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Bioinformatics analysis for structure and function of CPR of *Plasmodium falciparum*

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ABSTRACT

Objective: To analyse the structure and function of NADPH-cytochrome p450 reductase (CYPOR or CPR) from *Plasmodium falciparum (Pf)*, and to predict its′ drug target and vaccine target. **Methods:** The structure, function, drug target and vaccine target of CPR from *Plasmodium falciparum* were analyzed and predicted by bioinformatics methods. **Results:** *Pf*CPR, which was older CPR, had close relationship with the CPR from other *Plasmodium* species, but it was distant from its hosts, such as *Homo sapiens* and *Anopheles*. *Pf*CPR was located in the cellular nucleus of *Plasmodium falciparum*. 335aa-352aa and 591aa - 608aa were inserted the interior side of the nuclear membrane, while 151aa-265aa was located in the nucleolus organizer regions. PfCPR had 40 function sites and 44 protein-protein binding sites in amino acid sequence. The teriary structure of 1aa-700aa was forcep-shaped with wings. 15 segments of PfCPR had no homology with *Homo sapien* CPR and most were exposed on the surface of the protein. These segments had 25 protein-protein binding sites. While 13 other segments all possessed function sites. **Conclusions:** The evolution or genesis of *Plasmodium falciparum* is earlier than those of *Homo sapiens*. *Pf*CPR is a possible resistance site of antimalarial drug and may involve immune evasion, which is associated with parasite of sporozoite in hepatocytes. *Pf*CPR is unsuitable as vaccine target, but it has at least 13 ideal drug targets.

1. Introduction

NADPH-cytochrome p450 reductase (CYPOR or CPR) in mammals can transport electron to cytochrome P450 monooxygenase system (CYPs), cytochrome C, other oxidation compounds and enzymes and molecular oxygen. Then it takes part in a series of oxidation-reduction reaction, and the catalysis of reductive metabolism of prodrugs[1], which may produce reactive oxygen species (ROS)[2]. Gene mutation of CPR could cause a series of dysfunctions[3-6]. *Plasmodium berghei (Pb)* NOS and similar proteins have been searched and predicted by bioinformatics. It is also found that *Pb*CPR is a possible resistance site of antimalarial drug, targets of antimalarial drug and vaccine and may involve immune evasion[7]. At present studies on *Plasmodium falciparum (Pf)* CPR are still scanty. This study aims to analyze the structure and function of *Pf*CPR, and predicted its potential drug target and vaccine target by bioinformatics methods.

2. Materials and methods

The amino acids sequences of CPR were obtained from http://www.ncbi.nlm.nih.gov. The molecule evaluation was analyzed by ClustalX2, The physiological-biochemical property, sub-cellular localization of the protein, signal peptide, secondary, topological and 3D structure were predicted based on http://www.expasy.org/tools/ protparam.html, http://psort.nibb.ac.jp/form2.html, http://www.cbs.dtu.dk/services/SignalP/, http://www.predictprotein.org/ and http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO 3D/ geno3d_ home.html, respectively.

3. Results

3.1.Analysis of amino acids sequences and molecular

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evolution

The accession number of amino acid of *Pf*CPR was XP_001348652.1, which included 865aa. Compariing with CPR from other organisms, *Pf*CPR was the more primitive and more close to CPR from other *Plasmodium*, and less related with CPR from the hosts, human and anopheles (Figure 1) .

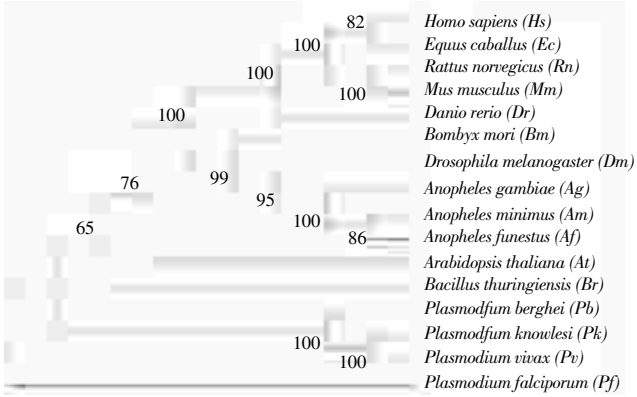

Figure 1. Molecular evolution analysis of *Pb*CPR.

3.2. Physiological and biochemical characters of PfCPR

The predicted isoelectric point (pI) of *Pf*CPR was 8.72 and the molecular weight was 103 074.0 Da. The estimated half-life of protein predicted in mammals, yeast and *Escherichia coli* were 30 h, >20 h, >10 h, respectively, and its instability coefficient was 43.71. Thus *Pf*CPR was predicted as a unstable protein. The total average hydrophobicity was −0.570.

3.3. Subcell localization and structure of PfCPR

PfCPR was localized in cell nucleus, with 335aa-352aa and 591aa-608aa insetting the interior side of the nuclear membrane, and 151aa-265aa locating in the nucleolus organizer regions (NORs). The secondary structure of *Pf*CPR included α-helics (37.69%), β strands (15.61%), loop (46.71%). A total of 52.37% amino acid was exposed on the surface of the protein. *Pf*CPR had two conservative structure domains, FNR_like superfamily and FMN_ red superfamily. 5aa-126aa lied on domain of FMN_red superfamily, and 250aa-433aa and 538aa-706aa lied on domain of FNR_like superfamily. It had 8 N-glycosylation sites, 7 protein kinase C phosphorylation sites, 14 casein kinase Ⅱ phosphorylation sites, 2 tyrosine kinase phosphorylation sites, 6 N-myristoylation sites, 1 vacuole target motif, 2 bileucine motif, and 44 protein-protein binding sites (Figure 2). The structure of 1aa-700aa was forceps-shaped with two wings, and two conserved domains formed a big and deep leak. 151aa-265aa and 434aa-537aa formed wing-liked structure, respectively, and protein-protein binding sites were exposed on the surface of the protein (Figure 3).

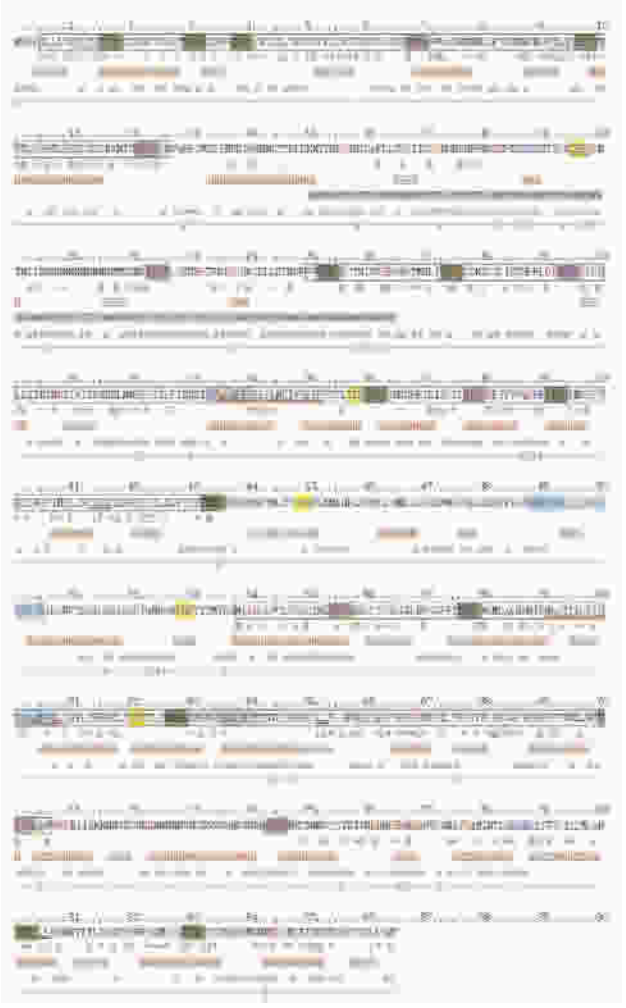

Figure 2. Secondary structure of *Pf*CPR.
Red alphabet represent conserved amino acid residue, □ =conserved domain, H= α helics, E= β strands, e = exposed, N=NORs, ▭= N-glycosylation site, ▭and▭= Protein kinase C phosphorylation site, ▭and▭= Casein kinase Ⅱ phosphorylation site, ▭= Tyrosine kinase phosphorylation site, ▭= N-myristoylation site.TLPN= vacuole target motif, ▭= tail bileucine motif, ▭= amino acid site inseted the interior side of the nuclear membrane, and P represents protein-protein binding site.

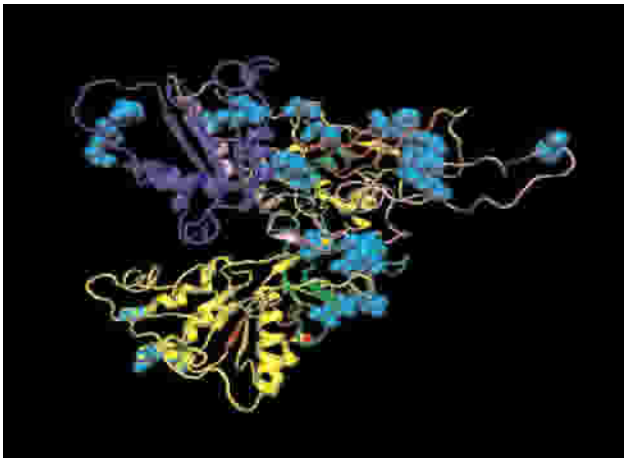

Figure 3. Space structure of *Pf*CPR 1aa-700aa.
Blue represents the sequences located in domain of FMN_red superfamily, green represents the sequences located in NORs, yellow represents the sequences located in domain of FNR_like superfamily, pink represents 434aa-537aa, red represents the sequences located in nuclear membrane, and cyan represents protein-protein binding site.

3.4. Comparison of PfCPR and Homo sapiens (Hs)CPR

*Pf*CPR was located in nucleus and had two segments insetting in the interior side of the nuclear membrane, but *Homo sapiens* jkl (*Hs*CPR) was located in cytoplasm. There were 151 highly conserved amino acid residues. The amino acid residue amount of FNR_like superfamily of *Pf*CPR was 60, less than that of *Hs*CPR. FNR_like superfamily of *Pf*CPR was divided into two segments by 434aa–537aa. 749aa–864aa, which did not belong to conserved domain, was highly similar with the end of FNR_like superfamily of *Hs*CPR (Figure 4). The results of sequences alignment *Pf*CPR and *Hs*CPR showed that *Pf*CPR 844aa–861aa, 5aa – 433aa, 538aa–706aa and 749aa–864aa were homologous with *Hs*CPR 21aa–38aa, 83aa–470aa, 470aa–620aa and 569aa–679aa. The number of conserved amino acid residue were 7, 87, 34 and 23, respectively; the consistency were 39%, 20%, 19% and 20%, respectively; and the similarity were 67%, 16%, 38% and 47%, respectively.

*Pf*CPR 126aa–136aa, 141aa–161aa, 177aa–214aa, 223aa–233aa, 248aa–255aa, 292aa–296aa, 328aa–335aa, 357aa–370aa, 374aa– 380aa, 434aa–537aa, 551aa–557aa, 619aa–631aa, 635aa–651aa, 701aa–705aa and 707aa–748aa all had no homology to *Hs*CPR, and most were exposed on the surface of the protein. They had 25 protein–protein binding sites and function sites except for 126aa–136aa and 141aa–161aa. There were 6 function sites in 434aa–537aa (Figure 2).

Figure 4. Conserved domain of *Pf*CPR and *Hs*CPR.

4. Discussion

The molecular evolution analysis of *Pf*CPR shows that CPR is a kind of the old proteins, and the evolution of *Pf*CPR is older than the evolution of not only *Plasmodium vivax* CPR but also CPR of bacterium, plant, insects and vertebrates retrieved. But it doesn't rule out the possibility that the origin of *Plasmodium falciparum*, including other plasmodium, may be earlier than the origin of other organisms retrieved. *Plasmodium falciparum* may experience non–parasitic life for quite some time before parasitic life. And there may be some reservoir hosts of *Plasmodium falciparum* that are not found, too.

The amino acid sequence comparison of *Pf*CPR and *Hs*CPR shows that *Pf*CPR has two segments (434aa – 537aa and707aa–748aa) in amino acid sequence, more than *Hs*CPR. FNR_like superfamily of *Pf*CPR, which is one of conserved domains, has 60 residue amino acid, less than that of *Hs*CPR. It is divided into two segments by a amino acid sequence that has no homology with *Hs*CPR. *Pf*CPR 749aa–864aa, which doesn't belong to conserved domains, is highly similar with the end of *Hs*CPR FNR_like superfamily. After entering into human body, *Plasmodium falciparum* infectes human hepatocyte firstly. *Hs*CPR are also found mostly in the hepatocyte. Hence it is possible that the evolution of *Pf*CPR has correlation with *Hs*CPR. Considering that human hepatocyte produces a mass of enzyme, such CYPs, which is an important drug metabolic enzyme, it is resumed that *Plasmodium* may escape host immune system and the antimalarial activities of drugs by enzymes in hepatocyte. In another words, *Pf*CPR possibly participates in the evasion of *Plasmodium falciparum* from immune system and antimalarial drugs activities. *Plasmodium* sporozoite makes parasite and schizogony in hepatocyte, which may be related to *Pf*CPR.

The subcellular localization prediction shows that both *Pf*CPR and *Pb*CPR[7] are located in cellular nucleus and have a segment of amino acid in NORs; while *Hs*CPR and *Mm*CPR[7] were located in cytoplasm. The subcellular localization of *Pf*CPR may be one of the immune evasion mechanisms of *Plasmodium falciparum*.

Regarding to the function and importance of CPR in mammal, *Plasmodium* CPR is a potential drug and vaccine target. *Pf*CPR is in cellular nucleus with two segments insetting into nuclear membranes. And drugs must inhibit and even kill plasmodium, but not human normal cells. So drug targets selected should be some amino acid sequence without homology with human. *Pf*CPR 177aa–214aa, 223aa–233aa, 248aa–255aa, 292aa–296aa, 328aa–335aa, 357aa–370aa, 374aa–380aa, 434aa–537aa, 551aa–557aa, 619aa–631aa, 635aa–651aa, 701aa–705aa and 707aa–748aa all have no homology with *Hs*CPR, but all have potential function sites. Therefore, they may be ideal drug targets.

Conflict of interest statement

We declare that we have no conflict of interest.

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