肿瘤防治研究 2010, Vol. 37 Issue (9): 1004-1009 DOI: 10.3971/j.issn.1000-8578.2010.09.008

siRNA沉默DNMT1对人乳腺癌细胞MCF-7生长的影响

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Influence of siRNA Induced Silencing DNMT1 Gene on Growth of Breast Cancer MCF-7 Cell Line

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全文: PDF (4441 KB) HTML (0 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 目的 靶向人DNMT1（DNA methyltransferase 1, DNMT1）构建RNA干扰载体，研究其对乳腺癌细胞周期、增殖及凋亡的影响。方法 靶向DNMT1基因设计三条短发夹状RNA(short hairpin RNA, shRNA) 的寡核苷酸片段，构建重组体pGCsi-DNMT1，转染至乳腺癌细胞株MCF-7，定量PCR 法检测DNMT1 mRNA表达水平；流式细胞技术分析细胞周期的变化；MTT 法检测细胞生长情况；Annexin V/PI双染法观察细胞凋亡情况；定量PCR 法分析沉默DNMT1基因后对RASSF1A、*p*16、*p*21、*p*27及ERβ基因表达的影响。结果 在构建的靶向DNMT1的shRNA重组质粒pGCsi-DNMT1中，成功筛选到pGCsi-T3能显著下调DNMT1的表达。实时定量PCR检测结果证实重组质粒pGCsi-DNMT1对乳腺癌MCF-7细胞中DNMT1基因的转录有明显的抑制作用。MCF-7细胞转染后，pGCsi-DNMT1可明显抑制乳腺癌MCF-7细胞的增殖；大量细胞发生凋亡；细胞周期分析可见S期明显减少，而G_1/G_0期细胞显著增加；定量PCR检测到乳腺癌细胞中RASSF1A、p16、p21及ERβ基因mRNA表达水平明显升高，而p27基因表达水平未见明显变化。结论 重组质粒pGCsi DNMT1能特异有效地抑制MCF-7细胞内DNMT1的表达、 抑制细胞增殖、促进细胞凋亡，并可通过抑制DNMT1的活性来解除相关基因启动子区的高度甲基化状态，从而促进肿瘤相关基因的表达，提示DNMT1可作为乳腺癌基因治疗的新靶标。

关键词： DNMT1 RNA干扰 乳腺癌 MCF-7

Abstract： Objective To construct the small interfering RNA(siRNA) expression vector targeting DNMT1 gene, and to investigate the effect of cell cycle, proliferation and apotosis of breast cancer cell line. Methods Three short hairpin RNA(shRNA) targeting coding sequence of *DNMT*1 were synthesized, and the cell three recombination plasmids were constructed pGCsi-*DNMT*1. After transfection into breast cancer MCF-7 cells, the mRNA expression level of *DNMT*1 gene was detected by real time quantitative PCR, and cell cycle were analyzed by flow cytometry; The growth status of cells was detected by MTT assay, and the cell apoptosis was analyzed by Annexin V/PI double-dyed. The influence of mRNA expression about *RASSF*1A、p16、p21、p27 and ERβ was analyzed by real time quantitative PCR after silencing DNMT1 gene. Results Three recombinant plasmids pGCsi-*DNMT*1 were successfully constructed.It was confirmed that pGCsi-T3 can markedly silence *DNMT*1 gene expression. Transfection of pGCsi-T3 significantly down regulated the DNMT1 mRNA expression in MCF-7 cells. The proliferation of MCF-7 cells were markedly inhibited after transfection with pGCsi-T3, A majority of cells has become apoptosis,The frequency of S phase of cell cycle obviously reduced while G1/G0 phase significantly increased in MCF-7 cells. From the real-time PCR dectction results,it showed that the expression of *RASSF*1A,p16,p21 and ERβ mRNA obviously raised while the expression of p27 mRNA had no change. Conclusion PGCsi-*DNMT*1 can efficiently and specifically inhibit the expression of *DNMT*1 gene in MCF-7 cells and the cell proliferation,and promote the cell apotosis. The tumor relate genes can be expressed by relieving the hypermethylation in promoter regions through inhibiting the expression of DNMT1 gene. It may provide a new target for gene therapy of human breast cancer.

Key words： *DNMT*1 RNA interference Breast cancer MCF-7

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收稿日期: 2009-04-22;

引用本文:

魏钦俊,鲁雅洁,曹新. siRNA沉默DNMT1对人乳腺癌细胞MCF-7生长的影响[J]. 肿瘤防治研究, 2010, 37(9): 1004-1009.

WEI Qin-jun,LU Ya-jie,CAO Xin. Influence of siRNA Induced Silencing DNMT1 Gene on Growth of Breast Cancer MCF-7 Cell Line[J]. CHINA RESEARCH ON PREVENTION AND TREATMENT, 2010, 37(9): 1004-1009.

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