[1]刘晓丽,杨海捷,苏勇,等.E2-2基因腺病毒载体的构建及其对EPCs生长、增殖及ID1表达的影响[J].第三军医大学学报,2014,36(16):1664-1669.
Liu Xiaoli,Yang Haijie,Su Yong,et al.Construction of adenovirus vector of E2-2 and its effects on expression of ID1, and growth and proliferation in endothelial progenitor cells[J].J Third Mil Med Univ,2014,36(16):1664-1669.

点击复制

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(1639KB)
立即打印本文/Print Now
查看/发表评论/Comments
导出

E2-2基因腺病毒载体的构建及其对EPCs生长、增殖

分享到:

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 36 期数: 2014年第16期 页码: 1664-1669 栏目: 论著 出版日期: 2014-08-30

Title: Construction of adenovirus vector of E2-2 and its effects on expression of ID1, and growth and proliferation in endothelial progenitor cells

作者: 刘晓丽; 杨海捷; 苏勇; 谭志胜; 朱琴; 王红
成都军区昆明总医院干部病房

Author(s): Liu Xiaoli; Yang Haijie; Su Yong; Tan Zhisheng; Zhu Qin; Wang Hong
Cadres' Wards, Kunming General Hospital of Chengdu Military Command, Kunming, Yunnan Province, 650032, China

关键词: EPCs; E2-2基因; ID1基因; 腺病毒

Keywords: endothelial progenitor cells; E2-2 gene; inhibitor of DNA binding/differentiation gene; adenoviral vector

分类号: R322.12;R329.28;R394.2

文献标志码: A

摘要: 目的 构建转录因子E2-2基因腺病毒载体，观测内皮祖细胞（endothelial progenitor cells, EPCs）过表达E2-2基因对DNA结合抑制因子-1（inhibitor of DNA binding/differentiation, ID1）表达的影响。 方法 分离、培养并鉴定小鼠骨髓EPCs。RT-PCR法扩增E2-2基因CDs全长DNA，克隆入载体pTG19-T后，亚克隆入腺病毒穿梭载体pAdTrack-CMV中，构建pAdTrack/E2-2重组载体,与pAdEasy-1骨架质粒同源重组形成重组病毒pAd/E2-2，经293细胞包装，获具高效感染力的重组pAd/E2-2病毒。将该病毒感染EPCs，倒置显微镜观测经感染的EPCs的GFP表达情况。CCK-8（cell count kit-8)法检测病毒pAd/E2-2对EPCs生长、增殖的影响。RT-PCR、Western blot分别检测经感染的EPCs中E2-2与ID1基因及其编码蛋白的表达情况，并予以定量分析。 结果 分离、培养并鉴定到小鼠骨髓EPCs。克隆到2013 bp的E2-2基因，并获得高效感染力的重组pAd/E2-2病毒。CCK-8法检测表明，与对照比较，过表达E2-2的EPCs的生长、增殖速度减慢，48 h开始变得尤为明显（$P<0.01$）；RT-PCR、Western blot及定量分析结果显示， E2-2能下调ID1的表达，与对照比较，差异具统计学意义（$P<0.01$）。 结论 分离、培养并鉴定小鼠骨髓EPCs，克隆出E2-2基因，证实E2-2

更新日期/Last Update: 2014-08-28

能明显抑制EPCs的生长、增殖，并能下调ID1基因的表达。

Abstract: Objective To construct an adenovirus vector of transcription factor E2-2 gene and investigate whether the adenovirus vector carrying E2-2 gene affects the expression of inhibitor of DNA binding/differentiation (ID1) in endothelial progenitor cells (EPCs). Methods Murine EPCs of bone marrow were isolated, cultured and identified. cDNA fragment encoding murine E2-2 gene was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). The PCR amplified fragment was first cloned into pTG19-T vector, and then sub-cloned into the shuttle vector pAdTrack-CMV in order to construct the recombinant plasmid pAdTrack/E2-2, which homologously recombined with the adenoviral backbone vectors Adeasy-1 to generate recombinant adenoviral plasmid Ad/E2-2. The recombinant adenoviruses Ad/E2-2 were generated by transfecting the recombinant adenoviral DNA into 293 cells, and then employed to infect EPCs. The expression of enhanced green fluorescent protein (EGFP) in the transfected EPCs were observed by invert microscopy. The growth and proliferation of transfected EPCs were tested *via* Cell Count Kit-8 (CCK-8), and the expression of E2-2 and ID1 in transfected EPCs were examined by RT-PCR and Western blotting respectively, and the expression of ID1 was semi-quantitated. Results Murine EPCs of bone marrow were isolated, cultured and identified. Murine E2-2 gene was successfully cloned. Adenoviral virus particle which possesses infectious competent was produced from Ad/E2-2. The results of CCK-8 demonstrated that the growth and proliferation of EPCs, which over-expressed E2-2, were slower than the control cells, especially from 48 h after transfection (P<0.01). The results of RT-PCR, Western blotting and quantitative analysis revealed that E2-2 down-regulated the expression of ID1 in EPCs when compared with control cells (P<0.01). Conclusion The recombinant adenoviral vector of E2-2 is constructed successfully. The vector containing E2-2 gene prevents the EPCs from growth and proliferation and down-regulates the expression of ID1 in the cells.

参考文献/References:

刘晓丽，杨海捷，苏勇，等. E2-2基因腺病毒载体的构建及其对EPCs生长、增殖及ID1表达的影响[J].第三军医大学学报,2014,36(16):1664-1669.