DOI: 10.3872/j.issn.1007-385X.2011.01.008

· 基础研究 ·

Snail 促进乳腺癌 MCF-7 细胞移植瘤对多柔比星的耐药及其机制

王琳[1],李洪利[2],赵修世[1], 赵云[1], 赵一诺[1], 李文通[1],张式暖[1](1. 潍坊医学院 病理学教研室,山东 潍坊 261041; 2. 潍坊医学院 医学中心实验室,山东 潍坊 261041)

[摘 要] 目的:探讨 Snail 在乳腺癌 MCF-7 细胞移植瘤对多柔比星耐药中的作用及其可能的机制。方法:构建 *Snail* 基因真核表达载体 pcDNA3.1-Snail,转染至 MCF-7 细胞,筛选稳定表达 Snail 的 MCF-7/Snail 细胞,以转染空质粒 pcDNA3.1 的 MCF-7 细胞(MCF-7/pcDNA)为对照。构建小鼠 MCF-7/Snail 及 MCF-7/pcDNA 细胞移植瘤模型,注射多柔比星,观测移植瘤生长,计算抑瘤率。免疫组织化学方法检测移植瘤组织中 Snail、多药耐药基因-1(multidrug resistance-1, MDR-1)和基质金属蛋白酶-9(matrix metalloproteinase-9,MMP-9)的表达。结果:成功构建 pcDNA3.1-Snail 表达载体,转染 MCF-7 细胞后获得 MCF-7/Snail 和 MCF-7/pcDNA 细胞,并制备小鼠移植瘤。多柔比星治疗后,MCF-7/Snail 细胞移植瘤的瘤重明显高于 MCF-7/pcDNA 细胞移植瘤[(1.413 ± 0.674)g *vs* (1.257 ± 0.576)g, $P < 0.05$],多柔比星对 MCF-7/Snail 移植瘤抑瘤率明显低于 MCF-7/pcDNA移植瘤(18.42% *vs* 30.18%, $P < 0.05$),MCF-7/Snail 细胞移植瘤的组织中 Snail、MDR-1、MMP-9 的表达均显著高于 MCF-7/pcDNA 移植瘤(408.08 ± 20.39 *vs* 67.67 ± 16.56,363.50 ± 26.56 *vs* 55.08 ± 12.23,396.25 ± 16.03 *vs* 56.92 ± 7.35;均 $P < 0.01$),且 Snail 与 MDR-1 和 MMP-9 的表达均呈正相关($r_1 = 0.89$, $P < 0.01$; $r_2 = 0.81$, $P < 0.01$)。结论:Snail 促进乳腺癌 MCF-7 细胞移植瘤对多柔比星的耐药,其机制与增强 MDR-1 和 MM9-9 表达有关。

[关键词] 乳腺肿瘤;多柔比星; Snail; MDR-1; MMP-9
[中图分类号] R737.9; R730.2 [文献标志码] A [文章编号] 1007-385X(2011)01-0038-05

Snail increases resistance of breast cancer MCF-7 cell transplanted tumors to doxorubicin and its mechanism

WANG Lin[1], LI Hong-li[2], ZHAO Xiu-shi[1], ZHAO Yun[1], ZHAO Yi-nuo[1], LI Wen-tong[1], ZHANG Shi-nuan[1](1. Department of Pathology, Weifang Medical College, Weifang 261041, Shandong, China; 2. Central Laboratory, Weifang Medical College, Weifang 261041, Shandong, China)

[**Abstract**] **Objective**: To study the effect of Snail on resistance of breast cancer MCF-7 cell transplanted tumors to doxorubicin and its possible mechanism. **Methods**: Snail eukaryotic expression vector pcDNA3.1-Snail was constructed and transfected into MCF-7 cells, and MCF-7 cells with stable Snail expression (MCF-7/Snail cells) were screened. MCF-7 cells transfected with blank pcDNA3.1 (MCF-7/pcDNA cells) were used as control. MCF-7/Snail- and MCF-7/pcDNA-cell transplanted tumor models were established. After doxorubicin injection, the growth of transplanted tumors was observed, and the inhibitory rate of doxorubicin was calculated. The expressions of Snail, MDR-1 and MMP-9 in transplanted tumor tissues were examined by immunohistochemistry. **Results**: pcDNA3.1-Snail expression vector was successfully constructed, and MCF-7/Snail and MCF-7/pcDNA cells were obtained. After doxorubicin therapy, the transplanted tumor weight in MCF-7/Snail group was significantly higher than that in the MCF-7/pcDNA group ([1.413 ± 0.674]g *vs* [1.257 ± 0.576]g, $P < 0.05$), and the inhibitory rate of doxorubicin was significantly lower (18.42% *vs* 30.18%, $P < 0.05$). The expressions of Snail, MDR-1 and MMP-9 in transplanted tumor tissues were significantly higher than those in MCF-7/pcDNA group (408.08 ± 20.39 *vs* 67.67 ± 16.56, 363.50 ± 26.56 *vs* 55.08 ± 12.23, 396.25 ± 16.03 *vs* 56.92 ± 7.35, all $P < 0.05$), and the expression of Snail was positively correlated with that of MDR-1 and MMP-

[基金项目] 山东省高等学校科技计划项目资助(No. J10LF64)。Project supported by the Science and Technology Program of Higher Institutions of Shandong Province (No. J10LF64)
[作者简介] 王琳(1984 -),女,山东省高密市人,硕士生,主要从事乳腺肿瘤分子生物学方面的研究
[通信作者] 张式暖(ZHANG Shi-nuan, corresponding author), E-mail: xfjt1234@sina.com
[网络出版] 2011-01-25; http://www.cnki.net/kcms/detail/31.1725.R.20110125.1120.024.html

9 ($r_1 = 0.89$, $P < 0.01$; $r_2 = 0.81$, $P < 0.01$). **Conclusion**: Snail can increase resistance of breast cancer MCF-7 cell transplanted tumors to doxorubicin, which might be related with the increased expressions of MDR-1 and MMP-9 in breast cancer MCF-7 transplanted tumors.

[**Key words**] breast neoplasms; doxorubicin; Snail; MDR-1; MMP-9

[Chin J Cancer Biother, 2011, 18(1): 38-42]

上皮-间质转化(epithelial mesenchymal transition,EMT)是指细胞由上皮表型向间质表型的转变,这种转变存在于多种上皮来源的恶性肿瘤中,与肿瘤细胞的浸润和远处转移密切相关[1]。Snail 蛋白是一种锌指 DNA 结合蛋白[2],通过结合钙黏蛋白 E 启动子的 E-box,下调 E-cadherin 的表达,诱导 EMT 的发生[3]。EMT 的发生也与肿瘤细胞多药耐药密切相关,癌细胞突变过程中或经药物诱导后,多药耐药基因(multidrug resistance,MDR)被激活,癌细胞获得或增强抵抗化疗药物(如多柔比星)的能力[4]。MDR 基因表达不仅是药物耐受的信号,而且是癌细胞生物学行为恶化标志[5]。基质金属蛋白酶-9(matrix metalloproteinase-9,MMP-9)是细胞外基质降解过程中最重要的蛋白水解酶,参与机体许多生理和病理过程,特别是与肿瘤的浸润及转移密切相关[6]。本研究观察荷乳腺癌小鼠癌组织中 Snail、MDR-1、MMP-9 的表达及其相关性,以期探讨乳腺癌组织中 Snail 表达与多柔比星耐药的关系及其可能的机制。

1 材料与方法

1.1 主要实验材料

收集潍坊医学院附属医院病理科 2009 年 6 月至 2009 年 10 月间乳腺癌切除标本 10 例,所有患者均未接受化疗及放疗。患者均女性,年龄 35 ~ 54 岁,中位年龄 45 岁。BALB/c 裸鼠 24 只[实验动物合格证号为 SCXK(沪)2007-0005],雌性,4 周龄,重量 15 ~ 19 g。乳腺癌 MCF-7 细胞由潍坊医学院基础医学实验中心提供。抗 Snail、MDR-1 和 MMP-9 一抗及 SABC 通用试剂盒购自北京中杉生物技术公司。RNA 提取试剂 TRIzol 购自北京索莱宝科技有限公司,pcDNA3.1(-)质粒和 Lipofectamine™ 2000 脂质体购自美国 Invitrogen 公司,限制性内切酶、DNA 聚合酶购自宝生物工程(大连)有限公司;cDNA 第一链合成试剂盒、PCR 扩增试剂盒购自宝生物工程(大连)有限公司,T/A 克隆试剂盒和 PCR 引物购自上海生工生物工程有限公司。

1.2 构建 pcDNA3.1-Snail 真核表达载体

TRIzol 法提取乳腺癌组织总 RNA,逆转录合成 cDNA,PCR 扩增获得 *Snail* 基因片段。将获得的 *Snail* 基因片段末端加 A 后连接 pUCm-T 载体,构建成载体 pUCm-T-Snail。克隆产物 pUCm-T-Snail 质粒均用 *Not* Ⅰ 和 *Bam* HⅠ 进行双酶切,将酶切产物连接到 pcDNA3.1(-)质粒,构建成 Snail 真核表达载体 pcDNA3.1-Snail,并酶切和测序鉴定。

1.3 pcDNA3.1-Snail 质粒转染 MCF-7 细胞

通过脂质体法将 pcDNA3.1-Snail 和 pcDNA3.1(-)质粒(对照)导入 MCF-7 细胞,并用 G418 筛选稳定株,分别命名为 MCF-7/Snail 和 MCF-7/pcDNA 细胞。

1.4 乳腺癌荷瘤鼠的制备及多柔比星的治疗

取 BALB/c 裸鼠 40 只,随机分为 4 组,其中 2 组每组 12 只,为实验组;另 2 组每组 8 只,为对照组。分别将 MCF-7/Snail 和 MCF-7/pcDNA 细胞以 1×10^6/只的数量接种于实验组和对照组裸鼠胸壁的乳腺脂肪垫内。接种后 7 d 在接种部位出现肿瘤结节,质地较硬。接种 MCF-7/Snail 和 MCF-7/pcDNA细胞后 15 d 开始给药,两组小鼠均经尾静脉注射多柔比星 1 mg/kg(按照成人每天 40 mg/m^2 换算),每 5 d 注射 1 次,共注射 3 次。

用游标卡尺测量肿瘤大小,分别记录肿瘤长径(a)和短径(b),计算肿瘤体积,V(mm^3) = 1/2ab^2。根据两组小鼠移植瘤的体积,制作移植瘤生长曲线。多柔比星注射 15 d 后,脱颈处死全部荷瘤鼠,取出瘤体并称重,计算抑瘤率。抑瘤率(%) =(对照组肿瘤质量 - 实验组肿瘤质量)/对照组质量 × 100%。

1.5 免疫组织化学法检测 Snail、MDR-1 和 MMP-9 在乳腺癌移植瘤组织中的表达

免疫组织化学采用 SABC 法,分别滴加一抗(兔抗 Snail,1∶80 稀释;兔抗 MMP-9 工作液;鼠抗 MDR-1 工作液)和生物素化二抗,在显色之前滴加试剂 SABC;DAB 室温显色,镜下控制反应1 ~ 15 min;苏木精轻度复染,显微镜下观察结果。

棕黄色颗粒为阳性表达信号。Snail 阳性信号主要定位于细胞核,MDR-1 阳性信号主要定位于细胞膜,MMP-9 阳性信号主要定位于细胞质。分别以 16D 目镜测微网在 400 倍下计数网格中的阳性细胞数,每例计数 5 个网格。

1.7　统计学处理

实验数据以 $\bar{x} \pm s$ 表示,采用 SPSS10.0 软件分析,采用 t 检验和 Pearson 相关分析,以 $P<0.01$ 或 $P<0.05$ 表示差异有统计学意义。

2　结　果

2.1　pcDNA3.1-Snail 真核表达载体的构建及其稳定转染细胞的获得

RT-PCR 获得特异性 *Snail* 基因片段,长约 799 bp(图 1)。胶回收纯化后,将该片段与 T 载体连接,转化后进行酶切鉴定,在 800 bp 附近见到特异性片段。测序后将所得序列在 Blast 上进行比对,其与原序列相一致。将该序列连接到酶切好的 pcDNA3.1(-)质粒,构建 pcDNA3.1-Snail 真核表达载体(图 2)。

以脂质体法将 pcDNA3.1-Snail 和 pcDNA3.1(-) 转染 MCF-7 细胞,获得 MCF-7/Snail 细胞和 MCF-7/pcDNA 细胞。

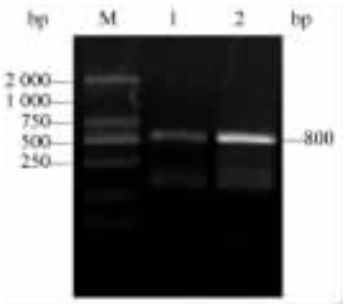

图 1　PCR 扩增 *Snail* 基因

Fig. 1　*Snail* gene amplified by PCR

M: Marker; 1, 2: *Snail* gene

2.2　Snail 促进乳腺癌细胞移植瘤对多柔比星的耐药性

以多柔比星分别治疗 MCF-7/Snail 和 MCF-7/pcDNA 细胞移植瘤,治疗结束后,MCF-7/Snail 细胞移植瘤的瘤重[(1.413 ± 0.674)g]明显高于 MCF-7/pcDNA 细胞移植瘤[(1.257 ± 0.576)g, $P<0.05$];多柔比星对 MCF-7/Snail 组的抑瘤率(18.42%)明显低于 MCF-7/pcDNA 组(30.18%, $P<0.05$)。结果表明,Snail 的表达能够明显促进乳腺癌 MCF-7 细胞对多柔比星的耐药性。

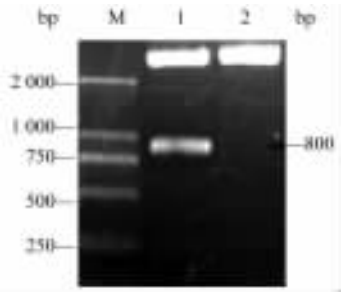

图 2　pcDNA3.1-Snail 真核表达载体的酶切鉴定

Fig. 2　Identification of eukaryotic pcDNA3.1-Snail expression vector by endonuclease digestion

M: Marker; 1: pcDNA3.1-Snail; 2: pcDNA3.1

2.3　Snail 和 MDR-1、MMP-9 在乳腺癌移植瘤组织中的表达及其相关性

Snail 阳性信号主要定位于细胞核,对照组 MCF-7/Snail 细胞移植瘤组织阳性细胞数明显高于对照组 MCF-7/pcDNA 细胞移植瘤组织[(408.08 ± 20.39) *vs*(67.67 ± 16.56), $P<0.05$;图 3A、D);MDR-1 阳性信号主要定位于细胞膜,实验组 MCF-7/Snail 细胞移植瘤组织阳性细胞数明显高于对照 MCF-7/pcDNA 细胞移植瘤组织[(363.50 ± 26.56) *vs* (55.08 ± 12.23), $P<0.01$;图 3B、E]。MMP-9 阳性信号主要定位于细胞质,实验组 MCF-7/Snail 细胞移植瘤组织阳性细胞数明显高于对照组 MCF-7/pcDNA 细胞移植瘤组织[(396.25 ± 16.03) *vs* (56.92 ± 7.35), $P<0.01$;图 3C、F]。

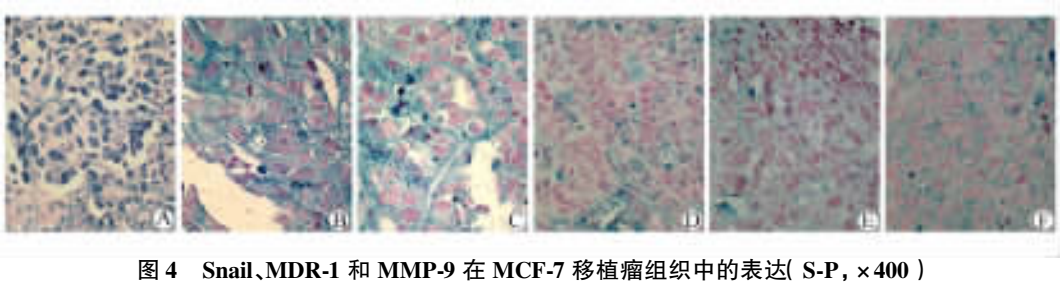

图 4　Snail、MDR-1 和 MMP-9 在 MCF-7 移植瘤组织中的表达(S-P, ×400)

Fig. 4　Snail, MDR-1 and MMP-9 expressions in tumor tissues of MCF-7-bearing mice(S-P, ×400)

A: Snail is strongly expressed in MCF-7/Snail tumor tissues; B: MDR-1 is strongly expressed in MCF-7/Snail tumor tissues; C: MMP-9 is strongly expressed in MCF-7/Snail tumor tissues; D: Snail is weakly expressed in MCF-7/pcDNA tumor tissues; E: MDR-1 is weakly expressed in MCF-7/pcDNA tumor tissues; F: MMP-9 is weakly expressed in MCF-7/pcDNA tumor tissues

与 MCF-7/pcDNA 移植瘤相比,实验组 MCF-7/Snail 细胞移植瘤组织中 Snail 的阳性细胞数增加时,MDR-1 和 MMP-9 的阳性细胞数也增加;Pearson 相关分析发现,两组乳腺癌组织 Snail 与 MDR-1、MMP-1 的表达均呈正相关($r_1 = 0.89$, $P < 0.01$; $r_2 = 0.81$, $P < 0.01$)。结果显示,Snail 的表达增加,可使 MCF-7 乳腺癌移植瘤组织中 MDR-1 和 MMP-9 的表达相应增加。

3 讨 论

大量研究[7-8]表明,转录因子 Snail 可以诱导 EMT 的发生,肿瘤的侵袭浸润和转移增强是 EMT 的特征之一[9],发生 EMT 的上皮细胞丧失了上皮细胞原有的细胞极性和细胞间连接,细胞的迁移和运动能力增强[1]。多药耐药(MDR)是导致恶性肿瘤患者化疗失败的重要原因之一。Li 等[10]的研究表明,多柔比星可以通过细胞周期依赖的方式诱导乳腺癌细胞发生凋亡和 EMT,发生 EMT 的乳腺癌细胞侵袭、转移能力和 MDR 均增强。金属基质蛋白酶(MMP)是一组锌肽酶超家族,能够降解细胞外基质成分,在肿瘤的浸润和转移过程中起着关键性的作用。目前已发现 MMP-2 和 MMP-9 是 MMPs 中与肿瘤浸润和转移关系最密切的两个因子。Davies 等[11]通过定量酶谱分析发现,MMP-2 和 MMP-9 的表达与乳腺癌的组织学分级及侵袭能力呈正相关。Savagner 等[12]研究发现,体外乳腺上皮细胞中的溶基质素(stromelysin)能够诱导 EMT 的发生。

以往的研究[13]发现,高表达 Snail 后可导致人乳腺癌细胞 P-gp 介导的多药耐药。为证明体内 Snail 高表达导致乳腺癌组织耐药性和侵袭力增强,本实验将构建的 Snail 真核表达载体 pcDNA3.1-Snail 转染至乳腺癌 MCF-7 细胞,并将稳定转染 MCF-7/Snail 细胞种植于 BALB/c 小鼠胸壁的乳腺脂肪垫内,构建小鼠乳腺癌移植瘤模型,观察 MCF-7/Snail 细胞对化疗药多柔比星的敏感性,并采用免疫组织化学方法检测移植瘤组织中 Snail、MDR-1 和 MMP-9 的表达。结果显示,MCF-7/Snail 移植瘤瘤重显著高于 MCF-7/pcDNA 组,其对多柔比星的敏感性显著低于 MCF-7/pcDNA 组;MCF-7/Snail 细胞移植瘤组织中 Snail、MDR-1 和 MMP-9 的阳性表达均显著高于 MCF-7/pcDNA 细胞移植瘤(均 $P < 0.05$)。相关性分析表明,在乳腺癌组织中 Snail 与 MDR-1 和 MMP-9 的表达成正相关($P < 0.01$)。EMT 与多药耐药的发生及侵袭浸润之间存在相互作用机制,因此,Snail 与乳腺癌的耐药、发生、演进关系密切,Snail 过表达可导致乳腺癌的预后变差。当然,EMT 与多药耐药的发生和侵袭浸润三者之间的关系机制复杂,尚需进一步的研究。

[参 考 文 献]

[1] Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression [J]. Curr Opin Cell Biol, 2005, 17(5): 548-558.

[2] Massoumi R, Kuphal S, Hellerbrand C, et al. Down-regulation of CYLD expression by Snail promotes tumor progression in malignant melanoma [J]. J Exp Med, 2009, 206(1): 221-232.

[3] Dohadwala M, Yang SC, Luo J, et al. Cyclooxygenase-2-dependent regulation of E-cadherin prostaglandin E2 inducest ranscriptional repressors ZEB1 and Snail in non-small cell lung cancer [J]. Cancer Res, 2006, 66(10): 5338-5345.

[4] 魏红梅, 阮一民, 张立成, 等. 热疗与阿霉素联合作用动物的实验研究[J]. 南方医科大学学报, 2007, 27(9): 1424-1426.

[5] Gotteman MM. How cancer cells evade chemotherapy: Sixteen Richard and Hinda Rosenthal Foundation Lecture [J]. Cancer Res, 1993, 53(4): 747-754.

[6] Orlichenko LS, Radisky DC. Matrix metalloproteinases stimulate epithelial-mesenchymal transition during tumor development [J]. Clin Exp Metastasis, 2008, 25(6): 593-600.

[7] Francí C, Gallén M, Alameda F, et al. Snail protein in the stroma as a new putative prognosis marker for colon tumors [J]. PLoS One, 2009, 4(5): e5595.

[8] Lundgren K, Nordenskjöld B, Landberg G. Hypoxia, Snail and incomplete epithelial-mesenchymal transition in breast cancer [J]. Br J Cancer, 2009, 101(10): 1769-1781.

[9] Geiger TR, Peeper DS. Metastasis mechanisms [J]. Biochim Biophys Acta, 2009, 1796(2): 293-308.

[10] Li QQ, Xu JD, Wang WJ, et al. Twist1-mediated adriamycin-induced epithelial-mesenchymal transition relates to multidrug resistance and invasive potential in breast cancer cells [J]. Clin Cancer Res, 2009, 15(8): 2657-2665.

[11] Davies B, Miles DW, Happerfield LC, et al. Activity of type Ⅳ collagenases in benign and malignant breast disease [J]. Br J Cancer, 1993, 67(5), 1126-1131.

[12] Savagner P. Leaving the neighborhood: Molecular mechanisms involved during epithelial-mesenchymal transition [J]. Bioessays, 2001, 23(10): 912-923.

[13] 刘传亮, 王辉, 陈伟娟, 等. Snail 增强乳腺癌细胞 MCF-7 中 P-gp 介导的多药耐药[J]. 中国药理学通报, 2010, 26(1): 87-90.

[14] Tsuruo T, Naito M, Tomida A, et al. Molecular targeting therapy of cancer: Drug resistance, apoptosis and survival signal [J]. Cancer Sci, 2003, 94(1): 15-21.

[15] Satoh H, Uesugi Y, Kawabata T, et al. Morphological classification of dental lesions induced by various antitumor drugs in mice [J]. Toxicol Pathol, 2001, 29(3): 292-299.

[收稿日期] 2010-10-28 [修回日期] 2010-12-05
[本文编辑] 韩 丹