[1]刘雪莉,宋敬东,郭小娟,等.重组腺病毒Ad5F11pTPEGFP的构建及其对UT-7/Epo细胞感染效率的检测[J].第三军医大学学报,2012,34(09):862-865.

Liu Xueli,Song Jingdong,Guo Xiaojuan,et al.Construction of a recombinant adenovirus Ad5F11p-TPEGFp and evaluation of its transfection efficiency to UT-7/Epo cells [J].Journal of Third Military Medical University,2012,34(09):862-865.

点击复制

重组腺病毒Ad5F11pTPEGFP的构建及其对UT-7/Epo细胞感染效率的

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 34 期数: 2012年第09期 页码: 862-865 栏目: 论著 出版日期: 2012-05-15

Title: Construction of a recombinant adenovirus Ad5F11p-TPEGFp and evaluation of its transfection efficiency to UT-7/Epo cells

作者: 刘雪莉; 宋敬东; 郭小娟; 王敏; 曾茁壮; 洪涛
中国疾病预防控制中心病毒病所

Author(s): Liu Xueli; Song Jingdong; Guo Xiaojuan; Wang Min; Zeng Zhuozhuang; Hong Tao
Institute of Viral Disease, Chinese Centre for Disease Control and Prevention, Beijing, 100052, China

关键词: 重组腺病毒; 端粒酶; 纤维顶球; UT-7/Epo细胞

Keywords: recombinant adenovirus; telomerase; fiber; UT-7/Epo cells

分类号: R394-33；R394.3；R733.7

DOI: -

文献标识码: A

摘要: 目的 构建携带报告基因绿色荧光蛋白（GFP）的重组腺病毒Ad5F11pTPEGFP，并且研究该病毒对人白血病细胞UT-7/Epo的感染效率及其在细胞中的复制。 方法 先通过分子克隆技术构建重组腺病毒质粒pAd5F11pTPEGFP，然后利用脂质体转染法，将其转染至HEK293细胞包装出重组腺病毒Ad5F11pTPEGFP，经酶切和PCR鉴定正确后，扩增并纯化得到滴度较高的重组腺病毒。以空载体病毒Ad5GFP作为对照，将纯化的重组腺病毒感染UT-7/Epo细胞，经流式细胞仪检测在不同感染复数（MOI）下荧光阳性细胞比例，即为Ad5F11pTPEGFP病毒对UT-7/Epo细胞的感染效率，同时将冻融后的重组腺病毒感染的UT-7/Epo细胞上清感染HEK293细胞，48 h后观察GFP在HEK293细胞中的表达。 结果 成功制备了重组腺病毒Ad5F11pTPEGFP，其对UT-7/Epo细胞的感染效率明显高于空载体对照病毒Ad5GFP，在MOI为200时，感染效率达98.2%，而空载体病毒在MOI为200时，对UT-7/Epo细胞的感染效率仅为29.7%。感染了重组腺病毒的UT-7/Epo细胞冻融上清感染新的HEK293细胞后，荧光显微镜下观察到GFP的表达。 结论 成功构建了重组腺病毒Ad5F11pTPEGFP，其对UT-7/Epo细胞有较高的感染效率，并能在其中复制。

Abstract: Objective To construct a recombinant adenovirus Ad5F11p encoding promoter of telomerase reverse transcriptase (TPEGFp) and evaluate its efficiency in the transfection of UT-7/Epo cells. Methods A novel plasmid pAd5F11p-TPEGFp was constructed by molecular cloning. The recombinant adenovirus Ad5F11p-TPEGFp was packaged in HEK293 cells after transfection. The infectious titer of the identified recombinant adenovirus was determined by limiting dilution assay on HEK293 cells after purified by double CsCl density gradient ultracentrifugation. The recombinant adenovirus Ad5F11p-TPEGFp encoding green fluorescence protein (GFP) gene was transferred into human leukemic cell lines UT-7/Epo cells. The gene transduction efficiency was determined by fluorescence-activated cell sorting assay by flow cytometry. The lysates of UT-7/Epo cells which infected with the recombinant adenovirus were prepared by three cycles of freeze and thaw and used to infect fresh HEK293 cells, and the expression of GFP was observed in 48 h after infection with flow cytometry. Results The recombinant adenovirus was successfully prepared and the infectious titer of the virus was 2×10^{10} IU/ml. The recombinant adenovirus was significantly more effective than control Ad5GFp in the infection of UT-7/Epo cells. At 200 MOI, Ad5F11p-TPEGFp transduced about 98.2% UT-7/Epo cells, while Ad5GFp only transduced less than 30% at 200 MOI. GFP was observed in UT-7/Epo cells when infected with Ad5F11p-TPEGFp, and GFP was also seen in the fresh HEK293 cells which infected by lysate of UT-7/Epo cells. Conclusion The recombinant adenovirus pAd5F11p-TPEGFp is successfully constructed which can infect UT-7/Epo cells effectively and replicate in UT-7/Epo cells.

导航/NAVIGATE
本期目录/Table of Contents
下一篇/Next Article
上一篇/Previous Article

工具/TOOLS
引用本文的文章/References
下载 PDF/Download PDF(670KB)
立即打印本文/Print Now
推荐给朋友/Recommend
查看/发表评论/Comments

统计/STATISTICS
摘要浏览/Viewed 42
全文下载/Downloads 22
评论/Comments

RSS XML

参考文献/REFERENCES

刘雪莉, 宋敬东, 郭小娟, 等. 重组腺病毒Ad5F11pTPEGFP的构建及其对UT-7/Epo细胞感染效率的检测[J].第三军医大学学报,2012,34(9):862-865.

备注/Memo: -

更新日期/Last Update: 2012-05-07