[1]党微旗,唐浩,曹红,等.可调控STAT3干扰载体抑制BIU-87细胞侵袭的体外研究[J].第三军医大学学报,2013,35(05):400-403.
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可调控STAT3干扰载体抑制BIU-87细胞侵袭的体外

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Title: Effect of CRE-dependent RNA interference targeting STAT3 on invasion and migration in human bladder cancer BIU-87 cells

作者: 党微旗; 唐浩; 曹红; 王林; 陈婷梅
重庆医科大学医学检验系临床检验诊断学省部共建教育部重点实验室

Author(s): Dang Weiqi; Tang Hao; Cao Hong; Wang Lin; Chen Tingmei
Key Laboratory of Laboratory Medical Diagnostics of Ministry of Education, College of Laboratory Medicine, Chongqing Medical University, Chongqing, 400016, China

关键词: STAT3; RNA干扰; BIU-87细胞; CRE; 侵袭

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摘要: 目的 探讨可调控RNA干扰载体通过抑制STAT3信号通路对膀胱癌细胞BIU-87侵袭性的影响。 方法 采用受CRE重组酶调控的RNA干扰载体pSico构建针对STAT3的shRNA表达载体,以pLVX-CRE作为CRE蛋白的表达载体,将BIU-87细胞分为pSico-shNeg、pSico-shNeg/CRE、pSico-shSTAT3、pSico-shSTAT3/CRE 4组,分别转染相应质粒,RT-PCR和Western blot法检测其干扰效率,Transwell实验检测其侵袭能力。 结果 双酶切及测序证实载体构建正确;各组细胞在转染重组质粒后EGFP的表达受CRE调控;RT-PCR结果显示STAT3的表达在干扰之后有显著降低。Western blot结果显示,在无CRE时,pSico-shSTAT3组与 pSico-shNeg组STAT3的表达无显著差异($P>0.05$),在有CRE时,pSico-shSTAT3组STAT3相对表达量下降为 pSico-shNeg组的(43±4.2)%($P<0.05$);体外侵袭实验显示pSico-shNeg组透膜细胞数为(203.33±12.42)/视野、pSico-shNeg/CRE组(196.33±11.85)/视野、pSico-shSTAT3 组(201.00±16.64)/视野,而pSico-shSTAT3 /CRE 组(42.00±3.00)/视野,较其他3组显著降低($P<0.01$)。 结论 由可调控RNA干扰载体介导的CRE依赖性STAT3表达载体能降低细胞内STAT3信号水平,并降低BIU-87细胞体外侵袭迁移能力。

Abstract: Objective To investigate the effect of CRE-dependent RNA interference targeting STAT3 on the invasion and migration of human bladder cancer BIU-87 cells. Methods RNA interfering vectors pSico was used to construct CRE-dependent shRNA expression plasmids targeting STAT3, and pLVX-CRE was used as an expression vector of CRE. BIU-87 cells were divided into 4 groups and were transfected with pSico-shNeg, pSico-shNeg/CRE, pSico-shSTAT3 and pSico-

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shSTAT3/CRE, respectively. RT-PCR and Western blot analysis were carried out to assess the efficiency of RNA interference. The abilities of invasion and migration of BIU-87 cells after CRE-dependent RNA interference of STAT3 were detected by Transwell chamber assay and wound-healing assay. Results Restriction analysis and DNA sequencing proved that the recombinant plasmid pSico-shSTAT3 was constructed successfully. CRE-dependent green fluorescent cells were detected after the transfection. The shRNA against STAT3 significantly inhibited STAT3 mRNA expression, and CRE and shSTAT3 transfection down-regulated the expression levels of STAT3 in BIU87 cells significantly (P<0.05). The number of migrating cells were significantly less in the pSico-shSTAT3 /CRE group (42.00±3.00) than in the pSico-shNeg (203.33±12.42), pSico-shNeg/CRE (196.33±11.85) and pSico-shSTAT3 (201.00±16.64) groups (P<0.01). Conclusion RNA interference of STAT3 mediated by CRE-dependent shRNA expression plasmid can down-regulate intracellular STAT3 signal level and reduce the abilities of invasion and migration of BIU-87 cells.

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