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论著

中国脑型疟患者恶性疟原虫分离株裂殖子表面蛋白MSP1 第16-17 区基因和MSP2 基因的分子克隆与鉴定

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摘要

目的: 为设计研制安全有效的人脑型疟疫苗提供理论依据。方法: 根据MAD20 株裂殖子表面蛋白1 (MSP1) 和FC27 株裂殖子表面蛋白2 (MSP2) 基因编码区高度保守碱基设计并合成两对引物, 应用多聚酶链反应(PCR) 技术对5 例脑型疟患者恶性疟原虫云南省勐腊县勐罕分离株CMH/YN 和云南省盈江县农场CYJ/YN 分离株基因组DNA MSP1 第13- 17 区基因和MSP2 基因进行扩增, 并将扩增产物分别经EcoRI 和KpnI, BamHI 和HindIII 双酶切后, 分子定向克隆M13mp18 和M13mp19 载体, 转染大肠杆菌(*E. coli*) TG1, 从含X-gal 和IPTG 的LB平板上, 将随机筛选得到的单个无色噬菌斑经*E. coli* JM 103 扩增, 用碱裂解法抽提重组子复制型DNA (RFDNA) 后, 再分别经EcoRI 和KpnI, BamHI 和HindIII 双酶切鉴定。结果: 证实重组子为编码脑型疟患者恶性疟原虫CMH/YN 和CYJ/YN 分离株MSP1 第16- 17 区基因和MSP2 基因分子克隆M13 载体。结论: 首次报道确证脑型疟患者恶性疟原虫CMH/YN 和CYJ/YN 分离株MSP1 第16- 17 区基因和MSP2 基因分别与MAD20 株MSP1 和FC27 株MSP2 相应基因完全一致。这些发现对研究预防人脑型疟疫苗和建立一种新型脑型疟恶性疟原虫检测方法具有重要意义。

关键词 脑型疟患者 恶性疟原虫 裂殖子表面蛋白1 裂殖子表面蛋白2 克隆 疫苗

分类号

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MOLECULAR CLONING AND IDENTIFICATION OF GENES ENCODING MSP2 AND REGIONS 16- 17 IN MSP1 FROM TWO ISOLATES OF *PLASMODIUM FALCIPARUM* FROM CHINESE PATIENTS WITH CEREBRAL MALARIA

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Abstract

AIM: To provide a theoretical basis for designing safe and effective vaccines of human cerebral malaria. METHODS: Genomic DNA samples of two P. falciparum isolates were prepared directly from 5 cases of cerebral malaria patients'blood in Mengla County, Yunnan Province(CMH/YN) and in Yingjiang County, Yunnan Province (CYJ/YN). The samples were used for polymerase chain reaction (PCR) amplification and the two pairs of oligonucleotides for the highly conserved genes encoding of FC27 merozoite surface protein 2 (MSP2) and the regions 12- 17 in MAD20 merozoite surface protein 1 (MSP1) of Papua New Guinea strain of *P. falciparum* were used as primers. The PCR products were digested with EcoRI and KpnI, BamHI and HindIII, respectively, and the generated fragments were cloned into M13mp18 and M13mp19 vectors and transfected into *Escherichia coli* (*E. coli*) TG1. A single colorless plaque on the LB agar plate containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and IPTG (isopropylthio-β-D-galactoside) was randomly picked and transformed into *E. coli* JM103. The replicative form (RF) DNA (RFDNA) of M13 recombinant DNA extracted from *E. coli* by the method of alkali lysis were digested with EcoRI and KpnI, BamHI and Hind III, respectively, and the generated fragments were identical with inserted foreign DNA 0.918 kb and 0.8 kb designed by ourselves. RESULTS: It is proved that M13 recombinant DNA consists of

M13 vectors with an insert of genes encoding MSP2 and the regions 16 - 17 in MSP1 from two isolates CMH/YN and CYJ/YN of *P. falciparum* from Chinese patients with cerebral malaria at its corresponding site. CONCLUSION: The results demonstrate for the first time that both isolates CMH/YN and CYJ/YN of *P. falciparum* from Chinese patients with cerebral malaria examined contain genes identical to those defined in known MAD20 MSP1 and FC27 MSP2 allelic dimorphic family. These findings provide valuable strategies both for the development of vaccines to prevent human cerebral malaria and for the establishment of a specific detection method of *P.falciparum* from patients with cerebral malaria.
Key words Cerebral malaria patient *Plasmodium falciparum* MSP1 MSP2 clone vaccine

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