山东大学学报(医学版) 2010, 48(11) 37-40 DOI: ISSN: 1671-7554 CN: 37-1390/R

论文

小鼠Tim-3真核表达载体pTARGET-Tim-3的构建

孟静1，刘晓立2，郭春3，马春红3

山东大学 1.第二医院检验科， 济南 250033； 2.第二医院肾移植科， 济南 250033；
3.医学院免疫学研究所， 济南 250012

摘要：

目的 构建小鼠Tim-3真核表达载体,并在黑色素瘤细胞系B16中表达小鼠TIM-3。方法 以脾细胞RNA为模板，逆转录PCR扩增鼠Tim-3编码区基因,T-A克隆至真核表达载体pTARGET,构建重组质粒pTARGET-Tim-3,采用脂质体转染法转染黑色素瘤细胞系B16细胞,以逆转录PCR和Western blot验证Tim-3在B16细胞中的表达。结果 利用酶切和测序的方法，筛选、鉴定pTARGET-Tim-3真核表达载体,转染黑色素瘤细胞系B16细胞,经逆转录PCR和Western blot证实TIM-3高效表达。结论 成功构建小鼠Tim-3真核表达载体, Tim-3在转染的小鼠B16细胞系中高表达。

关键词： 基因；克隆，分子；DNA，重组；真核表达；小鼠

Construction of the mouse T cell immunoglobulin mucin 3(Tim-3) eukaryotic expression vector

MENG Jing1， LIU Xiao-li2， GUO Chun3， MA Chun-hong3

1. Clinical Laboratory； 2. Department of Kidney Transplantation, The Second Hospital of Shandong University, Jinan 250033, China;
3. Institute of Immunology, School of Medicine, Shandong University, Jinan 250012, China

Abstract:

Objective To construct an eukaryotic expression vector of murine T cell immunoglobulin mucin 3(Tim-3), and express Tim-3 in the melanoma cell line B16. Methods The murine Tim-3 coding region was amplified by reverse transcription PCR （RT-PCR）, using RNA from spleen cells as a template. The Tim-3 cDNA was cloned into the eukaryotic expression vector pTARGET by T-A cloning. The recombinant vector was transfected into the melanoma cell line B16 by Lipofectamine. Tim-3 expression was analyzed by RT-PCR and Western blot. Results The murine Tim-3 expression vector was identified by enzyme digestion and sequencing. RT-PCR and Western blot results showed specific Tim-3 expression in B16 cells transfected with the recombinant vector. Conclusion The Tim-3 coding region was successfully cloned into the eukaryotic expression vector and highly expressed in the melanoma cell line B16.

Keywords: Genes; Cloning， molecular； DNA， recombinant； Eukaryotic expression; Mice

收稿日期 2010-07-09 修回日期 网络版发布日期

DOI:

基金项目:

山东省科技厅攻关计划资助项目（2007GG30002028）;山东省卫生厅青年基金资助项目（2007QZ011）。

通讯作者: 马春红(1970-)，女，博导，主要从事肿瘤免疫研究。E-mail:machunhong@sdu.edu.cn

作者简介: 孟静(1973-),女，硕士，主管技师，主要从事分子免疫和体液免疫的研究。 E-mail:mengjing189@126.com
作者Email:

参考文献：

本刊中的类似文章