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区基因的分子克隆及序列分析

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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进一步科学依据。方法:应用多聚酶链反应(PCR)技术对中国5 例脑型疟患者恶性疟原虫云南省勐腊县勐罕CMH/YN 分离株和云南省盈江县农场CYJ/YN 分离株基因组DNA 裂殖子表面蛋白1 (MSP1) 第13—17 区基因进行扩增,将扩增产物分别经EcoRI 和KpnI 双酶切后,回收的MSP1 第16—17 区基因分子定向克隆M13mp18 和M13mp19 载体,按Sanger 双脱氧链终止法进行DNA 序列测定,并与MAD20、K1 和Wellcome 株原型基因进行同源性分析比较。结果:发现脑型疟患者恶性疟原虫CMH/YN 和CYJ/YN 分离株MSP1 第16—17 区基因之间的序列完全相同,全长为918 bp,编码306 个氨基酸,含12个半胱氨酸组成的2 个表皮生长因子(EGF) 单体结构域;除了在核苷酸第4 869 位缺失1个碱基和散在分布5个碱基点突变之外,与MAD20、K1 和Wellcome 株相应基因之间的核苷酸同源性分别为98.6%、23.3%和22.8%。结论:本研究在世界上首次报道脑型疟患者恶性疟原虫分离株MSP1 第16—17 区DNA 序列测定分析结果,确证该基因与MAD20 株高度同源性,并发现在1 691—1 701位氨基酸存在TCTEEDSGSSR表位。

关键词 <u>脑型疟患者</u> <u>恶性疟原虫</u> <u>裂殖子表面蛋白1</u> <u>分子克隆</u> <u>序列分析</u> <u>疫苗</u> 分类号

MOLECULAR CLONING AND SEQUENCE ANALYSIS OF GENES ENCODING REGIONS16—17 IN MSP1 FROM TWO ISOLATES OF PLASMODIUM FALCIPARUM FROM CHINESE PATIENTS WITH CEREBRAL MALARIA

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AIM: To further provide scientific evidence for designing safe and effective vaccines of human cerebral malaria. METHODS: Genomic DNA samples of two P. falciparum isolates 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) were used for polymerase chain reaction (PCR) amplification and the two pairs of oligonucleotides for the highly conserved genes encoding 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, respectively, and the generated fragment regions 16—17 were cloned into M13mp18 and M13mp19 vectors and their DNA were analyzed as the templates for DNA sequencing by the dideoxy chain-termination method. RESULTS: Compared with the three other published-MAD20, K1 and Wellcome sequences, DNA sequences of regions 16—17 in MSP1 from two isolates CMH/YN and CYJ/YN of P. falciparum from Chinese patients with cerebral malaria examined contained identical genes which were composed of 918 bp, encoding 306 amino acid, and containing 12 cysteines, which consisted of 2 epidermal growth factor (EGF) —like domains, respectively, and they were highly homologous up to 98.6% with that of MAD20 strain except that it contained an additional single base deletion at positions 4869 nucleotides and 5 point mutations. CONCLUSION: The results demonstrate for the first time that both DNA sequence determined from two isolates CMH/YN and CYJ/YN of P. falciparum from Chinese patients with cerebral malaria belong to the MAD20 allelic dimorphic family, and the deduced amino acids 1691—1 701 have been found to be TCTEEDSGSSR epitopes defined by monoclonal antibodies in P. falciparum.

Key words <u>Cerebral malaria patient</u> <u>Plasmodium falciparum</u> <u>MSP1</u> <u>molecular cloning</u> <u>sequence analysis</u> <u>vaccine</u>

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