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# rAAV-Slug-siRNA载体的构建及其抗胰腺 癌的实验

崔海宁，余壮明，于飞，顾冠宏

517010海口，海南医学院附属医院普外科

# Construction and Identification of Recombinant Adeno-associated Virus-2 Expression Vector Mediated Slug-siRNA and Its Effect on Growth of Pancreatic Cancer

CUI Hai-ning,YU Zhuang-ming,YU Fei,GU Guan-hong

Department of General Surgery,The Affiliated Hospital of Hainan Medical College，Haikou 517010, China

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摘要 目的构建并制备携带目的基因的rAAV-EGFP-Slug-siRNA（Slug-siRNA）病毒载体，探讨Slug干扰抗胰腺癌作用。方法首先构建pDC316-EGFP-Slug-siRNA质粒,以PCR鉴定正确的pDC316-EGFP-Slug-siRNA克隆为模板扩增EGFP-Slug-siRNA片段,EcoRⅠ和SalⅠ双酶切回收PCR产物; 酶切pSNAV2.0-lacz-α载体质粒,并与上述产物进行连接；转化感受态大肠杆菌DH5α，得到重组质粒pSNAV2.0-EGFP-Slug-siRNA,酶切和测序对其进行鉴定。建立载体细胞株BHK/Slug-siRNA, 大规模制备rAAV2-EGFP-Slug-siRAN并对其纯化、鉴定和滴度测定。将体外培养的胰腺癌细胞株AsPC-1转染Slug-siRNA和pSNAV2.0-EGFP空载体。Western blot及RT-PCR方法鉴定转染前后AsPC-1细胞内PUMA及Slug蛋白及mRNA表达，MTT比色法检测细胞存活率，TUNEL检查细胞凋亡。结果携带编码靶向Slug的小发夹状干扰RNA序列的腺相关病毒载体质粒pSNAV2.0-EGFP-Slug-siRNA，经PCR、酶切和测序鉴定，质粒构建正确。将构建成功的载体质粒与重组Ⅰ型单纯疱疹病毒HSV1-rc/ΔUL2共转染包装细胞BHK-2，成功复制和包装出重组腺相关病毒Slug-siRNA，经检测目的片段插入成功，滴度为9.23×1010(puf)。Slug-siRNA有效抑制AsPC-1细胞内Slug表达，抑制体外细胞增殖，促进细胞凋亡，同时，伴随着Slug表达的下降，细胞内PUMA表达明显增加。结论成功制备出高滴度携带目的基因的Slug-siRNA病毒载体。干扰Slug表达抑制胰腺癌细胞增殖，促进细胞凋亡，其机制可能通过解除Slug对PUMA基因的抑制有关。

关键词： 关键词:Slug 小干扰 腺相关腺病毒 胰腺癌 凋亡

Abstract： ObjectiveTo conduct a recombinant adeno-associated virus-2 carrying Slug-siRNA (rAAV2-Slug-siRNA),and to study the effect and mechanism of Slug-siRNA on AsPC-1 cells. MethodsThe targeting sequence of Slug gene which can be effectively silenced in RNA inference was confirmed in our previous study. The DNA containing both sense and antisense Oligo DNA fragments of the targeting sequence was designed, synthesized and cloned into the pDC316-EGFP vector. The insert was confirmed by PCR and sequencing. EGFP-Slug-siRNA sequence was amplificated and PCR product was obtained by EcoRⅠand SalⅠ digestion. pSNAV2.0-lacz-α plasmids was digested,and ligated with EGFP-Slug-siRNA. Transformation of DH5α with recombinant plasmids and identification of bacterial colonies containing recombinant plasmids by LB-agar plate were conducted，and pSNAV2.0-EGFP-Slug-siRNA were extracted purified and yerified. pSNAV2.0-EGFP-Slug-siRNA was transfected into BHK cells by means of lipofectamine. Using G418 Selection from mixed cells，BHK-Slug-siRNA was isolated，which was capable of Slug-siRNA expression,and was subsequenily infected with recombinant herpes simplex virusl(HSV1-rc/ΔUL2),which was able to pack the rAAV2-Slug-siRNA to form a functional and infectiou virus. After purifiation，the packaged rAAV2-Slug-siRNA was obtained.Western blot and RT-PCR methods was used to detect the expression of slug and PUMA after being infected AsPC-1 cells by Slug-siRNA for 48h,MTT and Annexin V/EGF methods was

used to detect hibition rate and apoptosis of AsPC-1 cells.ResultsThe recombinant adeno-assoeiated virus-2 vector carring Slug-siRNA was constructed successfully, using RT-PCR and restriction anzyme digestion，the destination gene was dectected. BHK-Slug-siRNA was was subsequenily infected with recombinant herpes simplex virusl(HSV1-rc/ΔUL2) able to pack the rAAV2-Slug-siRNA to form a functional and infectiou virus.The recombine viral titer was 9.23×1010(puf).Slug-siRNA inhibited the expression of Slug, at the same time, the proliferation of AsPC-1 cells was suppressed and the apoptosis was increased,significantly. Otherwise, with the decrease of Slug,the expression of PUMAwas increased. ConclusionThe constructed，high titer，recombinant adeno-associated virus-2 vector carring Slug-siRNA was constructed successfully.RNAi-mediated Slug gene with rAAV silencing could transfect to pancreatic cancer cells and silence Slug effectively and selectively, which resulted in the growth and proliferation inhibition in pancreatic cancer cells. The mechanism of Slug silencing in the growth and proliferation inhibition may be through upregulating expression of PUMA.

Key words： Key words：Slug gene　Small intertering RNA　Recombinant adeno-associated virus　Pancreatic cancer　Apoptosis

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没有本文参考文献

[1] 郑浩;汤志刚. 5-Aza-dC对胰腺癌细胞系Panc-1中TFPI-2基因甲基化水平及表达的影响 [J]. 肿瘤防治研究, 2012, 39(2): 150-153.

[2] 牛国晓;李洁. 半枝莲抗肿瘤机制研究进展[J]. 肿瘤防治研究, 2012, 39(2): 231-233.

[3] 刘瑶;贺兴波;谢军;孟凡;杨建琼;黄才斌 . 5-氮杂-2′-脱氧胞苷对肝癌细胞HepG2凋亡及其PEG10基因表达的影响[J]. 肿瘤防治研究, 2012, 39(1): 9-12.

[4] 刘磊玉;赵彬佳惠;秦玮;陈媛媛;林锋;邹海峰;于晓光 . 转染PDCD5基因促进顺铂诱导前列腺癌细胞的凋亡作用[J]. 肿瘤防治研究, 2012, 39(1): 32-35.

[5] 孙建建;李胜棉;赵松;李光辉;王小玲 . Survivin和Caspase-3在胰腺癌组织中的表达及与预后的关系[J]. 肿瘤防治研究, 2012, 39(1): 62-67.

[6] 丁军利;夏钰弘;刘超英;许隽颖. M2型肿瘤相关巨噬细胞在胰腺癌中的表达及其临床意义[J]. 肿瘤防治研究, 2012, 39(1): 59-61.

[7] 周防震;张晓元;孙奋勇;郭勇 . 二氢杨梅素对人乳腺癌细胞MDA-MB-231的体外抗增殖作用[J]. 肿瘤防治研究, 2012, 39(1): 95-97.

[8] 方珏敏综述;王理伟审校 . 一氧化氮在胰腺癌发生发展中的作用[J]. 肿瘤防治研究, 2012, 39(1): 110-112.