.225, 2.691±0.120, P <0.05). SW480 cells in hTERT-shRNA group showed smaller cell size, nuclear condensation, uneven aggregation of chromatin along the nuclear membrane, and increased vacuolization. Conclusion: RNA interference can effectively silence hTERT expression, reduce telomerase activity, and induce apoptosis of SW480 cells.

Keywords:RNA interference colon cancer hTERT telomerase apoptosis

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