

实验研究

杜氏利什曼原虫四川分离株无鞭毛体蛋白基因的克隆及真核表达

李金福,陈建平,田玉,杨志伟,马莹,胡孝素

四川大学华西基础医学与法医学院寄生虫学教研室, 成都 610041

收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的 克隆、真核表达杜氏利什曼原虫四川株的无鞭毛体蛋白(amastin)编码基因。方法 PCR扩增杜氏利什曼原虫四川汶川人株L.d.SC10H2与四川南坪犬株L.d.SC7的无鞭毛体蛋白基因,将该基因导入pcDNA3.1(+),构建真核表达重组质粒,转染NIH3T3细胞,采用免疫荧光法鉴定重组质粒的瞬时表达;RT-PCR和Western blotting鉴定稳定表达。结果 2株杜氏利什曼原虫均扩增出552 bp的无鞭毛体蛋白基因,同源率为86%。转染后在NIH3T3细胞膜和细胞内观察到较强的绿色荧光,表明无鞭毛体蛋白基因在NIH3T3细胞中获得短暂表达。细胞裂解产物经Western blotting,在相对分子量(Mr)约20 000处检测到阳性杂交信号,表明无鞭毛体蛋白基因在NIH3T3细胞内获得了稳定表达。结论 获得了我国杜氏利什曼原虫四川分离株L.d.SC10H2和L.d.SC7的无鞭毛体蛋白基因序列,并在NIH3T3细胞中稳定表达。

关键词 [杜氏利什曼原虫](#) [无鞭毛体蛋白](#) [基因克隆](#) [表达](#)

分类号

Cloning of Amastin Gene of *Leishmania donovani* Isolates from Sichuan Province and its Expression in Eucaryotic System

LI Jin-fu, CHEN Jian-ping, TIAN Yu, YANG Zhi-wei, MA Ying, HU Xiao-su

Department of Parasitology, School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, China

Abstract

Objective To clone and express the amastin gene of two *Leishmania donovani* isolates from Sichuan Province of China. Methods Amastin gene was amplified from nuclear DNA of two *L. donovani* isolates, cloned into pcDNA3.1(+), and sequenced by the dideoxy chain termination. NIH3T3 cell was transfected by recombinant plasmid. Transient expression of amastin gene was detected by immunofluorescence and stable expression was detected by RT-PCR and Western blot. Results Amastin gene of both isolates was 552 bp. Sequence analysis showed that the similarity was 86% between the two isolates. A high green fluorescence was found on the cell membrane and inside the cell. The NIH3T3 cell was transfected by the recombinant plasmid successfully. Amastin gene was obtained by RT-PCR from the transfected NIH3T3 cells. Western blot analysis showed that there was a protein about Mr 20 000 in lysate of the transfected NIH3T3 cells, indicating that the amastin gene was expressed in the cells. Conclusion Amastin gene of the two *L. donovani* isolates has been cloned and the gene can be expressed stably in the NIH3T3 cell.

Key words [Leishmania donovani](#) [Amastin](#) [Gene cloning](#) [Expression](#)

DOI:

通讯作者 陈建平 jpchen007@163.com

作者个人主页 李金福;陈建平;田玉;杨志伟;马莹;胡孝素

扩展功能

本文信息

- ▶ [Supporting info](#)
- ▶ [PDF \(328KB\)](#)
- ▶ [\[HTML全文\]\(OKB\)](#)
- ▶ [参考文献\[PDF\]](#)
- ▶ [参考文献](#)

服务与反馈

- ▶ [把本文推荐给朋友](#)
- ▶ [加入我的书架](#)
- ▶ [加入引用管理器](#)
- ▶ [复制索引](#)
- ▶ [Email Alert](#)
- ▶ [文章反馈](#)
- ▶ [浏览反馈信息](#)

相关信息

- ▶ [本刊中 包含“杜氏利什曼原虫”的相关文章](#)
- ▶ 本文作者相关文章

- [李金福](#)
- [陈建平](#)
- [田玉](#)
- [杨志伟](#)
- [马莹](#)
- [胡孝素](#)