中国寄生虫学与寄生虫病杂志 2007 25 (4): 279-284 ISSN: 1000-7423 CN: 31-1248/R

论著

犬贾第虫病毒转染载体介导的锤头状核酶对KRR1基因体外转录体切割效果的研究

陈丽凤1, 2,李建华1 ,邹亚学2,赵月平1,曹利利1,张西臣1

1 吉林大学畜牧兽医学院, 长春 130062; 2 河北科技师范学院, 秦皇岛 066000

收稿日期 修回日期 网络版发布日期 接受日期

摘要

【摘要】 目的 检测犬贾第虫病毒介导的锤头状核酶对犬贾第虫滋养体核仁功能性蛋白KRR1基因体外转录体切割效率。 方法 将两端携带反义KRR1基因的锤头状核酶(ribozyme, R酶)插入犬贾第虫病毒（Giardia canis virus, GCV）转染载体中, 构建两个核酶嵌合型犬贾第虫病毒载体： 短反义序列, 上游及下游均为21个碱基, 形成重组质粒KRzS； 长反义序列, 上游为288个碱基, 下游为507个碱基, 形成重组质粒KRzL。 另设两种阴性对照, 即重组质粒TRzL(即R酶两端含虫体反义磷酸丙糖异构酶基因, 上游为324个碱基, 下游为380个碱基)和重组质粒PKR(即犬贾第虫病毒转染载体中仅有KRR1 基因的反义片段而无R酶功能区)。应用荧光实时定量RT?鄄PCR法检测嵌合型锤头状核酶体外对KRR1 基因mRNA切割活性。 结果 KRzS、 KRzL转录体对KRR1 基因体外转录RNA切割效率分别达到74.0%和81.1%。而PKR对KRR1 mRNA反转录的影响较小, RT-PCR效率仅降低12.0%。TRzL对KRR1 mRNA反转录几乎无影响。 结论 犬贾第虫病毒介导的锤头状核酶对KRR1基因体外转录体能进行有效切割, 为以后的体内锤头状核酶对KRR1基因表达抑制试验提供依据。

关键词 犬贾第虫病毒 转染载体 锤头状核酶 KRR1蛋白

分类号

The Cleavage Activity of GCV Transfer Vector-mediated Hammerhead Ribozyme for KRR1 *in vitro* Transcript

CHEN Li-feng1, 2,LI Jian-hua1 , ZOU Ya-xue2,ZHAO Yue-ping1, CAO Li-li1,ZHANG Xi-chen1

1 College of Animal Science and Veterinary Medicine, JiLin University, Changchun 130062, China； 2 Hebei Normal University of Science & Technology, Qinhuangdao 066000, China

Abstract

【Abstract】 Objective To detect the cleavage activity of *Giardia canis* virus (GCV) transfer vector-mediated hammerhead ribozyme for KRR1 *in vitro* transcript. Methods *Giardia*, a most primitive eukaryote, has KRR1 protein responsible for ribosome biosythesis. cDNA encoding hammerhead ribozyme flanked with various lengths of antisense RNA was cloned into a viral vector pGCV634/GFP/GCV2174 derived from the genome of GCV, KRzS flanked with 21 nt KRR1 antisense RNA on each arm, or KRzL flanked with 288 nt and 507 nt KRR1 antisense RNA. At the same time, two control groups were established: PKR without the inserted ribozyme, and TRzL flanked with 324 nt and 380 nt triosephosphate isomerase(Tim) antisense RNA. The cleavage activity of GCV transfer vector-mediated hammerhead ribozyme for KRR1 *in vitro* transcript was then analyzed by absolute real-time quantitative RT-PCR. Results The *in vitro* cleavage activities on KRR1 mRNA of the two ribozyme KRzS or KRzL were 74.0% and 81.1% respectively by the absolute real-time quantitative RT-PCR. The two control groups, PKR or TRzL, showed no effect on KRR1 mRNA *in vitro*. Conclusion The GCV transfer vector-mediated hammerhead ribozyme shows a high cleavage activity for KRR1 *in vitro* transcript, which demonstrates the feasibility of using a viral vector to express a ribozyme targeted at a specific mRNA in *Giardia* to reduce the expression of a specific gene.

Key words *Giardia canis* virus Transfer vector Hammerhead ribozyme KRR1 protein

DOI:

通讯作者 李建华 jianhuali7207@163.com

作者个人主页 陈丽凤1; 2;李建华1 ;邹亚学2;赵月平1;曹利利1;张西臣1