



Short communication

The identification of FERM domain protein in serum infected with *Plasmodium berghei*

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ARTICLE INFO

Article history:

Received 26 October 2015

Received in revised form 21 December 2015

Accepted 25 January 2016

Available online 28 January 2016

Keywords:

Malaria

Plasmodium berghei

FRMD5

Overexpression

Serum protein

Cytoskeleton linkers

ABSTRACT

Malaria continues to affect 500 million people worldwide every year. In this study, we compared the protein profile of uninfected and *Plasmodium berghei*-infected serum samples by one dimensional SDS-PAGE analysis, MALDI-TOF/TOF mass spectrometry and confirmed by semi-quantitative RT-PCR. Also the protein interacting networks were established using STRING protein–protein interaction analysis. We observed for the first time the upregulation of FERM domain during *P. berghei* infection. We predict that FRMD5 along with the other protein partners (identified by STRING analysis) are involved in the merozoites entry or protein trafficking where cell to cell interaction happens with the host erythrocyte; hence, upregulation.

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1. Introduction

Malaria continues to affect 500 million people worldwide with two-three million deaths per year and most of them are children below five years in Sub-Sahara Africa [1]. Emerging drug resistance in different species of *Plasmodium*, the parasite, has caused severe obstacle for eradication and management of malaria [2]. This situation is worse in underdeveloped and developing countries where availability of medical facilities is far from desired standards [3,4]. In recent years, proteomic studies have substantially contributed in understanding the host (human)-pathogen (malaria parasites) relationship [5], including infection-induced changes in host erythrocyte membrane proteins [6]. Identified potential disease related markers generally reflect rapid changes in expression pattern in response to disease conditions and show direct correlation with progression of disease [7]. In this direction, proteomic profile of serum can give insight into the systemic reaction to a disease state and can serve as a pool of differentially expressed proteins [8]. These proteins could potentially serve as an early infection indicators for diagnosis or therapeutic targets. *Plasmodium falciparum* is the major species involved in global malaria. Cerebral malaria pathogenesis caused by *P. falciparum* is poorly characterized and need to be better understood to identify

novel therapeutic targets. Certain components of cerebral malaria have been investigated in the mouse model of malaria caused by the infection with *P. berghei*. This infection in mice closely mimics *P. falciparum*-induced cerebral malaria in humans [9]. The analysis of the serum proteome can relate to a particular interested protein that changes in expression or abundance in response to a disease state. In this study, we have compared the serum protein profile of control and mice infected with *P. berghei* by one-dimensional (1D) sodium dodecyl sulfate- polyacrylamide gel electrophoresis (1D-SDS-PAGE) and identified the differentially expressed proteins by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) mass spectrometry. By this approach, we obtained differentially expressed protein profile of the *P. berghei*-infected and uninfected serum samples, which can identify potential biomarkers related to malaria infection.

Swiss mice were obtained from Central Animal Facility, Indian Institute of Science, Bangalore. All precautions were undertaken to minimize suffering. Animal experiments in mice and all experimental protocols were according to the Institutional Animal Care and Use Committee guidelines. For parasite propagation, fresh mice were injected intraperitoneally with *P. berghei*-infected blood. Infected mice died between 5 and 6 days and were confirmed by Giemsa-stained smears. Control and *P.berghei*-infected (day 5 post infection) blood were collected by cardiac puncture, allowed to clot for 2 h at room temperature and centrifuged at 5000 rpm for 15 min. The obtained serum was stored at -80°C until further analysis. 150 μg of protein from control and *P. berghei*-infected serum was separated on 10%

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SDS-PAGE gel. The gel was visualized by coomassie brilliant blue G-250 staining and differentially expressed band was identified by MALDI-TOF/TOF mass spectrometry. The MALDI mass spectra were obtained using an ULTRA FLEXtreme MALDI TOF/TOF (Bruker Daltonics). Thus obtained peptide masses were searched against SwissProt database employing Mascot (Matrix Science) database for protein identification. Protein matches with scores greater than 55 ($p < 0.05$) were considered significant. The identified protein was further analysed using STRING v9.1 (search tool for the retrieval of interacting genes), which is an application that aggregates available databases of known proteins. For the validation of FRMD5 protein identified by MALDI-TOF/TOF-MS, total RNA was isolated from control and *P. berghei*-infected blood by TRI reagent (Sigma) and semi quantitative RT-PCR was carried out with FRMD5-specific primers 5'-ACGGCCGTCTTCTCTGAAA-3' and 5'-ATGTTGCTGGTGTGGACCA-3', with a 204 bp PCR product (96 °C: 1 min; 60 °C: 1 min; 72 °C: 1 min; 30 cycles), visualized on 1.5% agarose gel with GAPDH as control. Statistical analysis of arbitrary units derived from densitometric measurements of protein bands from the study was carried out using SPSS software (Version 13.0, SPSS, Chicago). All the data are presented as mean \pm SEM ($n = 3$). Difference between groups was calculated by Student's *t*-test and $p < 0.05$ was considered significant.

Comparative study of serum protein profile of control and *P. berghei*-infected mice was done by one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). *P. berghei* infection in mice was confirmed by Giemsa staining of blood smears (Fig. 1a). Equal amount of serum proteins was run on a 10% SDS-PAGE gel, visualized by coomassie brilliant blue staining. Fig. 1b represents protein profile with differential expressions across control and infected mice. A student's *t*-test was performed of a selected protein band (marked by an arrow, Fig. 1b) to determine statistically significant difference between control and infected sample (Fig. 1c). Further, selected differentially expressed

protein band was analysed by MALDI-TOF/TOF mass spectrometry. The obtained mass spectra were analysed by SwissProt database search aided by Matrix Science for protein identification. Protein matches with scores greater than 55 ($p < 0.05$) were considered significant. The protein identified to be FERM domain containing protein 5 (FRMD5). Next, functional association networks with the FRMD5 were determined and predicted functional partners were identified by STRING v9.1 (Fig. 2). To correlate and validate differential expression pattern of FRMD5, total RNA was isolated from control and *P. berghei*-infected mice and a semi-quantitative RT-PCR was carried out with FRMD5 specific primers. A significant upregulation in FRMD5 expression seen in *P. berghei*-infected mice compared to control (Fig. 3).

In this study, we demonstrate the differential expression of protein in serum of *P. berghei*-infected mice. Earlier studies have shown the importance of highly abundant serum proteins in protection against various infections [10,11]. Therefore, we did not adopt any additional processes to remove such proteins, as we could risk losing important host serum proteins that, otherwise, could have an important role in protection against infection.

In this study, we have identified the overexpressed FERM domain present in the pooled sera of *P. berghei*-infected mice. This could be due to a host response to combat this particular parasitic infection. FERM domain is a known unique module involved in the linkage of cytoplasmic proteins to the membrane [12]. They form a family of proteins that mediate linkage of the cytoskeleton to the plasma membrane [13]. This domain was originally identified in band 4.1 (also known as protein 4.1) that was first isolated from human erythrocyte ghosts [14]. The presence of FERM domain in the cell-cell contact protein talin [15] suggests that these proteins link the membrane and the cytoskeleton in specific subcellular environments. The functional role of the FERM domain in maintaining cell integrity, motility and differentiation [11], and its involvement in human diseases are known. Recently, there is a

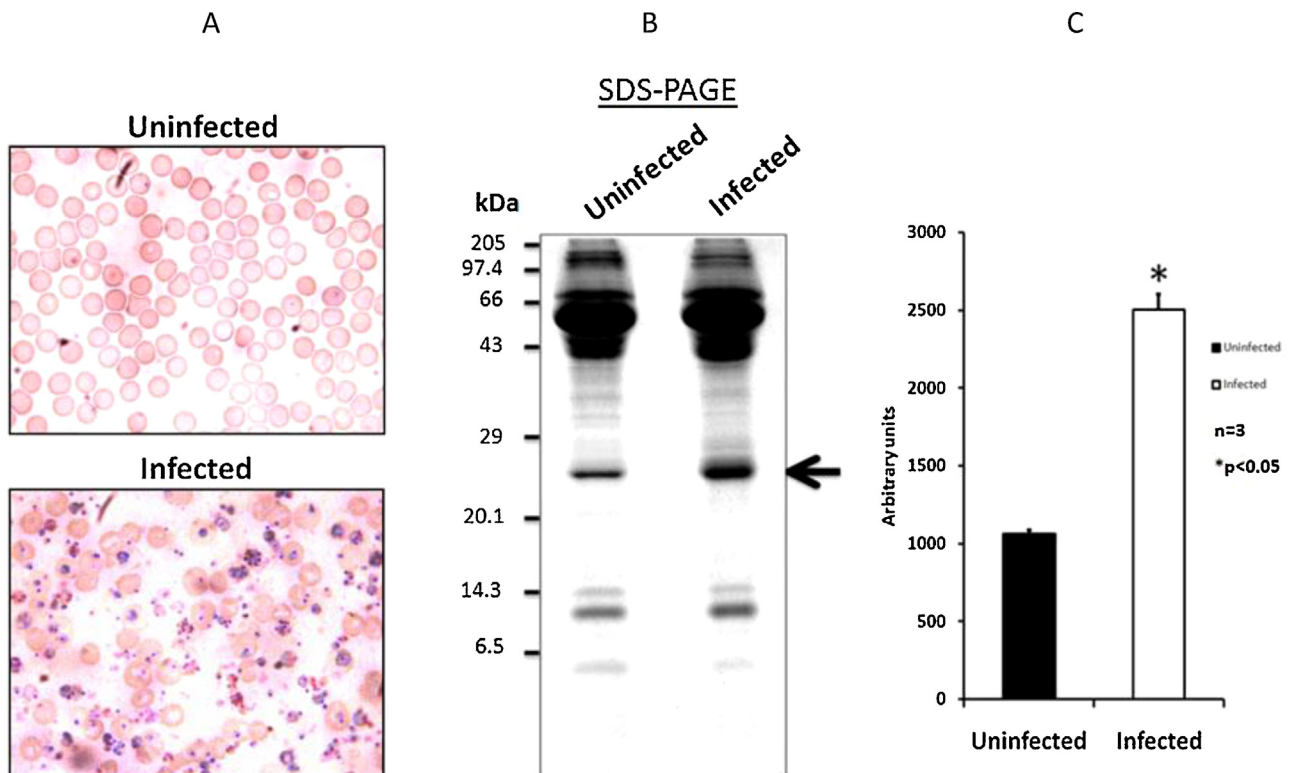


Fig. 1. (a) Giemsa-stained blood thin smears of control mice (uninfected) and mice infected with *P. berghei*. (b) SDS-PAGE of control (uninfected) and infected mice serum proteins on a 10% gel. (c) Student's *t*-test ($n = 3$, $p < 0.05$).

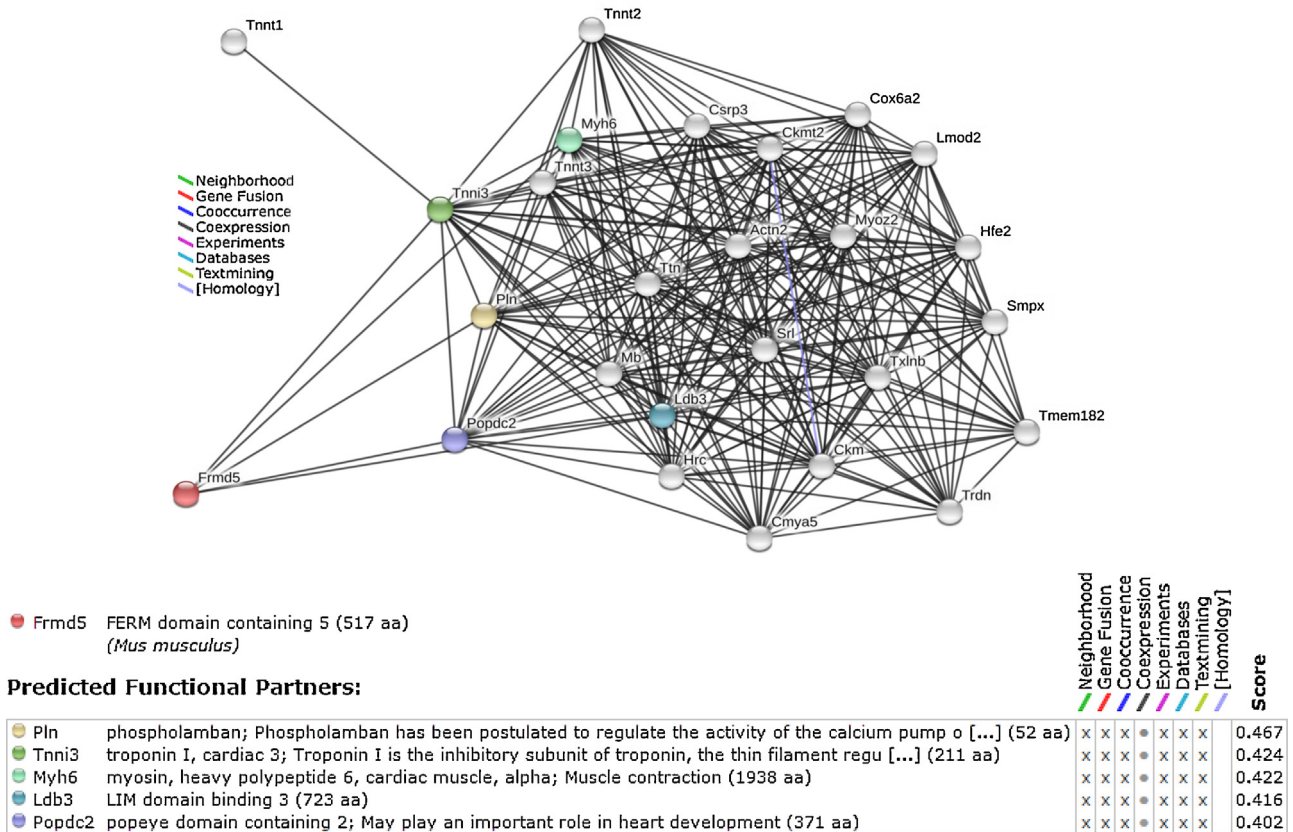


Fig. 2. The protein–protein interaction networks of FRMD5 constructed by STRING.

report that negative mutation in the FERM domain of Janus-associated kinase 3(JAK 3) were fully protected against cerebral malaria [16]. The intraerythrocytic parasites extensively remodel their host cells.

Plasmodium parasites achieve massive changes in erythrocyte structure by exporting proteins into the erythrocyte cytosol, some of which interact with components of the host cytoskeleton and plasma membrane [17]. Only a few of these exported proteins have been studied in detail, one of it is the mature parasite-infected erythrocyte surface antigen (MESA), which binds to the erythrocyte cytoskeleton associated protein Band 4.1, also known as 4.1R

[18] and another is Plasmodium helical interspersed subtelomeric (PHIST) domain proteins, which play a role in modulating the erythrocyte cytoskeleton [19]. Given the critical regulatory role that the 4.1R FERM domain plays in uninfected erythrocytes, it is not surprising that P. falciparum proteins also bind to it, and the 4.1R FERM domain is recognized by MESA [20] and PHIST [19].

Results of protein–protein interaction analysis of FRMD5 revealed several predicted protein partners. It is interesting to note that one of the interacting proteins phospholamban (Pln) has been shown to regulate the activity of the calcium pump, which is important for growth of P. falciparum [21]. Similarly, myosin heavy polypeptide 6

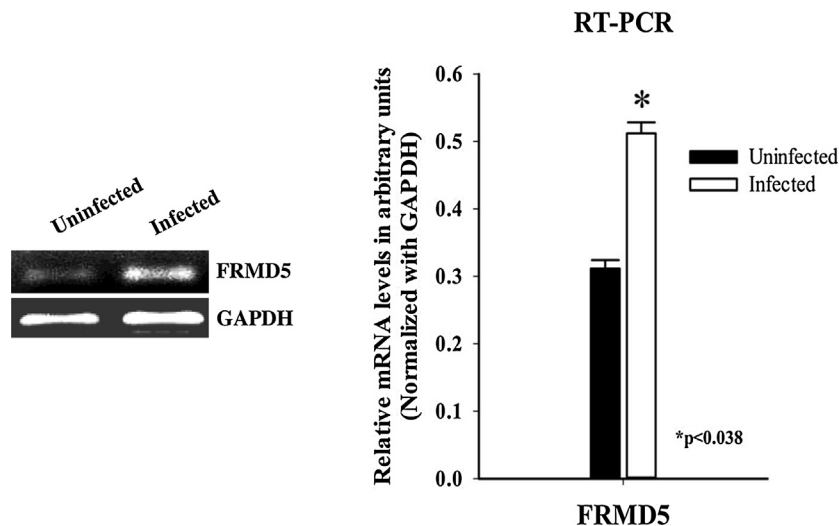


Fig. 3. FRMD5 mRNA expression patterns by semi-quantitative RT-PCR analysis.

(Myh6) also an Frmd5 interacting protein shown to be involved in gliding motility of *P. berghei* during host cell invasion [22]. These indicate that FERM domain has several protein partners to provide a link between cytoskeleton signals and membrane dynamics and hence upregulation of this FERM domain protein in case of *Plasmodium*-infected animal. Further studies identifying a mechanistic role of FRMD5 in malaria infection could be valuable in identifying the protein as possible diagnostic and pharmacological target.

Conflicts of interest

The author's declare that they have no competing interests.

Acknowledgments

This study was supported by Undergraduate Programme and funded by the Indian Institute of Science, Bangalore, India. Authors would like to thank Proteomics Facility, Molecular Biophysics Unit, Indian Institute of Science, Bangalore for MALDI-TOF analysis and thank Dr. Vijayakumar Govindaraj, Department of Biochemistry, Indian Institute of Science for critical-review of the manuscript.

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