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论著

CDK2-AP1基因过表达对乳腺癌MCF-7细胞增殖及周期的影响

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摘要:

目的:通过过表达手段上调细胞周期调节蛋白依赖性激酶2- 关联蛋白1(CDK2-AP1) 基因在乳腺癌细胞MCF-7 中的表达, 并观察其对MCF-7 细胞生长和细胞周期调控的作用。方法:将CDK2-AP1 基因的编码框构建于慢病毒表达载体, 导入MCF-7 细胞, 应用实时定量PCR 和Western 印迹验证CDK2-AP1 基因mRNA 和蛋白的表达效率。利用M法绘制生长曲线、克隆形成实验观察CDK2-AP1 基因过表达后MCF-7 细胞生长的变化, PI 染色流式细胞仪检测MCF-7 细胞周期的改变。通过Western 印迹检测CDK2-AP1 过表达后, 细胞周期相关蛋白(CDK2, CDK4, $P16^{Ink4A}$, $P21^{Cip1/Waf1}$) 的表达。结果:过表达CDK2-AP1 基因的慢病毒感染MCF-7 细胞可上调其mRNA 表达6.94 倍, 蛋白表达也十分显著地增高, 两者相一致。生长曲线显示MCF-7 细胞过表达CDK2-AP1 基因后, 增殖能力显著降低($P<0.05$);克隆形成实验表明, 其形成的克隆数目同样显著减少($P<0.05$);流式细胞仪检测证实MCF-7 细胞过表达CDK2-AP1 能够使细胞周期出现G_1期阻滞, 并且出现凋亡峰;CDK2-AP1 基因表达上调导致$P21^{Cip1/Waf1}$和$P16^{Ink4A}$蛋白表达上调, CDK2 和CDK4 蛋白表达下调。结论:CDK2-AP1 基因具有抑癌基因的功能, 在乳腺癌MCF-7 细胞过表达该基因能够抑制细胞的生长和克隆形成能力, 并且使细胞阻滞于G_1期。

关键词: CDK2-AP1 过表达 增殖 细胞周期

Effect of CDK2-AP1 gene over-expression on proliferation and cell cycle regulation of breast cancer cell line MCF-7

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Abstract:

Objective: To over-express cyclin-dependent kinase 2-associated protein 1 (CDK2-AP1) gene, andinvestigate its effect on the proliferation and cell cycle regulation in breast cancer cell line MCF-7. Methods: CDK2-AP1 gene coding region was cloned into lentivirus vector. Lentivirus particleswere infected into MCF-7 cells to upregulate the expression of CDK2-AP1 gene. The expressionlevel of CDK2-AP1 was detected at both mRNA and protein levels by real-time PCR and Westernblot. MTT assay, colony formatting assay, and flow cytometry were performed to detect the changeof proliferation and cell cycle in MCF-7 cells. We examined the expression of cell cycle associatedgenes (CDK2, CDK4, $P16^{Ink4A}$, and $P21^{Cip1/Waf1}$) followed by CDK2-AP1 over-expression byWestern blot.
Results: CDK2-AP1 gene was up-regulated significantly at both mRNA (6.94 folds) and proteinlevel. MTT based growth curve, colony formatting assay and flow cytometry showed that CDK2-AP1 over-expression lentivirus inhibited the proliferation of MCF-7 cells with statistical difference($P<0.05$). In addition, with CDK2-AP1 over-expression, MCF-7 cells were arrested in G1 phaseaccompanied by apoptosis. Western blot showed that the expression level of $P21^{Cip1/Waf1}$ and$P16^{Ink4A}$ was upregulated, while the expression level of CDK2 and CDK4, members of the CDKfamily, was downregulated.
Conclusion: CDK2-AP1 gene plays a cancer suppressor role in breast cancer. Its function includesinhibiting the proliferation of MCF-7 cells and arresting the cell cycle in G_1 phase.

Keywords: CDK2-AP1 over-expression proliferation cell cycle

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参考文献：

1. 彭卫军, 顾雅佳. 重视乳腺影像学检查技术和诊断水平的普及与提高为降低我国乳腺癌发病率而努力［J］.中华放射学杂志,2009, 43(5): 453-454.PENG Weijun, GU Yajia. Boost and generalization of breast imagingand diagnostic ability in order to reduce the incidence of breast cancerin our country［J］.Chinese Journal of Radiology, 2009, 43(5): 453-454.
2. 王深明, 林颖. 乳腺癌基因治疗进展［J］.中华外科杂志, 2006,44(1): 55-58.WANG Shenming, LIN Ying. Progress in gene therapy of breast cancer［J］.Chinese Journal of Surgery, 2006, 44(1): 55-58.
3. 陈嘉, 申良方, 钟美佐, 等. E1A抑制乳腺癌MCF-7细胞增殖及其分子机制［J］.中南大学学报:医学版, 2008, 33(7): 582-586.CHEN Jia, SHEN Liangfang, ZHONG Meizuo, et al. Molecularmechanism of proliferation of human breast cancer cell MCF-7inhibited by EIA gene［J］.Journal of Central South University. MedicalScience, 2008, 33(7): 582-586.
4. Figueiredo ML, Kim Y, St John MA, et al. P12 (CDK2-AP1) genetherapy strategy inhibits tumor growth in an in vivo mouse model ofhead and neck cancer［J］.Clin Cancer Res, 2005, 11(10): 3939-3948.
5. Shintani S, Mihara M, Terakado N, et al. Reduction of p12DOC-1expression is a negative prognostic indicator in patients with surgicallyresected oral squamous cell carcinoma［J］.Clin Cancer Res, 2001,7(9): 2776-2782.
6. Hiyoshi Y, Watanabe M, Hirashima K, et al. p12CDK2-AP1is associated with tumor progression and a poor prognosis inesophageal squamous cell carcinoma［J］.Oncol Rep, 2009, 22(1):35-39.
7. Choi MG, Sohn TS, Park SB, et al. Decreased expression of p12 isassociated with more advanced tumor invasion in human gastric cancertissues［J］.Eur Surg Res, 2009, 42(4): 223-229.
8. Dull T, Zu erey R, Kelly M, et al. A third-generation lentivirus vectorwith a conditional packaging system［J］.J Virol, 1998, 72(11): 8463-8471.
9. Gopinathan L, Ratnacaram CK, Kaldis P. Established and novelcdk/cyclin complexes regulating the cell cycle and development［J］.Results Probl Cell Differ, 2011, 53: 365-389.
10. Zolochevska O, Figueiredo ML. Cell-cycle regulators CDK2-AP1 and bicalutamide suppress malignant biological interactionsbetween prostate cancer and bone cells［J］.Prostate, 2011, 71(4):353-367.
11. Shintani S, Ohyama H, Zhang X, et al. p12(DOC-1) is a novel cyclindependentkinase 2-associated protein［J］.Mol Cell Biol, 2000,20(17): 6300-6307.
12. Todd R, McBride J, Tsuji T, et al. Deleted in oral cancer-1 (doc-1), a noveloral tumor suppressor gene［J］.FASEB J, 1995, 9(13): 1362-1370.
13. Tsuji T, Duh FM, Latif F, et al. Cloning, mapping, expression, function,and mutation analyses of the human ortholog of the hamster putativetumor suppressor gene doc-1［J］.J Biol Chem, 1998, 273(12): 6704-6709.
14. Matsuo K, Shintani S, Tsuji T, et al. p12(DOC-1), a growth suppressor,associates with DNA polymerase α/primase［J］.FASEB J, 2000,14(10): 1318-1324.
15. Hu MG, Hu G-F, Kim Y, et al. Role of p12CDK2-AP1 in transforminggrowth factor-b1- mediated growth suppression［J］.Cancer Res, 2004,64(2): 490-499.
16. Peng H, Shintaniy S, Kim Y, et al. Loss of p12CDK2-AP1 Expressionin human oral squamous cell carcinoma with disrupted transforminggrowth factor-b-smad signaling pathway［J］.Neoplasia, 2006, 8(12):1028-1036.
17. Yuan Z, Sotsky Kent T, Weber TK. Differential expression of DOC-1 in microsatellite-unstable human colorectal cancer［J］.Oncogene,2003, 22(40): 6304-6310.
18. Shin J, Yuan Z, Fordyce K, et al. A del T poly T (8) mutationin the 3' untranslated region (UTR) of the CDK2-AP1 geneis functionally significant causing decreased mRNA stabilityre sul t ing in dec rea s ed CDK2-AP1 e x pre s s ion in humanmicrosatellite unstable (MSI) colorectal cancer (CRC)［J］.Surgery, 2007, 142(2): 222-227.

本刊中的类似文章

1．张杰1,2， 周春山2， 刘韶3， 陈皓1， 杨超3.鬼臼毒素抗胃癌细胞株SGC 7901作用的实验研究[J]. 中南大学学报(医学版), 2008,33(08): 718-722
2．何晋1,谢秀梅1,姜德建2,方叶青1,陈晓彬1 .两种类型内皮祖细胞的体外分化及非对称性二甲基精氨酸对其增殖的抑制作用[J]. 中南大学学报(医学版), 2008,33(02): 138-145
3．黄垂学； 胡守兴； 陈兵； .脑星形细胞瘤中神经细胞黏附分子与PCNA的表达及其关系[J]. 中南大学学报(医学版), 2001,26(6): 543-
4．李琼； 何群； .2-氨基甾体对慢性粒细胞白血病细胞系K562细胞增殖的抑制作用[J]. 中南大学学报(医学版), 2002,27(5): 405-
5．罗洪英； 王海成； 冯德云； 郑晖；.肝细胞癌组织中PCNA和nm23的表达与转移的关系[J]. 中南大学学报(医学版), 2003,28(1): 17-
6．孙建军1, 刘勇2, 张蓬勃3, 陈新林2, 郭振宇1, 张建水2, 杨蓬勃2.大鼠脑出血后行为学改变和室管膜下区细胞增殖规律[J]. 中南大学学报(医学版), 2009,34(03): 236-241
7．何艳, 贺兴鄂, 孙会卿, 王文龙, 雷建华.RNA干扰HBx基因对肝癌细胞化疗效果的影响[J]. 中南大学学报(医学

版), 2009,34(05): 395-400
8. 孙国举， 谢秀梅， 邢莹， 鄢文海， 杨天<IMG height=20 alt= 非诺贝特对LPC诱导脐静脉内皮细胞增殖、凋亡eNOS基因表达的影响[J]. 中南大学学报(医学版), 2006,31(03): 373-378
9. 比拜·玉素甫， 哈木拉提·吾甫尔， 钟良军 四期依固酮液对LPS抑制人牙龈成纤维细胞DNA合成和细胞周期改变的影响[J]. 中南大学学报(医学版), 2006,31(04): 483-486
10. 刘映红， 刘伏友， 张浩， 彭佑铭， 袁芳， 刘虹， 成梅初， 卓莉 鼠耐受人腹膜间皮细胞的增殖和损伤及分泌细胞因子的影响[J]. 中南大学学报(医学版), 2006,31(04): 575-579
11. 尹雅玲， 何群.
氨基甾体H42648对K562白血病细胞系的抑制增殖和诱导分化作用
[J]. 中南大学学报(医学版), 2006,31(06): 853-857
12. 吴尚洁1， 李桂源2， 张晶1， 张艳1， 甘妍1， 刑西迁1， 陈平1， 赵水平3.阿托伐他汀对高胆固醇血症兔肺内炎性浸润的影响[J]. 中南大学学报(医学版), 2009,34(07): 608-615
13. 陈明亮1, 谭帅1, 张桂英2, 易梅1, 简丹1, 谢红付1, 陈翔1.雷公藤内酯醇对皮肤鳞状细胞癌A431细胞株增殖与凋亡的影响[J]. 中南大学学报(医学版), 2009,34(07): 638-641
14. 李跃辉， 刘妍， 王甲甲， 胡锦跃， 周国华， 谢平丽， 李官成.γ-氨基丁酸对肝癌细胞株HepG-2恶性表型的影响
[J]. 中南大学学报(医学版), 2009,34(08): 752-756
15. 徐灿霞1，齐艳美1，杨文斌1，王芬1，周建党2，沈守荣1.幽门螺杆菌CagA+菌株对BGC-823细胞系Cx43表达及细胞增殖的影响[J]. 中南大学学报(医学版), 2007,32(02): 288-294

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