



蓝氏贾第鞭毛虫alpha-8贾第素特异性锤头状核酶-GCV重组载体的构建

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Construction of GCV-Specific Hammerhead Ribozyme Recombinant Vector of Alpha-8 Giardin in *Giardia lamblia*

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摘要

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摘要 目的 构建蓝氏贾第鞭毛虫alpha-8贾第素(α -8 giardin)特异性锤头状核酶-GCV重组载体。方法 采用RNA draw软件对蓝氏贾第鞭毛虫 α -8贾第素基因序列(GenBank登录号为AY781323)的二级结构进行模拟分析,按照G:C比例和锤头状核酶设计原则,选取合适的核酶切割靶点,设计特异性锤头状核酶(H8)序列,并将其与犬贾第虫病毒(GCV)连接,获得 α -8贾第素特异性锤头状核酶-GCV重组载体(pGCV634/H8/1423)。将载体线性化体外转录产物电击转染至贾第虫滋养体细胞内。提取转染后24 h的各组虫体总RNA,并以其为模板采用RT-PCR验证转染效果及对靶mRNA的切割效果。结果 成功设计、合成了蓝氏贾第鞭毛虫 α -8贾第素mRNA锤头状核酶序列(H8),将其与犬贾第虫病毒载体(GCV)连接,成功构建了pGCV634/H8/1423;RT-PCR实验结果表明,重组载体pGCV634/H8/1423转染贾第虫细胞后24 h可检测到核酶RNA的存在,并实现了对 α -8贾第素mRNA高效、特异的切割作用。结论 构建的pGCV634/H8/1423能有效转染至贾第虫细胞内,并在其细胞内对 α -8贾第素基因的mRNA具有高效、特异的切割作用。

关键词: 蓝氏贾第鞭毛虫; 贾第素; α -8贾第素; 锤头状核酶; 犬贾第虫病毒

Abstract: Objective To construct a GCV-ribozyme recombinant vectors of α -8 giardin in *Giardia lamblia*. Methods The secondary structure of α -8 giardin mRNA (GenBank Accession No. AY781323) was analyzed with the RNA draw software. According to the proportion of G:C and principles of designing hammerhead ribozyme, suitable ribozyme cleavage points were chosen. A specific antisense-hammerhead ribozyme (H8) was designed and synthesized. The ribozyme was cloned into *Giardia canis* virus (GCV) vector to construct a recombinant viral vector-pGCV634/H8/1423. The vector was linearized and transcribed into the trophozoites of *G. lamblia* by electroporation method. The α -8 giardin mRNA level of the transfectants and normal trophozoites were analyzed 24 h after electroporation by RT-PCR. Results The recombinant vector of GCV-specific hammerhead ribozyme of α -8 giardin in *Giardia lamblia* (pGCV634/H8/1423) was constructed. RT-PCR assays showed the ribozyme (H8) mRNA can be detected 24 h after transfection and α -8 giardin mRNA was cleaved effectively by ribozyme (H8) intracellularly. Conclusion pGCV634/H8/1423 can transfect *Giardia* trophozoites and cleave mRNA of α -8 giardin intracellularly.

Keywords: *Giardia lamblia*; Giardin; α -8 giardin; Hammerhead ribozyme; *Giardia canis* virus (GCV)

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