·论 著·

小窝蛋白-1 与食管癌细胞迁移之间的关系

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摘要：目的 探讨小窝蛋白(Cav)-1 在食管癌细胞迁移中的作用，并探讨两者之间的关系。**方法** 体外培养人食管癌 TE13 细胞株，采用 Cav-1 小干扰 RNA(siRNA)转染 TE13 细胞(转染组)，以未转染细胞为对照(NC)组。采用蛋白免疫印迹(Western blot)实验检测细胞的 Cav-1 表达水平，采用划痕实验观察细胞迁移情况，探讨 Cav-1 和食管癌细胞迁移的关系。**结果** Western blot 实验结果显示，食管癌 TE13 细胞高表达 Cav-1，转染 siRNA 后细胞 Cav-1 表达水平较转染前明显下降，转染组和未转染组细胞的灰度值分别为(1.98 ± 0.34)和(3.15 ± 6.10)，两者比较差异有统计学意义($t = 4.26$，$P < 0.05$)。划痕试验结果显示，未转染组在划痕后 24 h 痕间距明显缩窄，部分痕区域细胞密集；而转染组细胞在划痕后 24 h 痕间距未见明显缩窄。表明转染后细胞的迁移能力较转染前明显下降。**结论** Cav-1 在食管癌的发生、发展过程中扮演着促癌基因的角色，抑制 Cav-1 表达可能抑制食管癌的进展。

关键词：食管癌；小窝蛋白-1；细胞迁移；细胞转染；划痕试验；蛋白免疫印迹实验

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Relationship between caveolin-1 and esophageal cancer cells migration in vitro

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Abstract: Objective To investigate the effect of caveolin -1 (Cav-1) on esophageal cancer cell migration and the relationship between them. **Methods** Human esophageal carcinoma cell line TE13 cells were cultured in vitro. The TE13 cells transfected by Cav-1 small interfering RNA (siRNA) were served as transfection group, and the no-transfection cells were served as control (no-transfection group). The Cav-1 expression level was detected by protein immunoblotting (Western blot) test, and the cell migration was observed by the scratch test. The relationship between Cav-1 and esophageal cancer cell migration was studied. **Results** The result of Western blot test showed that Cav-1 presented high-expression in the esophageal carcinoma TE13 cells, and the Cav-1 expression level after transfecting siRNA decreased significantly compared with pre-transfection. The grey value of transfection group and no - transfection group were 1.98 ± 0.34 and 3.15 ± 6.10, respectively, and there was significant difference between two groups ($t = 4.26$, $P < 0.05$). Scratch test showed that in the no-transfection group, the distance between scratch marks got obvious narrow 24 hours after scratching, and the cells were crowded together in partial scratch area, while there was no obvious narrow in the distance between scratch marks 24 hours after scratching in transfection group. These results showed that the migration ability of cells after transfection decreased significantly compared with pre-transfection. **Conclusions** Cav-1 plays a role of cancer-promoting gene in carcinogenesis and progress of esophageal cancer, and the progress of esophageal cancer might be inhibited by suppressing Cav-1 expression.

Key words: Esophagus cancer; Caveolin -1; Cell migration; Cell transfection; Scratch test; Western blot test

本课题组前期研究显示，Rho 激酶(ROCK)通路抑制剂 Y-27632 能间接调节小窝蛋白(caveolin, Cav-)1 的表达水平从而影响食管癌细胞的侵袭转移[1-2]。为了进一步明确 Cav-1 与食管癌发生、发展之间的关系，我们采取转染干扰的方法使食管癌细胞低表达 Cav-1，从而更为直接地了解 Cav-1 与食管癌之间的关系。

1 材料与方法

1.1 材料 人食管癌细胞系 TE13(由本院实验室提供)，RPMI 1640 培养液、胎牛血清(杭州四季青公司)，二甲基亚砜(DMSO)(美国 MPBIO 公司)，Cav-1 多克隆抗体(CST)，胰蛋白酶(碧云天生物技术有限公司)。

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1.2 方法

1.2.1 细胞转染 转染组：食管癌细胞 TE13 用含 10% 胎牛血清(FBS)的 DMEM 高糖培养基在 37℃、5% CO_2、饱湿条件下培养，细胞呈贴壁生长。选择指数生长期的食管癌细胞接种于培养皿中 18h，细胞融合度约为 70% 时进行转染。在 500 μl Opti-MEM 培养基中加入 10 μl(200 pmol)小干扰 RNA(siRNA)并轻柔混匀；在 500 μl Opti-MEM 培养基中加入 10 μl lipofectamin 试剂，混匀后室温放置 5min；稀释好的 siRNA 和 RNAi-Mate 试剂混合，室温放置 20 min 后将混合物加到培养皿中。细胞置于 37℃、5% CO_2 培养箱中培养 5 h 后进行下一步研究。以未转染 Cav-1 siRNA的食管癌细胞 TE13 为未转染组。

1.2.2 划痕实验 将 TE13 细胞以 1×10^5/ml 的密度接种于 6 孔培养板中，并设对照组；置于 37℃、5% CO_2 的培养箱内培养过夜，待细胞贴壁生长铺满皿底后，按照转染步骤进行转染，培养 5h 后进行划痕，继续用无血清培养基培养 24 h，观察细胞的迁移情况，并拍照。

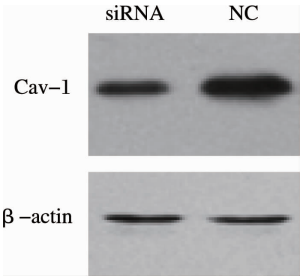
1.2.3 蛋白免疫印迹(Western blot)实验 将对数生长期的 TE13 细胞传代并置于 37℃、5% CO_2 的培养箱内培养，待细胞长至皿底的 80% ~90% 时进行转染处理，培养 5 h 后收集细胞，预冷 PBS 洗涤细胞 3 次，加细胞裂解液，超声破碎细胞，二喹啉甲酸(BCA)法测定蛋白浓度。十二烷基硫酸钠 - 聚丙烯酰胺凝胶(SDS-PAGE)电泳分离细胞总蛋白，将蛋白通过电转移印迹到 PVDF 膜上，5% 脱脂奶粉/TBST 液封闭 PVDF 膜；蛋白上样量分别为 30 μg，Cav-1 工作浓度为 1∶500，β-actin 工作液浓度为 1∶1 000，二抗工作浓度为 1∶10 000，ECL 试剂发光，X 胶片曝光，显影，定影，条带扫描灰度分析。

1.3 统计学处理 采用 SPSS 19.0 软件处理数据。计量资料以 $\bar{x} \pm s$ 表示，采用成组 t 检验进行统计分析。$P < 0.05$ 表示差异有统计学意义。

2 结 果

2.1 Western blot 检测结果 食管癌 TE13 细胞高表达 Cav-1，转染 siRNA 后细胞 Cav-1 表达水平较转染前明显下降，转染组和未转染组细胞的灰度值分别为 1.98 ±0.34 和 3.15 ±6.10，差异有统计学意义(t = 4.26，P <0.05)。见图 1。

2.2 划痕试验结果 划痕后 24 h 转染细胞的迁移情况较转染前明显下降。未转染组在划痕后 24 h 痕间距明显缩窄，部分痕区域细胞密集；而转染组细胞在划痕后 24 h 痕间距未见明显缩窄。见图 2。



注：siRNA：转染组；NC：未转染组。

图 1 转染 siRNA 后食管癌细胞 Cav-1 表达下降

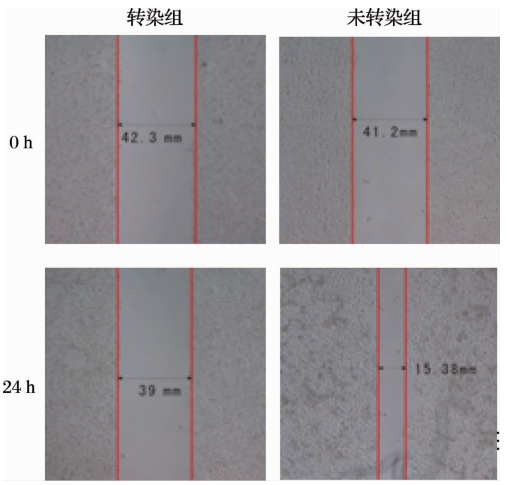


图 2 划痕试验检测细胞株 TE13 的迁移能力(×40)

3 讨 论

食管癌是消化系统常见的恶性肿瘤，在世界范围内，存在从土耳其东部开始，经伊拉克、伊朗、中亚，一直延伸到中国北部太行山南侧地区的食管癌高发地带[3-5]。我国一般地区食管癌发病率：男性高于女性，男性发病率为 27.54/10 万，女性发病率为 14.05/10 万。虽然国内外学者一直致力于提高食管癌临床疗效的研究，包括寻找各种肿瘤标志物提高早期诊断率，探索食管癌转移机制阻断早期转移，研讨致癌位点进行靶向治疗等，而且近年来食管癌的诊治水平也在不断提高，然而患者的预后仍不容乐观[4-8]。

Cav-1 是由 178 个氨基酸组成的整合膜蛋白，研究发现它可绑定多种信号分子如 Src 家族酪氨酸激酶、生长因子受体、内皮一氧化氮合酶、G 蛋白和 G 蛋白偶联受体等，而产生一系列生物学效应，从而参与细胞增殖、分化、凋亡、血管生成及肿瘤的发生、发展[8-13]。

近年来，Cav-1 在肿瘤中的表达水平及其扮演的角色已在多种肿瘤标本中被广泛研究。在胰腺癌[14]、膀胱癌[15]、胃癌[16]中，Cav-1 的表达水平和肿瘤发生淋巴结转移、脉管侵袭、根治术后肿瘤的局部复发率呈正相关。而在食管癌中，无论是在食管癌患

者标本或食管癌细胞中,关于 Cav-1 的研究较少。本研究组前期研究结果显示 Cav-1 可能参与食管癌的发生发展[1-2]。为了进一步证实这一假设,本实验通过对高表达 Cav-1 的食管癌细胞株 TE13 进行 RNA 干扰,结果显示经过转染 Cav-1 siRNA 后细胞的 Cav-1 表达水平明显下降;同时通过划痕实验发现,转染后细胞的迁移能力明显下降,这说明 Cav-1 可能在食管癌的发生、发展中起着促癌基因的作用。有关 Cav-1 磷酸化与食管癌的关系有待我们进一步深入研究。

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(上接第 984 页)

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