

论著

恶性疟原虫红内期不同发育阶段PfRON4基因转录水平分析

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摘要

目的 分析恶性疟原虫棒状体颈部蛋白4基因(PfRON4)在红内期不同发育阶段的转录水平。方法 用山梨醇结合等渗细胞分离液(Percoll)对实验室体外培养的恶性疟原虫进行均一化处理, 收集间隔为6 h不同发育阶段的疟原虫, 提取RNA。根据PfRON4基因及相关基因(PfAMA1和PfRhopH2)的序列设计特异性引物, 构建标准质粒并制作标准曲线, 对PfRON4及相关基因的mRNA进行定量检测分析。结果 纯化并同步后的疟原虫生长发育较为同步均一, 用于定量分析的标准曲线相关性较好, PfRON4、PfAMA1和PfRhopH2的相关系数(*r*值)分别为-1.00、-0.98和-0.98。产物溶解曲线分析结果均显示为单一波峰。定量分析结果显示, 在恶性疟原虫红内期发育过程中, PfRON4基因的转录水平在裂殖子入侵红细胞后36~40 h(即成熟裂殖体阶段)达到高峰。结论 恶性疟原虫PfRON4基因在成熟裂殖体阶段高表达。

关键词 [恶性疟原虫](#); [PfRON4](#); [实时定量PCR](#); [转录](#)

分类号

Transcription Profile of PfRON4 Gene in *Plasmodium falciparum* Erythrocytic Stage

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Abstract

Objective To reveal the transcription profile of PfRON4 gene in *Plasmodium falciparum* erythrocytic stage. Methods *P. falciparum* schizonts were purified by differential centrifugation on a Percoll-sorbitol gradient, after which the released merozoites were allowed to invade uninfected erythrocytes for 4 hours before the clearance of all remaining schizonts using 5% D-sorbitol. The cultured synchronous parasites were harvested for RNA assay immediately, 24 hours later, and then at every 6th hour. PfRON4 and related genes (PfAMA1 and PfRhopH2) were amplified by real-time PCR for establishing standard curves to evaluate the copy number of genes. Results *P. falciparum* parasites were well synchronized. Those quantitative analyses were reliable because the R value of standard curves were more than 0.98 and the melting curve showed a single peak. When parasites were in the schizont stage, PfRON4 gene transcription reached a peak in 36-40 hours after invasion. Conclusion The transcription of PfRON4 peaks at mature schizont stage, suggesting that the PfRON4 gene may involve in erythrocyte-invasion of *P. falciparum*.

Key words [Plasmodium falciparum](#); [PfRON4](#); [Real-time PCR](#); [Transcription](#)

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