世界华人消化杂志®
wcjd@wjgnet.com
世界华人消化杂志 2006年5月28日; 14(15): 1448-1452
ISSN 1009-3079 CN 14-1260/R

■背景资料
Caveolae是细胞表面胞膜内陷形成的膜结构. Caveolin为一蛋白家族, 是Caveolae的重要组成蛋白, 相对分子量为21-24 kDa, 其家族成员有Caveolin-1, 2, 3三种, 其中Caveolin-1是形成Caveolae所必不可少的结构蛋白. 研究表明, Caveolin-1与胆固醇的转运胞饮作用及信号转导密切相关, 另外, Caveolin-1与肿瘤的发生 发展密切相关.

罗红梅, 龙治峰, 刘月顺, 南华大学医学院组胚教研室 湖南省衡阳市 421001
唐圣松, 廖端芳, 严鹏科, 谭力铭, 汪煜华, 朱炳阳, 南华大学药物药理研究所 湖南省衡阳市 421001
罗红梅, 2004年南华大学病理学与病理生理学硕士, 讲师, 2005年南华大学博士研究生, 主要从事分子肿瘤学的研究.
国家自然科学基金资助项目, No. 30270684
唐圣松, 421001, 湖南省衡阳市, 南华大学医学院药物药理研究所. tangss111@yahoo.com.cn
电话: 0734-8282854 传真: 0734-8281305
收稿日期: 2006-03-07 接受日期: 2006-03-29

Effect of Caveolin-1 on growth of human gastric cancer cell Line MGC803

Hong-Mei Luo, Sheng-Song Tang, Duan-Fang Liao, Peng-Ke Yan, Li-Ming Tan, Yu-Hua Wang, Zhi-Feng Long, Yue-Shun Liu, Bing-Yang Zhu

Hong-Mei Luo, Zhi-Feng Long, Yue-Shun Liu, Department of Histology and Embryology, Medicial College of Nanhua University, Hengyang 421001, Hunan Province, China
Sheng-Song Tang, Duan-Fang Liao, Peng-Ke Yan, Li-Ming Tan, Yu-Hua Wang, Bing-Yang Zhu, Institute of Pharmacy and Pharmacology, Medicial College of Nanhua University, Hengyang 421001, Hunan Province, China
Supported by National Natural Science Foundation of China, No. 30270684
Correspondence to: Dr. Sheng-Song Tang, Institute of Pharmacy and Pharmacology, Medicial College of Nanhua University, Hengyang 421001 Hunan Province, China. tangss111@yahoo.com.cn
Received: 2006-03-07 Accepted: 2006-03-29

Abstract

AIM: To investigate the effect of Caveolin-1 on the proliferation and differentiation of gastric carcinoma cell line MGC803, and explore the probability that Caveolin-1 can be used for gene therapy.

METHODS: Caveolin-1 gene and Pcl-neo control plasmid were transfected into human MGC803 cell line by lipofectin, respectively. The positive clones were selected by G418. We also stabled a positive control group which was treated with PD98059 for 48 hours. Then the expression of Caveolin-1 in each group was detected by Western blot. Cell morphology was observed under optical microscope. Cell population doubling time was determined by cell counting method and cell cycle was analyzed by flow cytometry.

RESULTS: The expression of Caveolin-1 was significantly higher in the cells treated with Caveolin-1 or PD98059 than that in the empty controls ($P < 0.001$, $q = 23.067$ or 13.3376). Furthermore, Caveolin-1 expression was also markedly higher in the cells transfected with Caveolin-1 than that in the positive controls ($P < 0.001$, $q = 9.7294$). Under light microscope, marked changes occurred in cell morphous after gene transfection. Before transfection, the cells had a significant heteromorphism, with the features of large cell body, little cytoplasm, obvious karyokinesis. While in Caveolin-1-transfected MGC803 cells, the cell malignancy declined as the cellular heteromorphism diminished, with the ratio of nuclear-to-cytoplasm decreased, and the karyokinesis disappeared. Caveolin-1-transfected cells had an extended doubling time (65.46 h *vs* 46.67 h, $P < 0.05$, $q = 4.8695$). At same time, the population of Caveolin-1-transfected cells in G_0/G_1 phase was obviously increased ($P < 0.01$, $q = 9.1824$) while that in S phase was decreased ($P < 0.01$, $q = 7.827$). There were also notable differences in cell cycle distribution between Caveolin-1-transfected cells and the positive controls (G_0/G_1: $P < 0.01$, $q = 4.9323$; S: $P < 0.05$, $q = 3.3295$).

CONCLUSION: Caveolin-1 not only induces the differentiation of MGC803 cells, but also blocks them at in G_0/G_1 phase. Caveolin-1 can inhibit the proliferation of MGC803 cells *in vitro* by prolonging the cell doubling time.

Key Words: Caveolin-1; Gastric carcinomas; Cell proliferation; Cell differentiation; Gene therapy

Luo HM, Tang SS, Liao DF, Yan PK, Tan LM, Wang YH, Long ZF, Liu YS, Zhu BY. Effect of Caveolin-1 on growth of human gastric cancer cell Line MGC803. Shijie Huaren Xiaohua Zazhi 2006;14(15):1448-1452

摘要

研究Caveolin-1对胃癌细胞增殖 分化的影响, 以探讨Caveolin-1作为基因治疗的候

选基因的可能性.

运用基因重组技术, 将人全长Caveolin-1基因稳定转染胃癌细胞系MGC803, 建立能稳定表达Caveolin-1的胃癌细胞系, 同时建立空载体转染的MGC803细胞系作为空白对照, 另用PD98059处理48 h作为阳性对照. 以重组细胞系作为研究模型, 通过免疫细胞化学及Western blot确认Caveolin-1蛋白在被转染细胞中的稳定表达. 运用光学显微镜观察转染前后MGC803细胞形态的变化; 另运用细胞计数检测了Caveolin-1对MGC803细胞生长的影响, 流式细胞术分析了Caveolin-1对MGC803细胞周期分布的影响.

Western blot结果显示, 基因转染组及阳性对照组细胞中Vaveolin-1表达比未处理组细胞中明显增强($P<0.001$, q值分别为23.067与13.3376), 且基因转染组中Caveolin-1表达比阳性对照组更强($P<0.001$, $q = 9.7294$); 基因转染后的MGC803细胞形态发生明显变化, 由异形性明显 核大 胞质很少 核分裂明显变得形态较一致 胞质丰富 核/质比明显变小 核分裂相很少见; 基因转染后细胞的群体倍增时间明显延长, 由46.67 h延长至65.46 h, 差异有统计学意义($P<0.05$, $q = 4.8695$); 基因转染后细胞周期分布发生了明显变化, G_0/G_1期细胞数明显增多($P<0.01$, $q = 9.1824$), S期细胞数明显减少($P<0.01$, $q = 7.827$), G_2/M期细胞数无明显变化($P>0.05$), Caveolin-1基因转染组与阳性对照组间细胞周期分布的变化也有明显差异性(其中G_0/G_1期$P<0.01$, $q = 4.9323$; S期$P<0.05$, $q = 3.3295$).

Caveolin-1既能诱导MGC803细胞分化又能将其阻滞于G_0/G_1期, 通过延长细胞群体倍增时间而抑制胃癌细胞的体外增殖.

关键词

罗红梅, 唐圣松, 廖端芳, 严鹏科, 谭力铭, 汪煜华, 龙治峰, 刘月顺, 朱炳阳. Caveolin-1对胃癌细胞系MGC803细胞生长的影响. 世界华人消化杂志 2006;14(15):1448-1452
http://www.wjgnet.com/1009-3079/14/1448.asp

0 引言

Caveolae , Caveolin-1 Caveolae . , Caveolin-1 , (: [1-5] [6-8] [9] [10]) , , (: [11-13] [14]) , . pcl-neo Caveolin-1 MGC803 Caveolin-1 .

1 材料和方法

1.1 材料 pcl-neo-Caveolin-1 , G418 RPMI 1640 Lipofectamine™ 2000 Gibco , Caveolin-1 BCA Protein Assay Reagent Western blot Santa Cruz , SABC DAB , . MGC803 . 100 mL/L RPMI 1640 , , 37 50 mL/L, 2.5 g/L .

1.2 方法 MGC803 6 , 2×10^5 , 80% , . , Caveolin-1 cDNA pcl-neo-cav-1 pcl-neo , MGC803 , (MGC803) PD98059 48 h . 48 h 400 mg/L G418 , 15 d G418 . , (25 mmol/L MBS pH 6.5, 0.15 mmol/L NaCl, 10 g/L Triton X-100) , . PVDF Caveolin-1 MGC803 Caveolin-1 . β-actin β-actin . , , , . , PBS 3 950 mL/L 30 min, PBS 3 , HE . 24 , 3 , 7 d.

■研发前沿

Caveolin-1与多种信号分子存在反向调节关系. Caveolin-1的主要作用是减缓细胞增殖进程, 作为抑癌基因发挥作用, 但在前列腺癌等肿瘤中可能作为促癌剂, 这可能与Caveolin-1的磷酸化(Tyr14及Ser80)及突变(P132L)导致骨架区功能丧失有关.

■相关报道

(1)许多细胞内的癌基因可引起Caveolin-1表达下调, 如: c-Myc, HPV E6, v-Abl, Bcr-Abl, H-Ras, v-Src及Neu/ErbB2; (2)失去Caveolin-1的表达协同基因突变可导致INK4a的功能丧失, 而作为编码$p16^{INK4a}$与$p19^{ARF}$的细胞周期调节因子的INK4a基因一旦缺失就足以导致细胞永生化; (3)Caveolin-1存在着自然突变(P132L), 该突变能引起ERK-1/2的超活化; (4)在卵巢癌 乳腺癌及间充质肉瘤中, Caveolin-1表达均为下调.

■创新盘点
本文着眼于Caveolin-1与胃癌, 首次明确报道了Caveolin-1与肿瘤细胞分化的关系.

(T_D). : $T_D = t \times \lg 2/\lg(N_t/N_0)$, ($N_t$ t , N_0). , PBS 3 , 700 mL/L 5 mL 4 24 h. PBS 2 , PI (5 mg/L PI, 20 mg/L RNase A), 300 , .

mean ± SD , (one-way ANOVA) , Student Newman Keuls , SPSS 11.0 for Windows .

2 结果

2.1 细胞中Caveolin-1蛋白表达 Western blot Caveolin-1 Caveolin-1 (P<0.001, q 23.067 13.3376); Caveolin-1 Caveolin-1 (P<0.001, q = 9.7294); Caveolin-1 (P>0.05)(1). Caveolin-1 MGC803 .

2.2 细胞形态学变化 MGC803 , , , , (2A-B); , (2C); Caveolin-1 , , / , (2D).

2.3 肿瘤细胞群体倍增时间 (1), , Caveolin-1 (P<0.05, q = 4.8695); .

2.4 流式细胞仪检测结果 Caveolin-1 , : G_0/G_1 (P<0.01, q = 9.1824; P<0.05, q = 4.6105), S (P<0.01, q = 7.827; P<0.05, q = 4.9929), G_2/M (P>0.05). Caveolin-1 (G_0/G_1 P<0.01, q = 4.9323; S P<0.05, q = 3.3295); , (2).

■应用要点
若能进一步研究将Caveolin-1抑制胃癌细胞增殖机制阐明, 可望为胃癌的基因治疗提供理论依据及靶点.

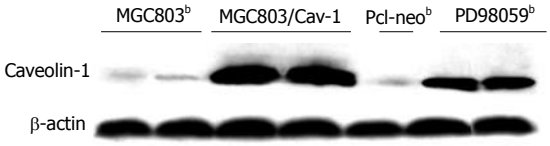

图 1 Caveolin-1在不同处理因素的MGC803细胞中的表达. [b]P<0.01 *vs* MGC803/Cav-1 (n = 4).

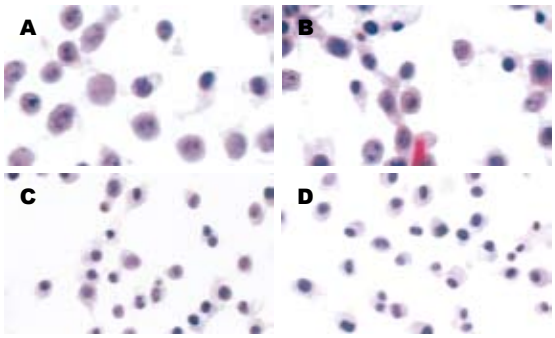

图 2 MGC803细胞的普通光镜观察 (HE, × 400). A: MGC803; B: MGC803/Pcl-neo; C: MGC803+PD98059; D: MGC803/Cav-1.

表 1 各处理组MGC803细胞的群体倍增时间

分组	初种细胞数 (× 10^4)	细胞计数 (× 10^4)	倍增时间 (h)
未处理组	2.50	30.10	46.67[a]
空载体对照组	2.50	28.78	47.32[a]
Caveolin-1基因转染组	2.50	14.79	65.46

[a]P<0.05 *vs* Caveolin-1基因转染组.

3 讨论

[1-5] , [6-8] [9] Caveolin-1 , Caveolin-1 [10]. , Caveolin-1 [2,6,9,15], [11-14,16], , Caveolin-1 , . , , , [17]. Caveolin-1 MGC803 , Caveolin-1 MGC803 . , Caveolin-1 MGC803 Caveolin-1 MGC803

表 2 各处理因素对MGC803细胞增殖周期的影响 (mean ± SD)

分组	G_0/G_1 (%)	G_2/M (%)	S (%)
未处理组	35.02 ± 2.41[a,c]	8.01 ± 0.93	56.97 ± 2.86[a,c]
阳性对照组	43.95 ± 1.39[a]	9.64 ± 1.12	46.41 ± 2.00[a]
空载体对照组	39.56 ± 0.61[a,c]	7.90 ± 1.16	52.54 ± 2.13[a,c]
Caveolin-1基因转染组	51.98 ± 2.67	8.22 ± 1.01	39.80 ± 2.05

[a]P<0.05 *vs* Caveolin-1基因转染组; [c]P<0.05 *vs* 阳性对照组.

■名词解释

1 Caveolin-1: 译名较多, 有窖蛋白 陷窝蛋白 囊泡素等, 与细胞的多种生命活动密切相关, 包括细胞内吞 胆固醇的转运 信号转导和肿瘤发生等密切相关, 一般认为他是一抑癌基因.

2 PD98059: 是MAPK通路中ERK的特异性抑制剂, 抑制ERK的活化, 阻遏细胞周期的进程.

, , Caveolin-1 MGC803 , Caveolin-1 . MGC803 Caveolin-1 , , , , , / , . , Caveolin-1 MGC803 .

, . , , , .

: G_1/S G_2/M , G_2/M ; G_1/S [17]. , Caveolin-1 G_1/S , G_0/G_1 [18]. Caveolin-1 , .

Caveolin-1 , : Caveolin-1 MGC803 : G_0/G_1 , S , G_2/M , ERK PD98059 . Caveolin-1 G_1/S , G_0/G_1 S , MGC803 ERK .

ERK1/2(p42/p44MAPK) , , [19-23]. , ERK [24-26]. , MAPK [27]. PD98059 MAPK ERK , ERK . , PD98059 MGC803 48 h , ERK 94.07% ± 3.01%.

: Caveolin-1 MGC803 ERK PD98059 .

: PD98059 MGC803 , Caveolin-1 , ERK1/2 , Caveolin-1 . , Caveolin-1 Ras/MAPK , , , ERK1/2 Caveolin-1 , .

4 参考文献

1 Fiucci G, Ravid D, Reich R, Liscovitch M. Caveolin-1 inhibits anchorage-independent growth, anoikis and invasiveness in MCF-7 human breast cancer cells. *Oncogene* 2002; 21: 2365-2375

2 Hino M, Doihara H, Kobayashi K, Aoe M, Shimizu N. Caveolin-1 as tumor suppressor gene in breast cancer. *Surg Today* 2003; 33: 486-490

3 Bouras T, Lisanti MP, Pestell RG. Caveolin-1 in breast cancer. *Cancer Biol Ther* 2004; 3: 931-941

4 Sloan EK, Stanley KL, Anderson RL. Caveolin-1 inhibits breast cancer growth and metastasis. *Oncogene* 2004; 23: 7893-7897

5 Sotgia F, Williams TM, Schubert W, Medina F, Minetti C, Pestell RG, Lisanti MP. Caveolin-1 deficiency (-/-) conveys premalignant alterations in mammary epithelia, with abnormal lumen formation, growth factor independence, and cell invasiveness. *Am J Pathol* 2006; 168: 292-309

6 Wiechen K, Diatchenko L, Agoulnik A, Scharff KM, Schober H, Arlt K, Zhumabayeva B, Siebert PD, Dietel M, Schafer R, Sers C. Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. *Am J Pathol* 2001; 159: 1635-1643

7 Bagnoli M, Tomassetti A, Figini M, Flati S, Dolo V, Canevari S, Miotti S. Downmodulation of caveolin-1 expression in human ovarian carcinoma is directly related to alpha-folate receptor overexpression. *Oncogene* 2000; 19: 4754-4763

8 Davidson B, Nesland JM, Goldberg I, Kopolovic J, Gotlieb WH, Bryne M, Ben-Baruch G, Berner A, Reich R. Caveolin-1 expression in advanced-stage ovarian carcinoma-a clinicopathologic study. *Gynecol Oncol* 2001; 81: 166-171

■同行评价
Caveolin是近年来热门研究的与肿瘤相关的细胞因子. 本文研究选题新颖 实验设计较合理 统计方法正确 结果可信 讨论较充分, 文章说明了Caveolin通过诱导细胞分化 阻滞分裂抑制肿瘤细胞的增殖, 为一种候选抑癌基因. 但要说明Caveolin通过抑制信号通路达到抑制细胞增殖尚需进一步研究探讨.

9 Wiechen K, Sers C, Agoulnik A, Arlt K, Dietel M, Schlag PM, Schneider U. Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *Am J Pathol* 2001; 158: 833-839
10 罗红梅, 张蒙夏, 唐圣松, 谭军, 刘月顺, 龙治峰. Caveolin-1表达及其与胃癌生物学行为关系的研究. 肿瘤防治杂志 2004; 11: 341-343
11 Yang G, Timme TL, Frolov A, Wheeler TM, Thompson TC. Combined c-Myc and caveolin-1 expression in human prostate carcinoma predicts prostate carcinoma progression. *Cancer* 2005; 103: 1186-1194
12 Williams TM, Hassan GS, Li J, Cohen AW, Medina F, Frank PG, Pestell RG, Di Vizio D, Loda M, Lisanti MP. Caveolin-1 promotes tumor progression in an autochthonous mouse model of prostate cancer: genetic ablation of Cav-1 delays advanced prostate tumor development in tramp mice. *J Biol Chem* 2005; 280: 25134-25145
13 Satoh T, Yang G, Egawa S, Addai J, Frolov A, Kuwao S, Timme TL, Baba S, Thompson TC. Caveolin-1 expression is a predictor of recurrence-free survival in pT2N0 prostate carcinoma diagnosed in Japanese patients. *Cancer* 2003; 97: 1225-1233
14 Fong A, Garcia E, Gwynn L, Lisanti MP, Fazzari MJ, Li M. Expression of caveolin-1 and caveolin-2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation. *Am J Clin Pathol* 2003; 120: 93-100
15 Capozza F, Williams TM, Schubert W, McClain S, Bouzahzah B, Sotgia F, Lisanti MP. Absence of caveolin-1 sensitizes mouse skin to carcinogen-induced epidermal hyperplasia and tumor formation. *Am J Pathol* 2003; 162: 2029-2039
16 Said J. Biomarker discovery in urogenital cancer. *Biomarkers* 2005; 10 Suppl 1: S83-86
17 卢建, 余应年, 徐仁宝. 受体、信号转导系统与疾病. 第1版. 济南: 山东科学技术出版社, 1999: 597-618
18 Galbiati F, Volonte D, Liu J, Capozza F, Frank PG, Zhu L, Pestell RG, Lisanti MP. Caveolin-1 expression negatively regulates cell cycle progression by inducing G(0)/G(1) arrest via a p53/p21(WAF1/Cip1)-dependent mechanism. *Mol Biol Cell* 2001; 12: 2229-2244
19 Maestroni A, Tentori F, Meregalli G, Gabellini D, Asnaghi V, Ruggieri D, Zerbini G. Inhibition of MAP-kinase cascade normalizes the proliferation rate of fibroblasts from patients with Type 1 diabetes and nephropathy. *J Diabetes Complications* 2005; 19: 291-296
20 Riddle RC, Taylor AF, Genetos DC, Donahue HJ. MAP kinase and calcium signaling mediate fluid flow-induced human mesenchymal stem cell proliferation. *Am J Physiol Cell Physiol* 2006; 290: C776-C784
21 Siriwardana G, Bradford A, Coy D, Zeitler P. Autocrine/paracrine regulation of breast cancer cell proliferation by growth hormone releasing hormone (GHRH) via Ras, Raf, and MAP Kinase. *Mol Endocrinol* 2006
22 Wilkinson MG, Millar JB. Control of the eukaryotic cell cycle by MAP kinase signaling pathways. *FASEB J* 2000; 14: 2147-2157
23 Qi M, Elion EA. MAP kinase pathways. *J Cell Sci* 2005; 118: 3569-3572
24 Torii S, Nakayama K, Yamamoto T, Nishida E. Regulatory mechanisms and function of ERK MAP kinases. *J Biochem (Tokyo)* 2004; 136: 557-561
25 Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004; 68: 320-344
26 Ebisuya M, Kondoh K, Nishida E. The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms for providing signaling specificity. *J Cell Sci* 2005; 118: 2997-3002
27 Kuno Y, Kondo K, Iwata H, Senga T, Akiyama S, Ito K, Takagi H, Hamaguchi M. Tumor-specific activation of mitogen-activated protein kinase in human colorectal and gastric carcinoma tissues. *Jpn J Cancer Res* 1998; 89: 903-909

电编 张敏 编辑 潘伯荣

ISSN 1009-3079 CN 14-1260/R 2006

·消息·

NERD及相关疾病国际研讨会

本刊讯 NERD 2006-08 , :

1 稿件要求及截稿日期

800 , E-mail, 2006-06-15 .

2 联系方式

41 ; : 100009; : 010-64002844; E-mail: NERD@gisummit.com.