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共表达ENDO-VEGI 151和survivin-siRNA双功能质粒的构建及其抗瘤活性 点此下载全文

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摘要：

目的： 构建pCDNA3.1-ENDO-VEGI 151/survivin-shRNA(pEV/si-survivin)双功能表达质粒，观察其对乳腺癌细胞MDA-MB-231和人脐静脉血管内皮细胞(human umbilical vein endothelial cell,HUEVC)增殖和凋亡的影响，探讨其治疗肿瘤的可行性。 方法： 利用MDA-MB-231细胞筛选获得survivin的高效siRNA序列，构建pEV/si-survivin表达质粒并分别转染MDA-MB-231和HUEVC，以real-time PCR和Western blotting检测转染细胞中ENDO-VEGI 151和survivin的表达；MTT法检测细胞增殖抑制情况，流式细胞术检测细胞周期和细胞凋亡。结果： 成功构建pEV/si-survivin双功能表达质粒，并能在MDA-MB-231和HUEVC中正确表达相应基因产物。该质粒可明显抑制MDA-MB-231细胞内survivin的表达，并抑制细胞增殖\[48、72 h的抑制率为（39.36±4.16）%、（48.43±3.49）%\]，促进MDA-MB-231细胞凋亡\[(18.33±1.48)% vs (4.80±1.01)%, P <0 01)和细胞周期阻滞（ P <0.05）；该质粒也明显抑制HUEVC的增殖\[（48、72 h的抑制率为（38.16±3.37）%、（53.75±4 53）%），并促进HUEVC凋亡和细胞周期阻滞（ P <0.05）。 结论： 成功构建的pEV/si-survivin双功能表达质粒可以同时发挥抑制新生血管生成和促肿瘤细胞凋亡的作用，提高抗肿瘤的效果。

关键词：乳腺癌 survivin 抗血管生成 凋亡 基因治疗

Construction of dual function plasmid co-expressing ENDO-VEGI 151 and survivin-siRNA and its anti-tumor activity Download Fulltext

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Abstract:

Objective : To construct a dual function expressional plasmid pCDNA3.1-ENDO-VEGI 151/survivin-shRNA (pEV/si-survivin), and study its effect on the proliferation and apoptosis of breast cancer MDA-MB-231 cells and vascular endothelial cells (HUEVCs), so as to evaluate its feasibility for gene therapy of cancer. Methods: The efficient siRNA sequences targeting survivin was screened in MDA-MB-231 cells; the pEV/si-survivin expression vector was constructed and transfected into MDA-MB-231 and HUEVC cells, and the expression levels of ENDO-VEGI 151 and survivin were detected by the real-time PCR and Western blotting analysis; MTT assay was used to detect the proliferation inhibition in the cells of the two groups after transfection; and flow cytometry was used to detect the changes of cell cycles and apoptosis. Results: The dual function recombinant plasmid pEV/si-survivin was successfully constructed and it was correctly expressed in both MDA-MB-231 and HUEVC cells. The plasmid significantly inhibited the expression of survivin and the cell proliferation (inhibition rate being \[39.36±4.16\]% at 48 h and \[48.43±3.49)% at 72 h); it also significantly promoted cell apoptosis (\[18.33±1.48\]% vs \[4.80±1.01\]%, P <0 01) and induced cell cycles arrest (P <0.05) in MDA-MB-231 cells. The plasmid also significantly inhibited cell proliferation (inhibition rate being \[38.16±3.37\]%) at 48 h and \[53.75±4 53\]% at 72 h), promoted apoptosis, and arrested the cell cycles (P <0.05) in HUEVC cells. Conclusion: The dual function expressional plasmid pEV/si-survivin possess both angiogenesis inhibition and apoptosis promotion functions, and is expected to exert synergistic effect in vivo to improve the therapeutic outcome for patients with cancer.

Keywords:breast cancer survivin anti-angiogenesis apoptosis gene therapy

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