DOI:10.13471/j.cnki.j.sun.yat-sen.univ(med.sci).2001.0114

Construction of Eukaryotic Expression Recombinant Plasmid and Sequence Analysis of p41-3 Gene of *Plasmodium Falciparum* Isolate FCC1/HN

SHAN Zhi-xin, YU Xin-bing, LI Xue-rong, MA Chang-ling, LU Jia-hai, XU Jin

(Department of Parasitology, Sun Yat-sen University of Medical Sciences, Guangzhou 510089, China)

Abstract: 【Objective】 To construct a eukaryotic expression plasmid containing a gene encoding a 41-3 kilodalton blood stage antigen (p41-3) of *Plasmodiu falciparum* isolate FCC1/HN, and to determine the sequence of p41-3 gene and analyze the homology of the sequences of p41-3 gene of different *P. falciparum* isolates. 【Methods】 Two pairs of primers were designed according to the known sequence of p41-3 gene. Using PCR technique, the p41-3 gene was obtained by amplification from genomic DNA of isolate FCC1/HN. By cloning target gene into a eukaryotic expression vector, pcDNA$_3$, a recombinant plasmid pcDNA$_3$-p41-3 was constructed and transferred into *E. coli* DH5α. The positive clones were screened and identified by agarose gel electrophoresis, endonuclease digestion and PCR technique. The correct recombinant plasmid pcDNA$_3$-p41-3 was used as template, and the nucleotide sequence of p41-3 gene was determined by the dideoxy chain termination method. Using softwares to analyze the structure and sequence homology of p41-3 gene between isolate FCC1/HN and FCBR. 【Results】 The p41-3 gene was specifically amplified from genomic DNA of *Plasmodiumm falciparum* isolate FCC1/HN, and the correct recombinant plasmid pcDNA$_3$-p41-3 was screened and identified. The result of sequence determination showed that the p41-3 gene of isolate FCC1/HN was 2 137 base pairs in full length, encoding 375 amino acids. Isolate FCC1/HN and isolate FCBR exhibited 98.98% homology in the nucleiotide sequences and 99.73% homology in the encoded amino acids of p41-3 gene. 【Conclusion】 The eukaryotic expression plasmid pcDNA$_3$-p41-3 is successfully constructed and nucleotide sequence of p41-3 gene of isolate FCC1/HN is determined. The p41-3 genes of isolate FCC1/HN and isolate FCBR share quite high homology.

Key words: *Plasmodium falciparum*; 41-3 kilodalton blood stage antigen; polymerase chain reaction; cloning, molecular; sequence analysis

CLC number: R382.31 **Document code**: A **Article ID**: 1000-257X(2001)05-0329-06

恶性疟原虫红内期 p41-3 基因真核表达重组质粒的构建及序列分析

单志新, 余新炳, 李学荣, 马长玲, 陆家海, 徐 劲

(中山医科大学寄生虫学教研室, 广东 广州 510089)

摘 要: 【目的】 构建恶性疟原虫海南(FCC1/HN)株 p41-3 基因真核表达重组质粒 pcDNA$_3$-p41-3; 测定 p41-3 基因序列, 并了解 FCC1/HN 株与其它分离株 p41-3 序列的差异。【方法】 根据 p41-3 基因已知序列设计合成 2 对引物, 用 PCR 技术从 FCC1/HN 株基因组 DNA 中扩增 p41-3 基因; 将 p41-3 基因定向克隆入真核表达载体 pcDNA$_3$, 转化大肠杆菌 DH5α 感受态细胞; 用酶切, PCR 扩增鉴定筛选到的重组质粒阳性克隆。以正确的重组质粒为模板, 用双脱氧链末端终止法测定 p41-3 基因序列, 应用软件辅助分析 p41-3 序列及进行同源性比较。【结果】 PCR 扩增得到特异的 FCC1/HN 株 p41-3 基因; 正确的 pcDNA$_3$-p41-3 重组质粒被筛选和鉴定。测序表明, FCC1/HN 株 p41-3 基因大小为 2 137 bp, 编码 375 个氨基酸。恶性疟原虫 FCC1/HN 株与 FCBR 株 p41-3 基因核苷酸序列同性为 98.98%, 编码氨基酸序列同源性为 99.73%。【结论】 从恶性疟原虫 FCC1/HN 株基因组 DNA 中获取 p41-3 基因, 成功构建真核表达重组质粒 pcDNA$_3$-p41-3, 并测定了 FCC1/HN 株 p41-3 基因的

Received date: 2001-05-08

Foundation item: Education Commission of China for doctoral-training units(93-186); Guangdong provincial natural science foundation(980089); Sun Yat-sen University of Medical Sciences-foundation(98169)

Biography: SHAN Zhi-xin(1972-), male, born in Heilongjiang province, Master degree, Post-graduate for PhD, working in Research Institute of Cardiology and Angiopathy, Guangdong Provincial People's Hospital.

序列;FCC1/HN 株与其它分离株 p41-3 基因有高度的同源性。

关键词: 疟原虫, 恶性; 红内期 p41-3 抗原; 聚合酶链反应; 克隆, 分子; 序列分析

41-3 kilodalton protein is a soluble antigen exported from the parasite in the blood stage of *Plasmodium falciparum*, and it localizes mainly within the erythrocyte cytoplasm as shown by immunoelectron microscopy[1]. The p41-3 gene has a complex structure consisting of nine exons, and it has been identified that at least three different splice products of the p41-3 precursor mRNA expressed *in vitro* during only one developmental stage, the late blood stage form of *P. falciparum*[1]. The p41-3 gene encodes 375 amino acids in total with a calculated molecular weight of 43 400. Provided that the N-terminal hydrophobic residues function as signal sequence which is cleaved off, the molecular weight of the p41-3 protein decreases to 41 200, as weight as the *P. falciparum* aldolase(p41)[2]. Up to date, the biological function of the p41-3 protein is yet unknown. No significant homology is observed to known protein sequences. The study on the structure and function of the p41-3 gene of chinese isolate *P. falciparum* has not yet been reported. In this study, using PCR and cloning technique, the p41-3 gene of *P. falciparum* isolate FCC1/HN was obtained and cloned into a eukaryotic expression vector, pcDNA$_3$, and a recombinant plasmid pcDNA$_3$-p41-3 was constructed. The nucleotide sequences of the p41-3 gene were determined, and the structure analysis and comparision of the homology of p41-3 genes between two different isolates were performed.

1 MATERIALS AND METHODS

1.1 *Plasmodium falciparum* isolate, bacterial strain and plasmid

P. falciparum isolate FCC1/HN, *E. coli* DH5α and the eukaryotic expression vector, pcDNA3, used in this study are kept in our laboratory.

1.2 Main reagents

*Taq*DNA polymerase, 4dNTP were purchased from TaKaRa (Dalian), *Bam*HⅠ, *Hin*d Ⅲ and λDNA/ *Eco*RⅠ + *Hin*dⅢstandard weight marker, PCR standard weight marker, T_4DNA ligase were purchased from SABC. Proteinase K and agarose were purchased from Sigma. HEPES and RPMI1640 were purchased from Gibcol.

1.3 Polymerase chain reaction primers

Two pairs of primers, representing P1, P2, P3 and P4, respectively, were designed based on the published sequence of p41-3 gene of *P. falciparum* strain FCBR (Columbia)[1]. Having been analyzed by the Pcgene software, the four primers were synthesized by Sangon (Shanghai). The sequence of P1 is 5′-GGC GGA TCC ATG TTG TTA CGA CAT AAT TC-3′, GGATCC is the digestion site of *Bam*HⅠ, GGC is added as protective nucleotides. P2 is 5′-AAC TCT TTC TCC AAG CTT CT-3′. P3 is 5′-ATT GTA CAT AGA AGC TTG GA-3′. P4 is 5′-GTA GAA TTC CTA AAA ATT TTT CAA AGT CT-3′, GAATTC is the digestion site of *Eco*RⅠ, GTA is added as protective nucleotides. P1 and P2 were used to amplify the positions from 1 to 1 606 of the original sequences of the p41-3 gene (p41-$3_{1\text{-}1606}$). P3 and P4 were used to amplified the positions from 1 578 to 2 135 of the original sequences of the p41-3 gene (p41-$3_{1578\text{-}2135}$). Both P2 and P3 have the AAGCTT digestion site of *Hin*d Ⅲ, and the two PCR products of p41-3 gene, p41-$3_{1\text{-}1606}$ and p41-$3_{1578\text{-}2135}$, can be digested by *Hin*d Ⅲ and ligased into a full length p41-3 gene.

1.4 Cultivation of *P. falciparum*

According to the methods described by Trager and Jensen[3], *P. falciparum* isolate FCC1/HN was cultivated and transmitted continously. Under the condition of 37 ℃, 4.5% CO_2, the "O" type human erythrocytes in the RPMI1640 media with φ= 10% serum of new-born calf were used to culvitate *P. falciparum*. The *P. falciparum* were collected when the concentration of parasites reached 5% ~ 10%.

1.5 Genomic DNA extration

P. falciparum genomic DNA of isolate FCC1/

HN was extracted as described previously[4].

1.6 PCR amplification of the p41-3 gene

The genomic DNA of *P. falciparum* isolate FCC1/HN used as template, the p41-3 gene was amplified by conventional PCR protocol. The reactions were carried out in a final volume of 50 μL solution containing 100 ng of genomic DNA, 20 mmol/L Tris-HCl (pH8.4), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 0.2 mmol/L dNTP, 2.5 units of *Taq*DNA polymerase and 0.5 μmol/L of each primer. The reaction mixture was initially heated at 97 ℃ for 5 min, followed by 30 cycles of amplification. The cycling profile of $p41\text{-}3_{1\text{-}1606}$ was: 1 min at 94 ℃, 1 min at 55 ℃ and 2 min at 72 ℃. The cycling profile of $p41\text{-}3_{1578\text{-}2135}$ was: 1 min at 94 ℃, 1 min at 48 ℃ and 2 min at 72 ℃. After 30 cycles of amplification, the reaction mixtures were kept at 72 ℃ for 7 min, then stopped at 4 ℃. the PCR products were analyzed on 12 g/L agarose gels and stained with ethidium bromide. The size of the PCR products were observed and recorded by UVP-GDS8000 Analyzer.

1.7 Construction of recombinant plasmid

$p41\text{-}3_{1\text{-}1606}$ was digested with *Bam*HⅠ and *Hin*dⅢ, $p41\text{-}3_{1578\text{-}2135}$ was digested with *Hin*dⅢ and *Eco*RⅠ, and $pcDNA_3$ was digested with *Bam*HⅠ and *Eco*RⅠ, then the products digested were collected by DEAE membranes (Schleicher & Schuell Company) and ligated with T_4 DNA ligase to construct the eukaryotic expression recombinant plasmid, $pcDNA_3$-p41-3. *E. coli* strain DH5α was then transfered with $pcDNA_3$-p41-3. the plasmid DNA of the positive clones were extracted and screened by agarose gel electrophoresis. Then the possible recombinant plasmids were digested with *Bam*HⅠ, *Eco*RⅠ, *Bam*HⅠ and *Hin*dⅢ, *Hin*dⅢ and *Eco*RⅠ, and amplified by using P1 and P2, P3 and P4 separately. The products above were identified by 10 g/L agarose gel electrophoresis.

1.8 Sequence determination and analysis of p41-3 gene

The recombinant plasmid $pcDNA_3$-p41-3 was used as template, T_7 and Sp6 promoter sequences, at the 5′ and 3′ boundaries of the multiple cloning sites of the $pcDNA_3$ vector, were used as primers separately, and the sequencing reactions were performed using ABI PRISM™ 377XL genetic Analyzer. In order to determine the full length sequence of p41-3 gene, the other two primers were required to be designed and synthesized based on the determined sequences. The sequences of p41-3 gene of isolate FCC1/HN were analyzed by DNAstar software and submitted to GenBank. The Blastn program (http://www.ncbi.nlm.nih.gov/blast) was used to compare the homology of the nucleotide and encoded amino acid sequences of p41-3 gene of the two different isolates, FCC1/HN and FCBR.

2 RESULTS

2.1 Amplification of the p41-3 gene

Using two pairs of primers, two specific p41-3 gene fragments were amplified specifically from the genomic DNA of isolate FCC1/HN. Theoreticaly, the fragment of p41-3 gene amplified with P1 and P2 was 1 606 bp in size, another one amplified with p3 and P4 was 558 bp in size. Agarose gel electrophoresis showed that the size of the amplified DNA fragments were consistent with the theoretical values (Fig.1).

Fig. 1 PCR amplification of p41-3 gene (12 g/L agarose gel electrophoresis)

Lane M: PCR standard marker; Lane 1: Amplified product of $p41\text{-}3_{1\text{-}1606}$; Lane 2: Amplified product of $p41\text{-}3_{1578\text{-}2135}$

2.2 Construction of recombinant plasmid $pcDNA_3$-p41-3

The screened recombinant plasmid $pcDNA_3$-p41-3 was digested with *Bam*HⅠ and *Eco*RⅠ sepa-

rately, and the agarose gel electrophoresis showed that the two products digested were both larger than the pcDNA3 vector. Digestion with *Bam*HⅠ and *Eco*RⅠ together confirmed that a size of 2.13 kb DNA fragment was produced, as the same size as the full length p41-3 gene in theory. The product digested with *Bam*HⅠ and *Hin*dⅢ of pcDNA3-p41-3 was 1.60 kb in size, which was consistent with the theoretical value of p41-3_{1-1606}. Another product digested with *Hin*dⅢ and *Eco*RⅠ of p41-3 gene was 0.56 kb, which was also consistent with the theoretical value of p41-$3_{1578-2135}$. The products amplified from recombinant plasmid pcDNA$_3$-p41-3 by using P1 and P2, P3 and P4 separately, were consistent with the corresponding p41-3 gene fragments amplified from genomic DNA too. Therefore, these results confirmed that the recombinant plasmid pcDNA$_3$-p41-3 was constructed correctly(Fig. 2).

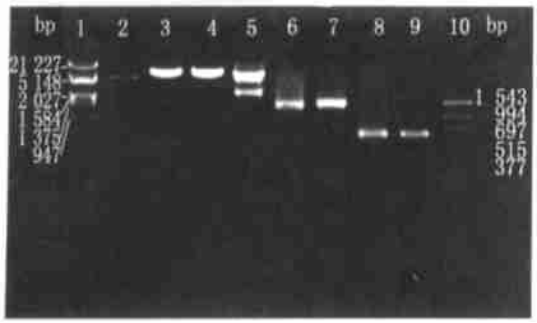

Fig. 2 Restrication analysis and PCR identification of recombinant plasmid pcDNA$_3$-p41-3 (10 g/L agarose gel electrophoresis)

Lane 1: λDNA/ *Eco*RⅠ + *Hin*dⅢ standard marker; Lane 2: pcDNA$_3$ digested by *Bam*HⅠ; Lane 3: pcDNA$_3$-p41-3 digested by *Bam*HⅠ; Lane 4: pcDNA$_3$-p41-3 digested by *Eco*RⅠ; Lane 5: pcDNA$_3$-p41-3 digested by *Bam*HⅠ + *Eco*RⅠ; Lane 6: PCR identification of p41-3_{1-1606} from recombinant plasmid pcDNA$_3$-p41-3; Lane 7: PCR product of p41-3_{1-1606} from *P. falciparum* genome; Lane 8: PCR identification of p41-$3_{1578-2135}$ from recombinant plasmid pcDNA$_3$-p41-3; Lane 9: PCR product of p41-$3_{1578-2135}$ from *P. falciparum* genome; Lane 10: PCR standard marker

2.3 Sequence determination and analysis of p41-3 gene

The nucleotide sequence of p41-3 gene was determined by the dideoxy chain termination method. The schematic representation of sequence determination the of p41-3 gene was shown in Fig. 3. In order to determine the full length of p41-3 gene, another two primers, P_{F1} and P_{F2}, were designed and synthesized based on the determined sequences. The sequence of P_{F1} is 5′-CTC TAA AAA TGA TGA CTG TGT-3′. The sequence of P_{F2} is 5′-GTT CTG TAT TGG TCT ATT TCT-3′. The result of sequence determination showed that p41-3 gene of *P. falciparum* isolate FCC1/HN was 2 137 bp in full length, with 71.54% in A+T and 28.46% in G+C. 375 amino acids were deduced from the coding regions of the p41-3 gene, and the molecular weight of p41-3 protein was about 43.41 kDa. At the *N*-terminal of the p41-3 protein, there was a inferred signal peptide, MLLRHNSFCICIILVLCSVQRCLS (Fig. 4). The p41-3 gene had nine exons, interrupted by eight introns, and the boundary sequences of the introns of p41-3 gene were shown in Table 1. The nucleotide and encoded amino acid sequences of p41-3 gene of *P. falciparum* isolate FCC1/HN have been accepted by GenBank, with the accession number AF220352.

The comparison of sequence homology of p41-3 gene between two different isolates has been analyzed by using softwares. Isolate FCC1/HN and isolate FCBR exhibited 98.98% homology in the nucleotide sequences and 99.73% homology in the encoded amino acids of p41-3 gene. In contrast to isolate FCBR, isolate FCC1/HN contained 22 sites of different nucleotides, 20 sites existing in the introns. The p41-3 genes of isolate FCC1/HN and FCBR encoded 375 amino acids, with only one amino acid different, at the site of amino acid 204, E displaced by D.

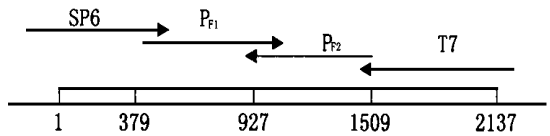

Fig. 3 Schematic representation for determing the sequence of p41-3 gene of *P. falciparum* isolate FCC1/HN

3 DISSCUSSION

The nucleotide and encoded amino acid sequences of p41-3 gene of *P. falciparum* isolate FCBR were

```
1    ATGTTGTTACGACATAATTCCTTCTGCATTTGTATCATTTTGGTGTTGTGCTCTGTTCAA
1    M L L R H N S F C I C I I L V L C S V Q
61   AGATGTTTATCTGATGAAgtacacaaaaaaaaataaaataaaataaaatatgaagagaata
21   R C L S D E
121  aaaataataagaagaaaaaacttcagatacataaaacaattactctcttcatgtatatac
181  cataacagtttaatatacaaatataattctccttcttttccatatttagCAAAATATAA
27                                                   Q N I
241  ATGATTGGCCTATAGACTTTGAATATAATTCTAAGTCTTTACCATCAATAGAAGTTAAAT
30   N D W P I D F E Y N S K S L P S I E V K
301  TAAGTCCTCCAGAAAATgttaaaaaaaaaaaaaaataaaataaaaataaaataaaaaaat
50   L S P P E N
361  aattaagtatctaaaaatgtagaaatatatatcataatatatatttacaaaaaagtaatg
421  taatttaatagaaaaatgtatgttggaatagaacagtgaagaattttatattatattaata
481  tataaatttttttttttttttttttttttttttaaacttagCCGTTACCACAAGTTTCTGC
56                                                   P L P Q V S A
541  AGAAATTAAAATTTTGGAATCTGCAAGACTTAAATTGGAAGAGgtgagaaaaaaatataa
63    E I K I L E S A R L K L L E
601  agattacaaiaatacaaaatgagaaaaattttatggacactatttatttaattcattata
661  tatttatagGGAATGATGCAAAAACTCGAAGATGAATATAATAAATCCTTGTCCATGGCA
77             G M M Q K L E D E Y N K S L S M A
721  AAGTTGAAAATAAAAGATACGGTTGAAAATTCATTAAGCATTTTTAATGACCCAAATATT
94   K L K I K D T V E N S L S I F N D P N I
781  TTAAGTTCTGTTATTTCTAATTCAGTAAAAATTTTAAAAAAAAAGAAAAATTTAAGAAAA
114  L S S V I S N S V K I L K K K K N L R K
841  ATAAAGGAAACCACTGATGAAGAGAAAACTTCAGATAATGTTTCTCAAATGTATGAAAGA
134  I K E T T D E E K T S D N V S Q M Y E R
901  AAAGGTGGACCATTACCACCACCCGAACTTAGAAAACACACATTATTCTTAGAGCAAAAT
154  K G G P L P P P E L R K H T L F L E Q N
961  TATTTAAACAAAACAATTCCTTCTGTAAAAATATCGgtatggaaaatataaaaaaaataa
174  Y L N K T I P S V K I S
1021 aataaaataaaatatttaacttaatatataaatatatgtgttatatacacaatatatttt
1081 aaaatgtgattgaaaatgggaaaacgtacaatttttgaacttctttttttttgaaatac
1141 ttcctcaagTTAACTGAAATAAGTGAACCTAGCGTTTTAATAAAGGAAAAAATTGAAGAA
186           L T E I S E P S V L I K E K I E E
1201 ATAGACCAATACAGAACAGATGAAGAAGTAACTgtaatatttataagaataaaaaaaatc
203  I D Q Y R T D E E V T
1261 gaatataatgttataatatatcgttttaataatatatgaaaaaaccccctttttttttt
1321 ttttttttttttttttttaatttcctagATGTTTGAAACGGCTATATCTGAATTAGATA
214                              M F E T A I S E L D
1381 TATTGACAGATATAACAATGCTAGAATTAGAAAAACAAATGCAACTTCAATTGAATCCAT
224  I L T D I T M L E L E K Q M Q L Q L N P
1441 TTTTAGTTGATAAACAGgtaaataaataaataaatatatatatatatatatatatata
244  F L V D K Q
1501 tatatgtgca*tatatttatataagaacatttacatattttaatcacgtatgttattaca
1561 taatgacattttgatacagATTGTACATAGAAGCTTGGAGAAAGAGTTGAAGGAAATGGG
250                    I V H R S L E K E L K E M G
1621 AAAAGCCGAACAAAGGGAAAATGTGAATgtacattcctatat-tatatttaacaaaaaaag
264   K A E Q R E N V N
1681 agatgagataataatatataaataatatttatttttatttatttatagAAAAGTTCTCA
273                                              K S S Q
1741 AACACAGTCATCATTTTTAGAGCAAGAAGAAAATGAAAATACAGGAAATATCTTAAATGT
277  T Q S S F L E Q E E N E N T G N I L N V
1801 AAAAATAAGCCAAACGGATTATAGgtatttgtttaaaaatagaattataaacttatgtag
297  K I S Q T D Y S
1861 ttaaaaaaaaatatacataatataatattctatttatttatatttttttacttatgatag
1921 TTATCCAACTATAGATGAATTGGTTATGCAAATGCAAAAAAAAAAGGGACATTACGGAAAA
305  Y P T I D E L V M Q M Q K K R D I T E K
1981 ATTAGAAAGACAAAAAATTTTAGAATTACAAATGAAATTATTAAAAGCACAAAGTGAAAT
325  L E R Q K I L E L Q M K L L K A Q S E M
2041 GATAAAGGATGCTCTACATTTTTCTATTTCAAAGGTTATAGCTCAATATTCACCCATAGT
345   I K D A L H F S I S K V I A Q Y S P I V
2101 CGAAACATTAAAATTACAGACTTTGAAAAATTTTTAG  2137
365   E T L K L Q T L K N F *   375
```

Fig. 4 Nucleotide and deduced amino acid sequence of the p41-3 gene of *P. falciparum* isolate FCC1/HN

The nucleotide sequences of the coding regions were shown in capital letters, while the sequences of the noncoding regions indicated in lower case, stop codons were denoted by asterisks. The predicted signal peptide was underlined, and the nine exons E1 to E9 were indicated by boxes. The sequences of p41-3 gene reported here is available in GenBank with the accession number AF220352

firstly reported by Knapp and cooperators[11]. In this study, sequence determination shows that p41-3 gene of isolate FCC1/HN also contains nine exons

Table 1 Boundary sequences of the introns of the p41-3 gene
The consensus splice sites were underlined

Intron	Exon/Intron	Intron length	Intron/Exon
1	GAA/GTACAC	152	CCTTCTTTTTCCATATTTAG/CAA
2	AAT/GTTAAA	203	TTTTTTTTTTTTAAACTTAG/CCG
3	GAG/GTGAGA	86	AATTCATTATATATTTATAG/GGA
4	TCG/GTATGG	153	TTTTGAAATACTTCCTCAAG/TTA
5	ACT/GTAATA	116	TTTTTTTTTTAATTTCCTAG/ATG
6	CAG/GTAAAT	122	ATAATGACATTTTGATACAG/ATT
7	AAT/GTACAT	81	TTATTTTTATTTATTTATAG/AAA
8	TAG/GTATTT	96	ATATTTTTTACTTATGATAG/TTA

interrupted by eight introns. All eight introns start with GT and end with AG, upstream to which pyrimidine residues are enriched. This sequence pattern is in agreement with the splice site consensus sequences found at the 5′ and 3′ boundaries of eukaryotic introns[5]. p41-3 gene of *P. falciparum* isolate FCC1/HN is 2 137 bp in full length, encoding 375 amino acids. The character of the structure of p41-3 gene of isolate FCC1/HN is consistent with what Knapp and cooperators reported previously[11].

The comparison of sequence homology of p41-3 gene between isolate FCC1/HN and FCBR was performed in this study. The homology analysis showed that isolate FCC1/HN and FCBR exhibit quite high homology in the nucleotide and encoded amino acids sequences of p41-3 gene. The positions and components of the exons and introns of p41-3 gene were quite similar between the two isolates, except a few nucleotides different existing in the introns. The comparison showed that the p41-3 gene was quite conserved among different isolates, and p41-3 protein seems to be a potential blood stage vaccine candidate for malaria.

p41-3 protein and aldolase (p41) are two components of 41-kDa protein band of *P. falciparum*, and the p41 protein was shown to be the main component of the 41-kDa protein band[2,6]. However, they are two quite different moleculars. The p41-3 gene contains eight introns[11], while p41 gene has no introns[2]. The amino acids of the two proteins show no homology either (not shown in this paper). In contrast to the p41, the p41-3 protein is expressed at

a low level as demonstrated by the different exposition times necessary for the detection of the p41 or the p41-3 specific mRNAs on northern blots[1]. Initially, a 41-kDa protein band of *P. falciparum* was shown to confer protective immunity to Saimiri monkeys[7]. However, the following research showed that the recombinant p41 protein failed to protect Aotus monkey[8]. It indicates that the protective 41-kDa protein may be the p41-3 protein, not the p41 protein. Because of the biological function of the p41-3 protein is yet unknown, further studies are required to be performed.

In this study, the eukaryotic expression recombinant plasmid pcDNA3-p41-3 was constructed. So the p41-3 gene with introns can be expressed in eukaryotic cells, the product expressed can be similar to the nature protein in structure, physical and chemical character and biological function. And the full length p41-3 gene of *P. falciparum* isolate FCC1/HN was obtained and sequenced. Our work lays a foundation for the eukaryotic expression of p41-3 gene and understanding the function and protective immunology of p41-3 protein.

REFERENCES:

[1] Knapp B, Nau U, Hundt E, *et al*. Demonstration of alternative splicing of a pre-mRNA expresed in the blood stage form of *Plasmodium falciparum* [J]. J Biol Chem, 1991, 266(11): 7148.

[2] Certa U, Ghersa P, Dobeli H, *et al*. Aldolase activity of a *Plasmodium falciparum* protein with protective properties[J]. Science, 1988, 240(4855): 1036.

[3] Trager W, Jensen J B. Human malaria parasites in continous culture[J]. Science, 1976, 193(4254): 673.

[4] Luo S H, Yu X B, Li X R, *et al*. *In vitro* amplification of the encoding sequences of the transmission blocking antigen genes, pfs25 and pfs48/45 of *Plasmodium falciparum* [J]. Chin J Zoonoses, 1998, 14(1): 3.

[5] Mount S M. A catalogue of splice junction sequences[J]. Nucleio Acids Res, 1982, 10(2): 459.

[6] Knapp B, Hundt E, Kupper H A. *Plasmodium falciparum* aldolase: gene structure and location[J]. Mol Biochem Parasitol, 1990, 40(1): 1.

[7] Perrin L H, Merkli B, Gabra M S, *et al*. Immunization with a *Plasmodium falciparum* merozoite surface antigen induces a partial immunity in monkeys[J]. J Clin Invest, 1985, 75(5): 1718.

[8] Herrera S, Herrera M A, Perlaza B, *et al*. Immunization of Aotus monkeys with *Plasmodium falciparum* blood-stage recombinant proteins[J]. Proc Natl Acad Sci USA, 1990, 87(10): 4017.

(编辑 张敏瑞)

·简 讯·

治疗性角膜移植挽救角膜溃疡患者眼球
——中山医科大学中山眼科中心取得重大成果

中山医科大学中山眼科中心陈家祺教授等人自1969年开始对治疗性角膜移植手术的若干重点问题进行了系列研究,历时40年,治疗1万例以上各种原因(感染、外伤、自身免疫性疾病)引起的角膜溃疡,取得重大成果,在国内外权威杂志发表论文近40篇,撰写多部专著,产生显著社会效益。2000年获广东省科学技术进步一等奖。

各种原因引起的角膜溃疡是我国最主要致盲角膜病,每年患者在百万以上。角膜由于其特殊的组织结构(无血管),发生病变以后,药物治疗效果不佳,许多患者因此而发生角膜穿孔,进而引起失明,甚至需摘除眼球。角膜移植手术是治疗此类患者最主要的办法,也是挽救此类患者眼球及复明的唯一措施。但治疗性角膜移植手术的时机选择、方案设计、手术技巧及术后处理因不同病因,不同溃疡大小而不同,直接影响治疗能否成功及效果。研究者就上述重要问题开展了历时40年的研究,通过全身及局部的免疫学观察,在国际上率先提出了蚕蚀性角膜溃疡是以体液免疫为主,细胞免疫参与的自身免疫性疾病新观点;对蚕蚀性角膜溃疡提出完全切除病变结膜及角膜组织,根据病变程度、范围进行半圆形或新月形或指环形或全板层角膜移植,术后局部使用FK506免疫抑制剂的综合治疗措施,使蚕蚀性角膜溃疡的治愈率提高到93.5%,明显高于国际治愈率40%~60%及国内治愈率50%。研究小组还自行设计及制作了分别用于有晶体眼及无晶体眼的人工角膜,用于眼球前、后段联合手术,治疗角膜混浊或角膜穿破致毁坏并同时伴有视网膜脱离患者。此手术开展前此类患者基本无复明的希望,经过大量临床应用证明他们设计制作的人工角膜较目前国外生产的人工角膜视野宽、清晰、手术成功率高。此外,最先在国内开展带巩膜瓣的眼前段重建术,发明叠加缝合术,使术后青光眼发生率明显减少。通过大量病例研究,系统地提出角膜移植手术治疗严重眼化学伤、热烧伤引起的角膜严重毁坏患者及感染性(细菌、真菌、病毒、及棘阿米巴感染)角膜溃疡的手术处理时机、原则、技巧及术后处理。上述已作为治疗性角膜移植的经典理论,在全国各级医院广泛应用并列入教科书内容。

(冯世容)