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论著

恶性疟原虫AMA-1基因变异区在大肠杆菌中的诱导表达

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摘要

目的 恶性疟原虫 (P.f .) AMA- 1蛋白抗原在大肠杆菌中的表达。 方法 以 FCC1/ HN基因组DNA作为模板 PCR扩增 AMA- 1基因变异区 ,扩增产物以 Bam H 和 H ind 双酶酶切后作为插入片段 ,与具有相同粘性末端的表达质粒 p QE- 40连接 ,并用 DNA自动测序仪测定 AMA- 1DNA片段的序列。取含重组表达质粒的重组菌株以 IPTG进行诱导表达 ,表达产物以 SDS- PAGE电泳和以兔抗 AMA- 1抗血清进行 Western blotting分析鉴定。 结果 FCC1/ HN AMA- 1基因变异区 DNA序列长度为 5 0 6 bp,预计编码 16 8个氨基酸。 Westernblotting分析确认诱导后的 SG130 0 9/ AMA- 1表达产物在分子量约 2 3.0 k Da处出现 1条与兔抗 AMA - 1抗血清特异反应的条带。 结论 FCC1/ HN AMA- 1基因变异区在大肠杆菌中获得表达 ,Western blotting分析表明该蛋白片段含有特异抗原表位

关键词 恶性疟原虫 AMA-1基因变异区 PCR 克隆 大肠杆菌 基因表达

分类号

Induced Expression of the Variable Region of AMA-1 from Plasmodium falciparum

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Abstract

Objective To express the variable region of AMA-1 gene from Plasmodium falciparum in Escherichia coli . \ Methods Genomic DNA of FCC1/HN was used as template and the variable region of AMA-1 gene was amplified by polymerase chain reaction(PCR). The PCR products were digested by endonuclease Bam HⅠ and Hin dⅢ, cloned into pBlu2KSP. The nucleotide sequences of the variable region of AMA-1 gene were determined by sequencing. The AMA-1 gene fragment was subcloned into plasmid pQE, expressed in E.coli and induced by IPTG. The fusion product as identified by SDS-PAGE gel electrophoresis and Western blotting were proceeded with anti-AMA-1 sera from rabbit.\ Results The size of the variable region of AMA-1 gene from FCC1/HN was 506 bp and encoded 168 amino acids. On SDS-PAGE gel dyed with Coomassie brilliant blue R250, no specific protein band can be discerned, but Western blotting proceeded with anti-AMA-1 sera from rabbit demonstrated that the specific protein band was about 23.0 kDa.\ Conclusion The variable region of AMA-1 gene from FCC1/HN was able to be expressed in E.coli and analysis of Western blotting demonstrated that the AMA-1 fussion protein contained specific antigenic epitopes.

Key words Plasmodium falciparum variable region of AMA-1 PCR cloning Escherichia coli gene expression

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