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论著

siRNA特异性沉默hTERT mRNA对人舌癌Tca8113细胞体内外生长的影响

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摘要 目的: 探讨靶向端粒酶逆转录酶(hTERT)基因的siRNA抑制舌癌Tca8113细胞的效果和机制。方法: 构建hTERT基因的siRNA(siRNA-hTERT1),阳离子脂质体法转染舌癌Tca8113细胞,以无同源性的siRNA-hTERT2作阴性对照,以未转染的细胞为空白对照。MTT法检测细胞生长率,流式细胞术分析细胞凋亡的情况。建立裸鼠舌癌皮下种植瘤模型,瘤内注射法将siRNA-hTERT1转导,观察3组种植瘤的大小,细胞凋亡-Hoechst染色试剂盒检测种植瘤内细胞凋亡的情况。逆转录聚合酶链反应 (reverse transcription-polymerase chain reaction, RT-PCR)技术检测细胞株和种植瘤内hTERT mRNA表达的变化。结果: 细胞株转染siRNA-hTERT1 72 h后,抑制率达47.2%,显著高于阴性对照组(2.6%),P<0.01;细胞凋亡率也明显增加,达27.30%±0.18%,显著高于阴性对照组和空白对照组(P<0.01)。种植瘤转染siRNA-hTERT1 14 d后,种植瘤体积为(298.8±138.7)mm3,显著小于阴性对照组的(495.1±151.6)mm3和空白对照组(506.8±207.4)mm3,抑瘤率达到40.0%(P<0.01);同时可见转染组的细胞凋亡数目增加,显著多于阴性对照组和空白对照组(P<0.01)。检测hTERT mRNA的表达,可见siRNA-hTERT1转染后,细胞株和种植瘤内的表达显著减少(P<0.01),而阴性对照和空白对照间无显著差异。结论: siRNA-hTERT1在体内外均能有效抑制舌癌Tca8113细胞的生长,抑制hTERT基因的表达和促进细胞的凋亡是其主要机制。

关键词 口腔肿瘤 端粒酶逆转录酶 RNA干扰

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Effect of specific hTERT-siRNA on growth of human tongue cancer cells in vivo and in vitro

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Abstract

<FONT face=Verdana>AIM: To investigate the effect and mechanisms of siRNA-hTERT-induced inhibition of Tca8113 tongue cancer cells in vitro and in vivo. METHODS: A small interference RNA (siRNA) targeting to hTERT mRNA (siRNA-hTERT1) was constructed. The siRNA was transfected into Tca8113 tongue cancer cells in vivo and in vitro with cationic liposome. A non-specific siRNA (siRNA-hTERT2) and non-treatment were used as negative control group and blank group. The cell growth in vitro was detected by MTT method. The cell apoptosis in vitro was analyzed by flow cytometry. The effect of siRNA-hTERT1 on xenografts in nude mice was observed by determining the tumor size. The cell apoptosis in xenografts was analyzed by Hoechst staining. The expressions of hTERT mRNA in vitro and in vivo were detected by RT- PCR. RESULTS: The inhibition rates of cell growth in vitro 72 h after siRNA-hTERT1 treatment was 47.2%, significantly higher than that in siRNA-hTERT2 treatment group (2.6%, P<0.01). The cell apoptosis rate was 27.30% ±0.18% in vitro, significantly increased at 48 h after transfection of siRNA-hTERT1, compared to negative control group and blank group (P<0.01). The size of xenografts in siRNA-hTERT1 treatment group was (298.8±138.7)mm3, significantly smaller than that in siRNA-hTERT2 treatment group and blank group (495.1±151.6) mm3 and (506.8±207.4)mm3, the inhibition rate was 40.0% (P<0.01). The numbers of apoptotic cells in xenografts significantly increased after transfection of siRNA-

hTERT1, compared to negative control group and blank group (P<0.01). Compared to negative control group and blank group, the expression of hTERT mRNA in Tca8113 tongue cancer cells in vitro and in vivo was inhibited by siRNA-hTERT1. CONCLUSION: siRNA-hTERT1 powerfully inhibits the growth of Tca8113 tongue cancer cells in vitro and in vivo. The specific inhibition of hTERT mRNA expression and cell apoptosis may be its main mechanisms.</FONT>

Key words Mouth neoplasms Telomerase reverse transcriptase RNA interference

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