中国医学科学院学报
ACTA ACADEMIAE MEDICINAE SINICAE
Medline收录 核心期刊
ISSN 1000-503X CN 11-2237/R

中国医学科学院学报 » 2011, Vol. 33 » Issue (6) :632-637 DOI: 10.3881/j.issn.1000-503X.2011.06.010

蛋白激酶Nemo样激酶基因重组腺病毒载体的构建与鉴定

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Construction and Identification of Nemo-like Kinase Gene Recombinant Adenovirus Vector

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摘要 目的构建含有蛋白激酶Nemo样激酶（NLK）的重组腺病毒载体。 **方法** 采用RT-PCR方法在人胚肾细胞系HEK293T细胞中扩增NLK基因及其突变体K155M、T286V和C425Y，同时在目的基因的C端添加便于蛋白表达检测的FLAG tag，经与载体pAdTrack-CMV连接、转化、筛选、鉴定后，将Western blot检测表达正确的重组过渡质粒pAdTrack-CMV-NLK及其突变体再经酶切、电转入BJ5183感受态细胞（已包含腺病毒骨架质粒pAdEasy-1）同源重组，并筛选、鉴定、扩增。通过*PacI*酶切后，以快速简便的乙醇沉淀法替代传统的酚-氯仿-异戊醇法回收酶切产物，用高效低毒高稳定性的FuGENE HD转染试剂替代传统的lipofectamin 2000转染低代数的HEK293A包装细胞，进行病毒包装。包装后的重组腺病毒感染结直肠癌细胞HCT-116，Western blot检测以确认NLK及其突变体蛋白的表达。 **结果** 经PCR、测序及Western blot检测证实，成功构建了携带NLK基因及其突变体的腺病毒载体，PCR及限制性内切酶酶切鉴定重组过渡质粒pAdTrack-CMV-NLK及其突变体与腺病毒骨架质粒pAdEasy-1同源重组成功，重组表达克隆转染HEK293A细胞获得病毒原液，扩增后感染结直肠癌细胞HCT-116能正确表达NLK蛋白及其突变体蛋白。 **结论** 成功制备NLK基因及其突变体重组腺病毒，可进一步用于NLK生理功能的研究。

关键词： 蛋白激酶Nemo样激酶 重组腺病毒载体 同源重组

Abstract: Objective To construct the nemo-like kinase (NLK) gene recombinant adenovirus vector. Methods The AdEasy system was used to construct the recombinant adenovirus vector. Using reverse transcriptase polymerase chain reaction (RT-PCR), the full-length gene of NLK and its mutants (K155M,T286V, and C425Y) were amplified from HEK293 cells. The FLAG tag was appended at the C-terminal of NLK. After ligation and transformation, the NLK gene and its mutants were cloned into the pAdTrack-CMV vector. It was detected by PCR, sequencing, and Western blot analysis. Using DNA recombination and homogenous recombinantion, the normally expressed plasmids were linearized by the restriction enzyme-*PmeI* and *PacI*, then the enzyme-digested products were recycled by using ethanol precipitation. The purified product was transfected to HEK293A packaging cells with FuGENE HD transfection reagent. After amplification of the recombinant adenovirus, Western blot analysis was performed to detect the expression of NLK gene and its mutants. Results The successful construction of pAdtrack-CMV-NLK (and mutants) was confirmed by PCR and sequencing. Western blot analysis showed that the target genes and the recombinant adenovirus were obtained. This recombinant virus was able to express NLK protein and its mutants correctly in HCT 116 cells. Conclusion The NLK gene recombinant adenovirus vector was successfully constructed and identified.

Keywords: nemo-like kinase adenovirus vector homogenous recombination

Received 2011-10-18;

Fund:

国家重大研究计划项目(2011CB944302)、国家重点实验室专项基金(2060204)和北京协和医学院青年科研基金(2010004)

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引用本文:

程筱雯,梁俊波,缪时英,王琳芳.蛋白激酶Nemo样激酶基因重组腺病毒载体的构建与鉴定[J] 中国医学科学院学报, 2011,V33(6): 632-637

CHENG Xiao-wen, LIANG Jun-bo, MIAO Shi-ying, WANG Lin-fang.Construction and Identification of Nemo-like Kinase Gene Recombinant Adenovirus Vector[J] CAMS, 2011,V33(6): 632-637

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http://www.actacams.com/Jwk_yxkxy/CN/10.3881/j.issn.1000-503X.2011.06.010 或