December 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.3 • 23-36

A REVERSE VACCINOLOGY APPROACH IN THE IDENTIFICATION OF POTENTIAL VACCINE CANDIDATES FROM *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN 1

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Abstract

Plasmodium falciparum is the most fatal species of *Plasmodium*, a unicellular human parasite which causes malaria. The transmission is through the bite of the female *Anopheles* mosquito. *P. falciparum* causes about 50% of malaria cases ever known. Intense research works has been committed into the development and design of malaria vaccine over the years, yet there is no commercially-available vaccine against malaria at the moment. More than 20 vaccine candidates are at the moment undergoing clinical trial evaluation. This study described a reverse vaccinology approach to the development and design of a potent vaccine candidate which works against *P. falciparum* by exploring the instability of its erythrocyte membrane protein 1 and using a combination of B-cell and T-cell epitope predictions, followed by molecular docking and simulations through molecular dynamics approach. The sequence of amino acids of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) was downloaded from the NCBI database and examined to determine the most immunogenic segments of the protein. This *in silico* approach includes the prediction of the allergen presence, physicochemical characteristics of the protein, sub-cellular localization, percentage sequence identity with the human and rat homologue, binding efficiency to the human major histocompatibility complex (MHC) class I and II etc using numerous web-based prediction tools. The molecular docking of the predicted best binding peptides with the human MHC was also carried out using pepATTRACT.

The *Pf*EMP1 was predicted to be an unstable protein with an instability index of 42.50. Results of the sequence alignment with the human and rat homologues gave a percentage identity of 20.07% and 19.81% respectively. Four potent B-cell epitopes have been predicted from the sequence of protein. The peptides are KEDNEDEEEEGE, PDASPFGGGQPR, GNEEDPPDDDYI and KILNNTSNGSLE at position 726, 1897, 1385 and 2694 respectively. LLDQLNIKY and IPHSAGEPL interacted with the HLA-A0101 and HLA-B0702 alleles of the MCH class I with the highest binding energy while the predicted highest binders with the DRB1_0101 and DRB3_0101 alleles of the MCH class II were TIPFGIALALSSIAF and NVKKYIEDNNKQISI respectively. The NRFDTNIIW peptide sequence also showed the highest TAP binding affinity with an IC50 value of 49.25nM. Molecular docking of candidate antigens with the MHC class I receptor was performed and the TIPFGIALALSSIAF sequence showed the highest binding energy, with a score of -17.28Kcal/mol. The visualization and dynamic approach simulation from the results revealed four B-cell epitopes. The peptide with the strongest affinity for transporter associated with antigen processing (TAP) was also revealed alongside the best binding T-cell epitopes. The TIPFGIALALSSIAF was shown to be the best vaccine candidate, with the best binding energy score against the MCH class I. The above results are from *in silico* experiments which should be validated using experiments by wet lab.

Keywords: *Plasmodium falciparum erythrocyte membrane protein 1; Molecular docking; Reverse vaccinology; Epitope*

Introduction

Plasmodium falciparum is the causative protozoa for malaria, a widely known infectious disease. *P. falciparum* remains the commonest strain of all malaria species which results into almost every recorded deaths as a result of malaria [9]. *P. falciparum* causes the infected red blood cell to stick to blood vessels by changing its surface [12]. In severe cases, it results into an obstruction of microcirculation which leads to the dysfunction of many organs. Symptoms depends on infection severity and can be accompanied with such signs as flu-like symptoms, intense vomiting, shock and headache, failure of the kidney, coma, and eventual death. *P. falciparum* infection is prevalent among children below 5 years of age as well as pregnant women [13].

The multidomain variant antigen, *Plasmodium falciparum* erythrocyte membrane protein-1 (*Pf*EMP-1), known to be expressed on the surfaces of red blood cells that has been infected by *P. falciparum*, can direct the binding to molecules such as CD36 and the intercellular adhesion molecule-1. The vascular cell adhesion molecule, chondroitin sulfate A (CSA), P-selectin, E-selectin and CD31 which are located on vascular endothelial cells are also bound, thus enabling the parasite to avoid clearance by the spleen [37]. The *var* gene family which can be subdivided into 3 main groups namely group A, B, and C, based on genomic location and upstream sequence codes for *Pf*EMP-1. *Pf*EMP-1 is proposed to modulate host immune responses through CD36-dependent interactions with antigen-presenting cells while it successfully in evades antibody responses [33]. Infected erythrocytes causes the dendritic cells maturation inhibition thereby, reducing their capacity to activate T cells [50]. This is as a result of the binding of *Pf*EMP-1 to CD36 [51].

Scientists have continually evaluated different vaccine candidates and formulations that are designed to make the immune system more active in order to destroy the malaria causing parasite [36]. The desired action of a vaccine designed for malaria can take place at several points during the parasite's life cycle [22]. The most known problem faced by scientists who specializes in designing malaria vaccines is their inability to understand the responses of the immune system which is associated with protecting the host against the [2]. The complexity of the malaria causing parasite has caused scientists to pursue a diversity of vaccine development approaches. It is believed by many that the malaria vaccine will need to cut across a series of different approaches to reach an optimum degree of efficacy [49].

Reverse vaccinology is the utilization of information obtained from the genome of an organism coupled with high throughput computation in preparing [20]. The antigenic determinant with an important role in the immunity of an organism is what is referred to as an epitope. Epitopes are present on the cellular surface of organisms which makes it easy for antibodies to detect them [6]. Reverse vaccinology utilizes analysis of the genome through computation to predict the surface protein epitopes of an organism [8]. Epitopes are therefore important entities in the development of candidate vaccines. The immune system is aided by the B and T lymphocytes. These lymphocytes play important roles in the immune system of an oganism [53]. The B cells recognizes the epitopes which the antibody paratopes has identified [14]. The T cells are the mediators of cell immunity. They achieve this by their interaction with antigenic peptides that has been processed [15].

The purpose of this study is to design a potent epitope-based malaria vaccine from *Plasmodium falciparum* erythrocyte membrane protein 1(*Pf*EMP1) that can bind to the MHC

for T-cell presentation, using the reverse vaccination approach.

Materials and Methods

Sequence retrieval

The *Pf*EMP1 sequence was downloaded from the National Center for Biotechnological Information database (NCBI) [26]. The protein was assigned an accession number of ABM88777.1. The physicochemical properties, sub-cellular localization and antigenicity sites were all predicted using various computational tools and databases.

Protein preparation

Identification of the protein sub-cellular localization was done using the Psort server (http://psort.nibb.ac.jp/form2.html) [39, 40, 41].

Allergenicity prediction

The protein sequence was checked for allergens presence using the AllergenFP v.1.0 server which can make accurate predictions for the presence of both allergens and non-allergens, by making comparison between the input sequence and the sequence of known allergens [28].

Sequence alignment

The alignment of the *Pf*EMP1 amino acid sequence with its human and rat homologues was achieved with the ClustalW software [23, 32, 35]

Prediction of the proteasome cleavage sites

The NetChop 3.1 server which works by predicting neural networks for the human proteasome cleavage sites was implemented to predict the cleavage sites of proteasomes for the *Pf*EMP1 sequence [30].

B cell epitope prediction

A docking analysis was conducted to ensure that MHC molecules interacted with our

The crystalized structure of the human MHC class I, was obtained from the Protein Data Bank, PDB 5TEZ (Figure 1). The protein 3D structure was subjected to a refinement protocol using the Pymol viewer [16].

Physiological-Biochemical Characterization

The physiochemical characterization, total number of negative and positive residues, molecular weight, aliphatic index, extinction coefficient, instability index, theoretical isoelectric point (pI) and grand average hydropathicity (GRAVY) of the PfEMP1 [21] were all predicted using the Expasy Protparam server [27].

Sub-cellular localization and signal peptide

The BCPREDS prediction server which uses the antigenicity scale of amino acid pair as the method of implementation was used to predict B cell epitopes [11].

Binding efficiency of peptides to MHC I and II

The NetMHC 4.0 server [5] which uses artificial neural networks (ANNs) for prediction was used to predict the binding efficiency of peptides to the MHC I while the peptide binding to the MHC II was predicted with the use of the NetMHC II 2.3 server [29]. Predictions are given in the nM IC50 values.

Prediction of peptide affinity to transporter associated with antigen processing (TAP)

TAPREG is an on-line service used for the prediction of peptide binding affinity to the transporter associated with antigen processing (TAP). The importance of this prediction is associated with the identification of the T cell epitopes restricted by the MHC class-1. The Prediction utilizes amino acid sequence and their properties with links to a cascade based on support vector machine (SVM) [18].

Docking simulation study

targeted epitopes by implementing the pepATTRACT web server [17]. This was

performed by considering the protein to be the MHC molecule while the epitopes that has been identified will be considered as ligands.

Results and Discussion

By the adoption of *in silico* analysis, 5 peptides which show high potential for being potent candidates in vaccine development were identified from the *Pf*EMP1. The protein was first considered for sub-cellular localization prediction using the **PSORTb** sub-cellular localization prediction server [39, 40, 41]. Proteins can be localized in various cell parts. This includes the cytoplasm, the cell membrane, or can be secreted out of the cell to become extracellular proteins. Proteins localized on the cell membrane or secreted as extracellular proteins are better antigens because their exposure to the host's immune cells [10].The *Pf*EMP1 (accession no: ABM88777.1) was predicted as an extracellular protein with a score of 8.91; hence, it has a great probability of containing peptide sequences that generates a protective response [34] (Figure 2).

The *pI* of the *Pf*EMP1 by the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 6.59 [45]. The hydrophobicity scale produced values with relativity to the amino acid residues hydrophobicity. The high the positive value, the hhgher the hydrophobicity the amino acids located that protein region [31]. The GRAVY calculator used in predicting the hydrophobicity assigned a value of -0.989to the protein.

The stability of a protein in a test tube is a function of the instability index value. Instability indices with values lower than 40 denote that such a protein is stable and values than 40 is estimated as an unstable protein [31]. The *Pf*EMP1 is therefore an unstable protein with an instability value of 42.50.

The aim of predicting B cell epitopes is to facilitate the process of identifying the B cells in order to replace foreign antigens with the production of antibodies [24]. Studies based on the structure and function of target proteins can also be carried out since any solvent-exposed region in the antigen can be subject of recognition by antibodies [25]. The BCPREDS [Chen] was used in predicting possible B cell epitopes in the membrane protein but only the highest scoring peptides were carefully selected for the purpose of this study. The peptides selected were KEDNEDEEEEGE, PDASPFGGGQPR, GNEEDPPDDDYI and KILNNTSNGSLE at position 726, 1897, 1385 and 2694 respectively.

The binding efficiency of *Pf*EMP1 peptides to the HLA-0101 and HLA-B0702 alleles of the human MHC class I was implemented in predicting potential T-cell epitopes, using the NetMHC 4.0 tool [3, 47]. 17 high binders and 47 weak binders resulted from the binding of a total of 2708 peptides to the HLA-0101 allele while a total of 6 high binders and 12 weak binders resulted from the peptide binding to the HLA-B0702 allele, setting the threshold of strong and weak binders at 0.500 and 2.000 respectively. LLDQLNIKY and IPHSAGEPL peptides at position 2168 and 2418 respectively were selected for the docking study because they exhibited the highest binding energy with the alleles.

The binding of *Pf*EMP1 peptides to MHC class II molecules was achieved using the NetMHC II 2.3 tool [29]. The selection of the T cell epitopes were based on such a fact that peptides that exhibit high affinity for MHC/HLA molecules are easily and most likely recognized by the receptors of specific T cells. Two MHC class II alleles were selected (DRB1_0101, DRB3_0101) and the best binding peptide were selected for the docking study. TIPFGIALALSSIAF peptide (position 2298) was selected from 10 high binders to the DRB1_0101 allele (45 weak binders) while the

NVKKYIEDNNKQISI peptide (position 765) was selected from a total of 11 high binders to the DRB3_0101 allele (109 weak binders).
The TAP is most important for the processing and presentation of MHC class I restricted antigens [38]. The transportation of cytosolic peptides into the endoplasmic reticulum and the conduction of match sequences and length to respective MHC class I molecules is a function of the TAP transporter [46]. Information about peptides affinity to the TAP transporter is important as it might contribute to the understanding of the basis of immune escape mechanisms in diseases and also to optimize the treatment for TAP defective patients [48]. The binding of the NRFDTNIIW peptide (position 1201) to the TAP transporter was the highest with an IC50 value of 49.25nM.

The proteasome is crucial in vertebrate's immune response [43]. Production of peptides by proteasome is achieved by the degradation of intercellular proteins from self and non-self and these are presented in complex with MHC to the cytotoxic T cells (CTL) [42]. 746 proteasome cleavage sites were predicted from the *Pf*EMP1, using the NetChop 3.1 server.

Epitopes were subjected to allergenicity analysis by the AllergenFP v.1.0 server [28]. The prediction tool pointed out the *Pf*EMP1 peptides as probable non-allergen. This implies that vaccines developed by utilizing these peptides are not likely to elicit any allergic reaction [1].

The selected peptides were also checked for molecular mimicry or cross reactivity between self and pathogen epitopes to avoid auto-immune responses [52]. Similarity analysis can alternatively be used in searching for molecules with similar functions and also providing ideas of their antigenicity and virulence [4, 7]. The result of the sequence alignment between *Pf*EMP1 and its human (accession no: NP_000110.2) and rat (accession no: NP_001099990.1) orthologs showed a percentage identity 20.07 and 19.81 respectively. The low similarity degree observed between the proteins suggests an ideal option in protein selection for the development of malaria vaccines since only molecules with a high similarity degree could generate auto-immune responses [7].
pepATTRACT [17] predicted the binding mode for the LLDQLNIKY, IPHSAGEPLTIPFGIALALSSIAF, NVKKYIEDNNKQISI, NRFDTNIIW epitopes with MHC class I molecule. pepATTRACT is a novel docking protocol for protein-peptide blind docking [19]. The binding energy of the selected epitopes with MHC class I receptor was found to be-12.82, -15.71, -17.28, -13.82 and -15.38kcal/mol. The 3D structure of the human MHC class I protein shown in Fig. 1was visualized and captured with the Pymol molecular graphics system [44].

Conclusion

The main objective of this study was to predict potential candidates for the development and design of potent malaria vaccine, using peptides obtained from *Pf*EMP1. The predictions were made using various computational algorithms. 5 vaccine candidates were selected as the best MHC I, MHC II and TAP transporter binders. The docking of these peptides with the human MHC I protein showed that the TIPFGIALALSSIAF peptide seems to be the most potent vaccine candidate as revealed by the reverse vaccinology analysis and the predicted binding energy score. However, verification of these peptide candidates as potent malaria vaccines is to be justified through the confirmation made by wet lab analysis.

Abbreviations: *Pf*EMP1; *Plasmodium falciparum* Erythrocyte Membrane Protein 1, ANNs; Artificial Neural Networks, MHC; Major Histocompatibility Complex, TAP; Transporter

associated with Antigen Processing, SVM; Support Vector Machine, pI; Isoelectric Point, GRAVY; Grand Average Hydropathicity.

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Figure 1: Crystal structure of the Human MHC class I protein. PDB 5TEZ

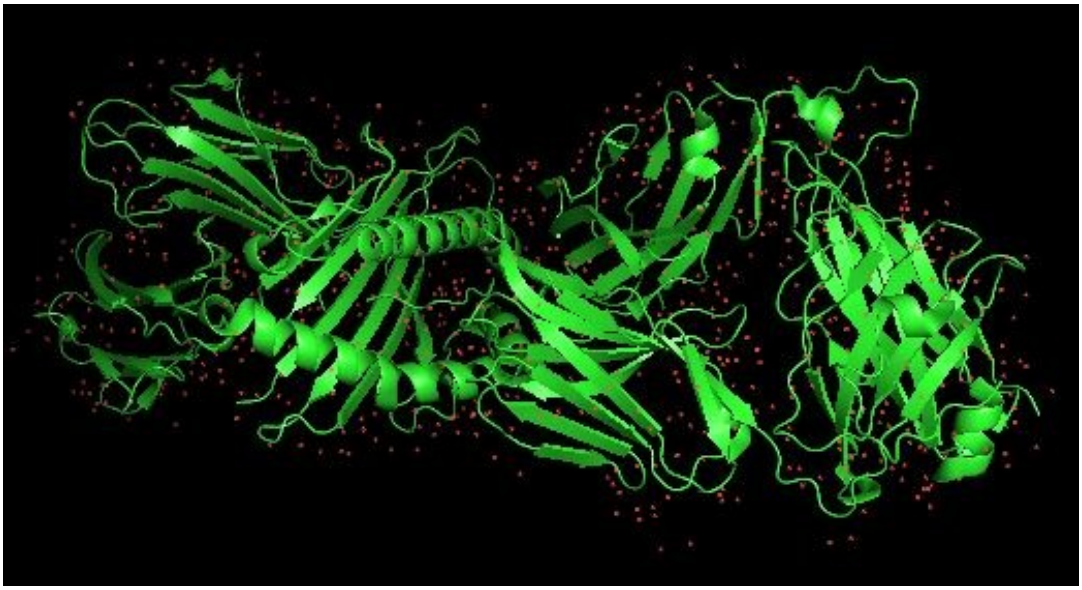

Figure 2: Sub-cellular localization prediction by the Psortb server

psortb

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PSORTb Results (Click here for an explanation of the output formats)

```
SeqID: ABM88777.1 erythrocyte membrane protein 1 [Plasmodium falciparum]
  Analysis Report:
    CMSVM+            Unknown                    [No details]
    CWSVM+            Unknown                    [No details]
    CytoSVM+          Unknown                    [No details]
    ECSVM+            Extracellular              [No details]
    ModHMM+           Unknown                    [No internal helices found]
    Motif+            Unknown                    [No motifs found]
    Profile+          Unknown                    [No matches to profiles found]
    SCL-BLAST+        Unknown                    [No matches against database]
    SCL-BLASTe+       Unknown                    [No matches against database]
    Signal+           Unknown                    [No signal peptide detected]
  Localization Scores:
    Cytoplasmic           0.24
    CytoplasmicMembrane   0.05
    Cellwall              0.80
    Extracellular         8.91
  Final Prediction:
    Extracellular         8.91
-------------------------------------------------------------------------------
```

Molecular docking results

Figure 3: LLDQLNIKY peptide of *Pf*EMP1 in complex with MHC.

Figure 4: IPHSAGEPL peptide of *Pf*EMP1 in complex with MHC.

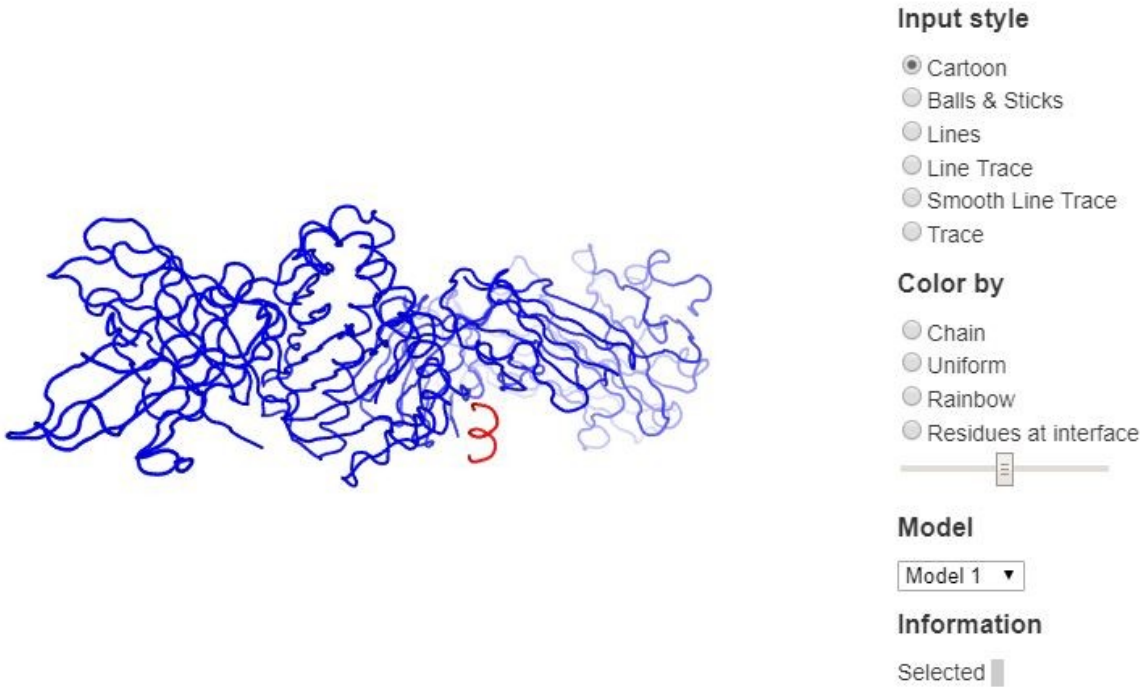

Figure 5: TIPFGIALALSSIAF peptide of *Pf*EMP1 in complex with MHC.

Figure 6: NVKKYIEDNNKQISI peptide of *Pf*EMP1 in complex with MHC.

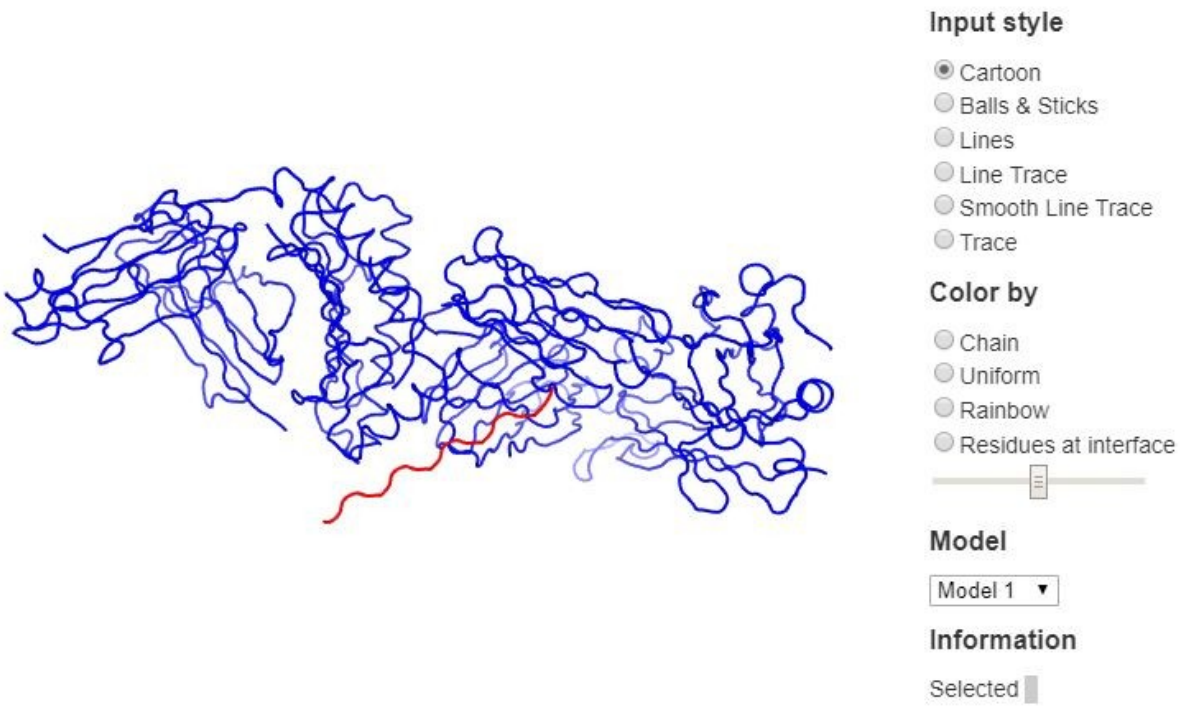

Figure 7: NRFDTNIIW peptide of *Pf*EMP1 in complex with MHC.