中南大学学报(医学版) 2011, 36(9) 836-843 DOI: 10.3969/j.issn.1672-7347.2011.09.004 ISSN: 1672-7347 CN: 43-1427/R

论著

Tet-On调控自杀基因治疗系统对乳腺癌细胞的DNA 损伤效应

李弘德, 向生光, 马楠, 胡维新, 曾赵军

中南大学生物科学与技术学院分子生物学研究中心,长沙 410078

摘要:

目的:研究重组腺相关性病毒(rAAV)介导的含有Tet-On 调控元件的HSV-TK/GCV 自杀基因调控治疗系统对人类乳腺癌细胞株MCF-7 DNA 损伤的影响及损伤应答的分子机制。方法:彗星实验检测HSV-TK/GCV自杀基因治疗系统对MCF-7 DNA 损伤的影响,并对主要的DNA 损伤应答的相关活性基因和表达蛋白进行RT-PCR和Western印迹检测,分析其表达变化情况。结果:与各对照组相比,实验组MCF-7细胞的彗星实验结果有明显的彗星拖尾现象,DNA 损伤应答的相关活性基因和活性蛋白(如ATM,p53和p27)的表达水平产生了明显的差异,而CyclinE和CDK2的表达水平没有明显变化。 结论:HSV-TK/GCV自杀基因治疗系统可以导致MCF-7的DNA 损伤反应,这种损伤反应可能是通过一种p53依赖的信号通路引起细胞阻滞导致死亡。

关键词: 乳腺癌 自杀基因治疗 彗星实验 HSV-TK/GCV DNA损伤

DNA damage caused by suicide gene therapy system under Tet-On regulation in breast cancer cells

LI Hongde, XIANG Shengguang, MA Nan, HU Weixin, ZENG Zhaojun

Molecular Biology Research Center, School of Biological Science and Technology, Central South University, Changsha 410078, China

Abstract:

Objective To determine the effect and molecular mechanism of DNA damage caused by suicide gene therapy system HSV-TK/GCV under Tet-On regulation in human breast cancer cell line MCF-7 infected by recombinant adeno-associated virus (rAAV). Methods We used comet assay to detect the effect of HSV-TK/GCV suicide gene regulation system on MCF-7 DNA damage, and analyzed the expression change of relative DNA damage response active genes and proteins with RT-PCR and Western blot. Results Compared with other control groups, the comet assay showed that MCF-7 cells with HSV-TK/GCV treatment had obvious comet tails, and the expression level of DNA damage response active genes and proteins changed obviously in the HSV-TK/GCV treatment group,such as ATM, p53 and p27,but CyclinE and CDK2 did not change. Conclusion DNA damage on MCF-7 cells is resulted from HSV-TK/GCV in suicide gene therapy system through a p53-dependent signal pathway, causing cell cycle arrest and cell death.

Keywords: breast cancer suicide gene therapy comet assay HSV-TK/GCV DNA damage

收稿日期 2011-04-20 修回日期 网络版发布日期

DOI: 10.3969/j.issn.1672-7347.2011.09.004

基金项目:

国家自然科学基金(30600753,81172154)。

通讯作者: 曾赵军, E-mail:zengzj71@hotmail.com.

作者简介: 李弘德,硕士,主要从事恶性肿瘤的自杀基因治疗研究。

作者Email: zengzj71@hotmail.com.

参考文献:

[1] Portsmouth D, Hlavaty J, Renner M. Suicide genes for cancer therapy［J］. Mol Aspects Med,2007,28(1):4-41.

[2] Moolten F L. Drug sensitivity ("suicide") genes for selective cancer chemotherapy［J］. Cancer Gene

Ther,1994,1(4):279-287.

[3] Wang J, Lu X X, Chen D Z, et al. Herpes simplex virus thymidine kinase and ganciclovir suicide gene therapy for human pancreatic cancer［J］. World J Gastroenterol, 2004,10(3):400-403.

[4] Ostling O,Johanson K J. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells［J］.Biochem Biophys Res Commun,1984,123(1):291-298.

[5] Tice R R, Agurell E, Anderson D, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing［J］. Environ Mol Mutagen,2000,35(3):206-221.

[6] McKelvey-Martin V J, Green M H, Schmezer P, et al. The single cell gel electrophoresis assay (comet assay): a European review［J］. Mutat Res,1993,288(1):47-63.

[7] Collins A R, Fleming I M, Gedik C M. In vitro repair of oxidative and ultraviolet-induced DNA damage in supercoiled nucleoid DNA by human cell extract［J］. Biochim Biophys Acta,1994,1219(3):724-727.

[8] Olive P L,Banáth J P. Sizing highly fragmented DNA in individual apoptotic cells using the comet assay and a DNA cross-linking agent［J］. Exp Cell Res,1995,221(1):19-26.

[9] Singh N P, McCoy M T, Tice R R, et al. A simple technique for quantitation of low levels of DNA damage in individual cells［J］. Exp Cell Res, 1988,175(1):184-191.

[10] Olive P L, Wlodek D, Banáth J P. DNA double-strand breaks measured in individual cells subjected to gel electrophoresis［J］. Cancer Res, 1991,51(17):4671-4676.

[11] Saha D T, Davidson B J, Wang A, et al. Quantification of DNA repair capacity in whole blood of patients with head and neck cancer and healthy donors by comet assay［J］. Mutat Res,2008,650(1):55-62.

[12] Olive P L, Banáth J P. The comet assay: a method to measure DNA damage in individual cells［J］. Nat Protoc,2006,1(1):23-29.

[13] Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters［J］. Proc Natl Acad Sci USA, 1992,89(12):5547-5551.

[14] Gossen M, Freundlieb S, Bender G,et al. Transcriptional activation by tetracyclines in mammalian cells［J］.Science,1995,268(5218):1766-1769.

[15] 陈迁, 李子博, 曾赵军, 等. Tet-On调控HSV-TK 表达的重组腺相关病毒载体的构建和感染活性的检测［J］. 生物工程学报, 2005, 21(3): 360-364. CHEN Qian,LI Zibo,ZENG Zhaojun,et al. The construction of recombinant AAV vector expressing HSV-TK gene controlled by Tet-On and the detection of its activity［J］.Chinese Journal of Biotechnology, 2005, 21(3):360-364.

[16] Li Z B, Zeng Z J, Chen Q, et al. Recombinant AAV-mediated HSV-TK gene transfer with direct intratumoral injections and Tet-On regulation for implanted human breast cancer［J］.BMC Cancer,2006,6(1):66.

[17] Beckman H J,Trego K S,Turchi J J. Cisplatin sensitizes cancer cells to ionizing radiation via inhibition of nonhomologous end joining［J］.Mol Cancer Res,2005,3(5):277-285.

[18] Siddik Z H. Cisplatin: mode of cytotoxic action and molecular basis of resistance［J］.Oncogene,2003,22(47):7265-7279.

[19] Petrini J H,Stracker T H. The cellular response to DNA double-strand breaks: defining the sensors and mediators［J］.Trends Cell Biol,2003, 13(9):458-462.

[20] Reinhardt H C,Aslanian A S,Lees J A,et al. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage［J］.Cancer Cell,2007,11(2):175-189.

[21] Colman M S,Afshari C A,Barrett J C. Regulation of p53 stability and activity in response to genotoxic stress［J］.Mutat Res,2000,462(2/3):179-188.

[22] Polyak K, Kato J Y, Solomon M J,et al. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth

factor-beta and contact inhibition to cell cycle arrest［J］.Genes Dev,1994,8(1):9-22.

本刊中的类似文章

1．邹文； 胡春宏； 周建平； 杨竹林； .E-钙粘素和α,β,γ连环素的表达与乳腺癌的转移和预后关系[J]. 中南大学学报(医学版), 2002,27(6): 499-

2．黄俊辉， 李洋， 刘亮， 杨怀才， 海健， 唐利立， 胡铁辉. VEGF-C介导的乳腺癌肿瘤淋巴管生成及定位[J]. 中南大学学报(医学版), 2006,31(01): 36-39

3．李建璜， 罗学港， 吉四他浜联合外照射诊断人乳腺癌MCF-7细胞凋亡[J]. 中南大学学报(医学版), 2006,31(05): 710-713

4．黄程辉1， 曹培国1， 谢兆霞2.MCF-7/Adr细胞mdr-1基因启动子甲基化和组蛋白乙酰化状态与多药耐药的关系[J]. 中南大学学报(医学版), 2009,34(05): 369-374

5．陈嘉， 申良方， 周蓉蓉， 姚蔚， 钟美佐， 朱虹， 曾珊.洛贝林逆转人乳腺癌细胞MCF-7/ADM耐药作用及其机制[J]. 中南大学学报(医学版), 2009,34(08): 738-743

6．邬玉辉, 陈飞宇, 欧阳慧英, 王守满.不可触及肿物乳腺癌的临床诊断[J]. 中南大学学报(医学版), 2008,33(09): 861-864

7．张建亭.多药耐药蛋白ABCG2的生物化学和药理学[J]. 中南大学学报(医学版), 2007,32(04): 531-541

8．吴芳，胡春宏，蒋少艾，卢放根，林绵辉，邓小戈.赫赛汀联合辅助化疗对人类表皮生长因子受体2阳性早期乳腺癌患者预后影响的Meta分析[J]. 中南大学学报(医学版), 2007,32(04): 684-689

9．丁波泥*，陈道瑾，吴君辉.Mammotome在早期乳腺癌保乳手术中的应用[J]. 中南大学学报(医学版), 2005,30(5): 618-619

10．黄俊辉1， 张轶2， 黄玉婷3， 张曦蓓3， 肖佳3 .MIF对耐ADM人乳腺癌细胞MCF-7/ADM体内外耐药逆转作用[J]. 中南大学学报(医学版), 2010,35(6): 576-

11．程瑞雪； 冯德云； 郑晖； 谭怡； .乳腺癌组织中p-MAPK活化对c-fos和c-jun的激活作用[J]. 中南大学学报(医学版), 2001,26(1): 10-

12．黄程辉， 曹培国， 谢兆霞， 等.不同方式加热联合甲基莲心碱对耐药乳腺癌MCF-7/Adr细胞γH2AX及mdr-1/P-gp表达的影响[J]. 中南大学学报(医学版), 2011,36(4): 317-

13．姜萍岚， 王曙红， 蒋冬梅， 等.乳腺癌术后化疗患者癌因性疲乏与应对方式的研究[J]. 中南大学学报(医学版), 2011,36(4): 323-

14．邓豪余； 段华新； 邱娟.乳腺癌患者腋窝淋巴结转移与骨转移关系分析[J]. 中南大学学报(医学版), 2001,26(3): 269-

15．姜萍岚, 王曙红, 蒋冬梅, 虞玲丽, 唐利立, 赖娟.长沙地区乳腺癌化疗患者癌因性疲乏与社会支持的相关性研究[J]. 中南大学学报(医学版), 2011,36(9): 844-848