肿瘤防治研究
ZHONGLIU FANGZHI YANJIU
Cancer Research on Prevention and Treatment
中华人民共和国卫生部主管
中国抗癌协会系列杂志

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

siRNA沉默DNMT1对人乳腺癌细胞MCF-7生长的影响

魏钦俊，鲁雅洁，曹新

210029南京，南京医科大学生物技术系

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

WEI Qin-jun, LU Ya-jie, CAO Xin

Department of Biotechnology, Nanjing Medical University, Nanjing 210029, China

- 摘要
- 参考文献
- 相关文章

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

服务
把本文推荐给朋友
加入我的书架
加入引用管理器
E-mail Alert
RSS
作者相关文章
魏钦俊
鲁雅洁
曹新

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.

没有本文参考文献

[1] 纪术峰;杨华锋;吴爱国 . PGRMC1参与调控乳腺癌细胞增殖及化疗敏感度的实验[J]. 肿瘤防治研究, 2012, 39(2): 123-126.

[2] 罗平;罗浩军;杨光伦;涂刚. 新型雌激素受体GPER在乳腺癌组织中的表达及与预后的相关性 [J]. 肿瘤防治研究, 2012, 39(2): 181-184.

[3] 王艳阳;折虹;丁喆;詹文华. Basal-like型乳腺癌临床特征与生存分析[J]. 肿瘤防治研究, 2012, 39(2): 177-180.

[4] 刘志容;吴诚义 . MMP-3、Vimentin联合检测与乳腺癌侵袭转移的关系[J]. 肿瘤防治研究, 2012, 39(2): 222-224.

[5] 潘翠萍;范威;马彪 . 乳腺癌干细胞研究进展[J]. 肿瘤防治研究, 2012, 39(2): 234-237.

[6] 裴新红;杨振;姜丽娜 . 淋巴结分类情况下不同类型三阴性乳腺癌的预后分析 [J]. 肿瘤防治研究, 2012, 39(1): 51-53.

[7] 黄东兰;谢菲;岑东芝;张积仁 . 2001—2010年乳腺癌预后基因临床研究文献的计量学分析[J]. 肿瘤防治研究, 2012, 39(1): 91-94.

[8] 周防震;张晓元;孙奋勇;郭勇 . 二氢杨梅素对人乳腺癌细胞MDA-MB-231的体外抗增殖作用[J]. 肿瘤防治研究, 2012, 39(1): 95-97.

[9] 周瑞娟;陈红风 . 中药影响乳腺癌细胞周期的研究进展[J]. 肿瘤防治研究, 2012, 39(1): 100-104.

[10] 卢洁;王春美;盛光耀 . FLT3靶向抑制诱导急性髓细胞白血病细胞凋亡的实验研究 [J]. 肿瘤防治研究, 2011, 38(9): 979-982.

[11] 刘先领;曾惠爱;马芳;杨农. 吉西他滨联合顺铂治疗复发转移性乳腺癌的疗效观察 [J]. 肿瘤防治研究, 2011, 38(9): 1055-1057.

[12] 金立亭;原俊;温固. 乳腺癌术中植入缓释氟尿嘧啶间质化疗的临床研究[J]. 肿瘤防治研究, 2011, 38(9): 1076-1077.

[13] 张兴梅;石玉生;陈明;夏许可;李树基;李晓文;曹东林 . EGFRvⅢ的siRNA对胶质瘤细胞凋亡和增殖的影响[J]. 肿瘤防治研究, 2011, 38(9): 975-978.

[14] 钟燕军;胡汉宁;杨桂;涂建成;喻明霞. NFAT在乳腺癌中的研究进展[J]. 肿瘤防治研究, 2011, 38(8): 960-962.

[15] 高炳玉;夏立平;刘玉;陈国平;郑武平 . X线照射后对乳腺癌细胞凋亡的影响及CDKN1A表达的变化[J]. 肿瘤防治研究, 2011, 38(8): 891-894.