中国普通外科杂志 2013, 22(3) 294-299 DOI: 10.7659/j.issn.1005-6947.2013.03.007 ISSN: 1005-6947 CN: 43-1213R

基础研究

14-3-3&sigma|对胰腺癌PANC-1 细胞侵袭能力的影响

江建新1|刘勇1|高珊2|孙诚谊1

（贵阳医学院附属医院 1. 肝胆外科 2. 消化内科|贵州 贵阳 550001）

摘要：

目的：探讨14-3-3σ过表达对胰腺癌PANC-1细胞侵袭能力的影响。方法：以人基因组为模板，通过PCR扩增出14-3-3σ基因的编码序列，定向插入真核表达载体pEGFP-N1中，构建重组质粒pEGFP-14-3-3σ。经酶切和测序验证后，首先将pEGFP-14-3-3σ质粒转染HEK293T细胞观察转染效率；然后用脂质体法稳定转染PANC-1细胞，并以转染空载体及未转染的PANC-1细胞分别作为阴性对照和空白对照，用实时荧光定量PCR和Western blot法分别检测目的基因mRNA和蛋白表达情况，Transwell法检测细胞侵袭能力。结果：酶切和序列测定证实14-3-3σ基因正确插入pEGFP-N1载体中，pEGFP-14-3-3σ对HEK293T细胞的转染效率达65%。PANC-1细胞转染pEGFP-14-3-3σ后，14-3-3σ mRNA与蛋白表达水平均明显增高；Transwell侵袭实验结果显示，转染pEGFP-14-3-3σ的PANC-1细胞穿膜数较转转染空载体及未转染的PANC-1细胞明显增多（129.4±19.6 vs. 76.4±17.7，78.7±16.7）（均P<0.05）。结论：14-3-3σ基因过表达能增强胰腺癌PANC-1细胞的侵袭能力。

关键词： 胰腺肿瘤/病理学；14-3-3蛋白质类；肿瘤侵润

14-3-3σ overexpression enhances invasive ability of pancreatic cancer PANC-1 cells

JIANG Jianxin1, LIU Yong1, GAO Shan2, SUN Chengyi1

(1. Department of Hepatobiliary Surgery 2. Department of Gastroenterology, the Affiliated Hospital, Guiyang Medical College, Guiyang 550001, China)

Abstract:

Objective: To study the effect of 14-3-3σ gene overexpression on the invasive ability of pancreatic cancer PANC-1 cells. Methods: The coding sequence of 14-3-3σ gene was amplified by PCR using human genomic cDNA as a template and inserted into the eukaryotic expression vector pEGFP-N1 to construct the recombinant pEGFP-14-3-3σ plasmid. After identification by restriction endonuclease digestion and nucleotide sequencing, the constructed plasmids were firstly transfected into the HEK293T cells to assess the transfection efficiency, following which they were transfected into the pancreatic cancer PANC-1 cells mediated by liposome and subsequently, the mRNA and protein expression of the target gene in the PANC-1 cells and invasive ability of these cells were detected by real time fluorescence quantitative PCR, Western blot analysis and Transwell invasion assay, respectively. The PANC-1 cells transfected with empty plasmid or without transfection were used as negative and blank control, respectively. Results: Restriction enzyme digestion and DNA sequencing demonstrated that 14-3-3σ gene was correctly inserted into the pEGFP-N1 vector, and the transfection efficiency of pEGFP-14-3-3σ for HEK293T cells reached 65%. Both the 14-3-3σ mRNA and protein expression were increased significantly in the PANC-1 cells after transfection with pEGFP-14-3-3σ. The results of Transwell invasion assay showed the number of the pEGFP-14-3-3σ transfected PANC-1 cells that invaded through the membrane was significantly higher than that of the PANC-1 cells transfected with empty plasmid or without transfection (129.4±19.6 vs 76.4±17.7, 78.7±16.7) (both P<0.05). Conclusion: Overexpression of 14-3-3σ gene can enhance the invasive ability of pancreatic cancer PANC-1 cells.

Keywords: Pancreatic Neoplasms/pathol 14-3-3 Proteins Neoplasm Invasiveness

收稿日期 2012-09-19 修回日期 2013-02-05 网络版发布日期 2013-03-15

DOI: 10.7659/j.issn.1005-6947.2013.03.007

: