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论著

约氏疟原虫孢子增殖特异18S核糖体DNA部分序列及其检测应用

徐晓春,瞿逢伊,宋关鸿

第二军医大学病原生物学教研室!上海200433

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摘要

目的 测定约氏疟原虫 By2 6 5株 S型 18S r DNA序列 ,并用于蚊体内疟原虫的分子检测。 方法 根据伯氏疟原虫 S型 18S r DNA序列 ,合成一对保守区引物 ,从感染约氏疟原虫 7d后的斯氏按蚊中肠组织逆转录 -聚合酶链反应 (RT- PCR)扩增约氏疟原虫 S型 18S r RNA片段并测序 ,据此序列合成约氏疟原虫 S型 18S r D-NA种型特异引物 ,与保守的逆转录引物配伍 ,对感染后 1～ 7d蚊体内约氏疟原虫 S型 18S r RNA表达量进行RT- PCR检测。 结果 扩增的约氏疟原虫 S型 18S r DNA片段长度为 920 bp,与伯氏疟原虫 S型和约氏疟原虫A型的同源性分别为 95 .3%和 94.0 %。 RT- PCR检测结果显示卵囊密度与扩增带强度明显一致 ,且敏感性高于解剖镜检方法。感染后 3d即可检出蚊体内疟原虫 ,而此时卵囊在光镜下尚难识别。 结论 测定了约氏疟原虫S型 18S r DNA的部分序列 ,将其作为分子指标 ,可早期、敏感、特异和半定量检测蚊体内感染的疟原虫。

关键词 约氏疟原虫 18S核糖体DNA 检测 逆转录-聚合酶链反应

分类号

Partial Sequence of Sporogony Stage-specific 18S Ribosomal DNA of Plasmodium yoelii and Its Application for Detection of Parasites

XU Xiao-chun,QU Feng-yi,SONG Guan-hong

Department of Etiologic Biology;Second Military Medical University;Shanghai;200433

Abstract

Objective To determine sequence of sporogony stage-specific (S type) 18S ribosomal RNA gene of Plasmodium yoelii (P.y) By265 strain, and by using it to detect the malaria parasites within vector mosquito. Methods A pair of conserved DNA primers, universe primer (Pu) and reverse transcription one (Pr), was designed and synthesized according to sequence of the 18S rRNA gene of Plasmodium berghei (P.b). The segment of the S type 18S rDNA of P.y was amplified by reverse transcript-polymerase chain reaction (RT-PCR) from dissected midguts of Anopheles stephensi infected with P.y on the 7th day after infective blood-meal, and its sequence was then determined. One P.y sporogony stage-specific primer (Pys) was selected according to the sequence. Using this primer and Pr, the parasites within mosquitoes were semi-quantitatively detected through RT-PCR between 1-7 d post-infection. Results The length of the amplified segment was 920 bp. Alignment in match region of the 18S rDNA among S type of P.y (PyS), S type of P.b (PbS) and asexual blood stage-specific one of P.y (PyA) revealed that the similarity between the former and the latter two reached 95.3% and 94.0% respectively. The density of amplified band was significantly concordance with the intensity of oocyst in the midgut. Sensitivity of RT-PCR method was higher than that of the traditional dissection and oocyst observation also. The assay could detect the 18S rRNA molecule of the parasites on the third day post-infection while their oocysts were difficult to be recognized under an optical microscope at that time. Conclusion This S type 18S rDNA sequence in P.y species was first reported (AF266261). As a molecular marker, it could be applied to monitoring the parasite development in its vector at an earlier stage semi-quantitatively with an adequate sensitivity and specificity.

Key words Plasmodium yoelii 18S ribosomal DNA detection reverse transcript-polymerase chain reaction

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通讯作者

作者个人主页 徐晓春;瞿逢伊;宋关鸿