Original Article

Functional prediction of human erythrocytic miR-451a on *Plasmodium falciparum* 3D7 transcriptome—an in-silico study

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Background: *Plasmodium falciparum* (*P. falciparum*) is one of the deadliest species responsible for the major deaths related to malaria. Recently emerging research has focused on small noncoding RNAs especially mature microRNAs (miRNAs) (19-25 nts) which play regulatory role chiefly by targeting mRNAs' cleavage or translational repression. During infection, parasitic *Plasmodium* invades and takes command of the host cell for its survival and develops into its various life stages. Seeing that host, miRNAs considered as chief regulators of various physiological functions of the cell itself. During erythrocytic stages, possible interactions between the erythrocytic miRNAs and *Plasmodium* mRNAs may affect parasite biology and its survival inside the host cell. It has been also reported that during erythrocytic stages of *P. falciparum*, erythrocytic miRNAs may translocate into the parasite phosphorous vacuole, some of them may forming a chimera with *Plasmodium* mRNAs to get involved in post transcriptional regulation.

Methods: In this study, one of the most abundant human erythrocytic miR-451a retrieved from miRbase and targeted on *P. falciparum* 3D7 transcriptome to find out the target genes and followed by annotation studies performed of targeted genes.

Results: We identified crucial gene targets of *P. falciparum* using PSRNA target tool. Functional annotation of targeted genes and their protein products help us to understand the possible protein-protein interaction during the course of infection as well as analysis of their functions at molecular/cellular/biological level shows the significant contribution from the cytoskeletal genes which codes for the proteins like *Plasmodium falciparum 3D7 erythrocyte membrane protein 1* (*PfEMP1*), *rifin*, etc. which are involved in the host membrane modifications.

Conclusions: Computational approach to elucidate the erythrocytic microRNA (miRNA) targeted genes and their functional annotation studies will help to understand the possible interactions and miRNA regulatory network in malaria.

Keywords: Malaria; *Plasmodium falciparum* 3D7 (*P. falciparum* 3D7); hsa-miR-451a; *Plasmodium falciparum* transcriptome (*P. falciparum* transcriptome)

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Introduction

Globally, an estimated 229 million malaria cases were recorded in 2019, in 87 malaria endemic countries including India. Among the five species, which mainly infect human beings complicated malaria is caused only by the parasitic protozoan *Plasmodium falciparum* (*P. falciparum*). *P. falciparum* is the most widespread malaria parasite not only in the African Region, but also in the South-East Asian Region (1). The virulent nature of *P. falciparum* contributes largely to malaria related deaths (2), in which India, accounts for about 86% of cases in the South-East Asia Region, including pregnant women and children (<5 years). Cytoadherence, sequestration and rosetting, like characteristics make it more dangerous (1,3).

In the case of malaria, to minimize transmission, risk of development of parasite resistance and adverse reactions to anti-malarial drugs, rapid and accurate diagnosis as well as identification of *Plasmodium* spp. is mandatory (4). The diagnostic tools have evolved from conventional microscopic observation of Giemsa-stained blood films to rapid diagnostic tests (RDTs), serology, fluorescence microscopy and molecular diagnostic tools like PCR, qPCR and loop-mediated isothermal amplification (LAMP) (5,6). Yet newer, improved diagnostics are an essential prerequisite to obtain easy-to-use low-cost tests, to enhance identification of the drug resistant profile of the patients. The emergence and spread of drug resistant strains of *P. falciparum* over a period, has contributed to the re-emergence of complicated malaria in the world (7,8). To overcome this problem, priority should be given to identify novel therapeutic approaches in global malaria research.

Since last, few years' researchers have been focussing on small noncoding RNA families in infectious diseases to understand novel perceptions of host parasitic interactions. Small noncoding RNA families mainly microRNAs (miRNAs) have emerged as one of the key players in the stringent, highly coordinated control of gene expression (9,10). miRNA molecules of ~21–25 nucleotides in length, synthesized in the nucleus and with the help of ribonucleoprotein complexes like RNA Induced Silencing Complex (RISC) directed towards target mRNAs to facilitate binding at 5' UTR region on it are mainly involved in transcriptional suppression through mRNA cleavage (10,11). They have also been found to bind on 3' UTR regions, promoters and translated regions of DNA sequences (12). There are reports that miRNAs also affect rate of translation and activation of genes etc. (10). As reported in earlier studies miRNAs are also secreted extracellularly in form of exosomes or bound with proteins and are involved in cell-cell communications (13).

Erythrocytes cannot synthesize miRNAs via the canonical pathway as, they are enucleated. They may retain miRNAs populations since enucleation (14). In addition, it has been observed that mature erythrocytes are proved as potential repositories of miRNAs in the circulating system (15). miRNAs can affect erythropoiesis by down regulation of miR-221, -222, -150 and -155 from transition of reticulocytes to mature erythrocytes (16,17). miR-451, -144 are reported to be upregulated during maturation of erythrocytes by *GATA-1* (14,15,18). miR-451 increases the activity of *FOXO3*—which is involved in the control of oxidative stress and promotes synthesis of antioxidants. miR-451 with miR-223 and let-7i are found to be elevated in Sickle Cell Anemia traits HbSS as well as in HbAS (19,20).

Protozoans have a slightly different mechanism with regard to the miRNA biogenesis pathway as compared to metazoans. It has been found that miRNA biogenesis is independent of Drosha and Argonaute like subfamilies. According to research reports on miRNA pathways in *P. falciparum*, lacks the main candidates of Dicer complex and RISC in genome (21,22). Bioinformatics analysis of small RNA libraries could not identify *Pf* specific miRNAs in *Pf*-infected erythrocytes (21-23). Hence, to date there is not a single report on *Plasmodium* miRNAs.

Mobility of miRNAs within the original cell or to other cells, as well as within the own body is well known. As most of the miRNAs are conserved, in recent studies it has been observed that exchange/interactions of small RNA molecules/miRNAs that occur between the cells belong to different Kingdom. This phenomenon has been discovered during intracellular infection of pathogen, parasites, and symbionts (20,24). *P. falciparum* alters host cell physiology by remodulation of membrane proteomics. Expression of proteins such as *Plasmodium falciparum 3D7 erythrocyte membrane protein 1* (*PfEMP1*), *MSP* family, Knob associated family and adhesive proteins occur at intervals to modify the host surface (25,26), which results in altered properties of the infected erythrocytes like cytoadherence, morphology and these molecules take over the overall charge of host cell machinery (26,27).

To find out the role of miR-451a, we targeted them on *P. falciparum* 3D7 transcriptome via *in silico* approach. This study revealed numerous interactions of miR-451a on crucial gene targets of *P. falciparum*, required for its growth,

survival, and its pathogenesis.

Methods

Erythrocytic miRNAs found in *Pf*-infected erythrocytes (iEs) and its connection with the pathogenesis caused by *P. falciparum* were identified from the literature studies on PubMed and Scopus database. Among the various erythrocytic miRNAs, miR-451a focused (15,19-21,23,28-31) to study its regulatory role during host parasitic interactions.

Retrieval of data

Identified erythrocytic miRNA hsa-miR-451a-5p (Accession No.: MI0001729) was retrieved using miR Base-the miRNA database 22.1 (http://www.mirbase.org/). *P. falciparum* 3D7 transcriptome retrieved from PlasmoDB 8.0 (RefSeq assembly accession: GCF_000002765.5 latest).

Potential target prediction - cross kingdom approach

The selected miR-451a sequence was analysed against the *P. falciparum* transcriptome using psRNATarget tool (http://plantgrn.noble.org/psRNATarget/) (32) with default parameters including maximum expected threshold: 3, length of complementarity scoring: 20, maximum energy to unpair target site: 25 and translation inhibition will set between 9 and 11 nts, to identify the plausible human target genes. The Input method followed will be searching user-submitted small RNAs against the user-submitted transcripts.

Functional annotation and disease association of targeted Plasmodium Genes

Determination and prediction of the function(s) of miRNA-451a, which targeted the transcriptome of *P. falciparum*, gene ontology study carried out using g:Profiler (https://biit.cs.ut.ee/gprofiler) (33) software using the Gene Ontology (GO) terms. Functional enrichment analysis of the predicted *Plasmodium* target genes conducted in order to identify their role in molecular function (MF), biological process (BP) and cellular component (CC). To find protein class details encoded by *P. falciparum* targeted genes, PANTHER online tool (http://pantherdb.org/) (34) was performed. These parameters were statistically significant with the threshold P value <0.05.

Identification of protein-protein interactions and hub proteins

Protein-protein interactions were examined in The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (http://string-db.org/) database (35), which is a well-known biological database and web resource to predict protein-protein interactions. Cytoscape software was also applied to visualize the protein interactions and analyse the hub proteins.

Pathway prediction analysis

In addition to gene ontology, pathways may be the important factors to investigate the role of the genes at cellular component synthesis, molecular functioning, biological processes and their downstream signalling. KEGG pathway enrichment analysis tool and literature review were used to identify the involvement of these targeted genes in various diseases/pathophysiological conditions.

Statistical analysis

It is not applicable in identification of erythrocytic miRNA, it's retrieval, its potential target genes' identification, protein-protein interactions of targeted genes and pathway prediction analysis.

Provisions of the Declaration of Helsinki (as revised in 2013)

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

On analysing the various datasets, it was observed that erythrocytic miRNAs predominantly interact with the *Plasmodium* transcriptome (20). During the infection, miRNAs translocate intracellularly from its cytoplasm to the parasitophorous vacuole (PV) and their enrichment in the *P. falciparum* infected erythrocytes shows strong correlation between them (20). In order to identify the possible interactions between miR-451a and *P. falciparum* mRNAs' computational prediction software PSRNA tool was performed. Bioinformatics analysis revealed novel target genes through chimeric fusions of (hsa-miR-451a-5p: mRNAs of *P. falciparum*), which down-regulate the genes responsible for the invasion, pathogenesis, and survival of *P.*

Table 1 Predicted *P. falciparum* mRNA targets by erythrocytic hsa-miR-451a-5p

Target_Desc	miRNA_start	miRNA_end	Target_start	Target_end	Alignment	Inhibition	Expectation
Transcription factor with AP2 domain(s) (PF3D7_1007700)	1	22	3794	3815	.:::::.:::::..::: :	Cleavage	5.0
Proteasome subu beta type-5 (PF3D7_1011400), partial mRNA	1	22	554	575	:.:::::: .::::::..:::	Cleavage	5.0
Duffy binding-like merozoite surface protein (PF3D7_1035700)	1	22	353	374	.:.:.. :.:::::::..: :::	Cleavage	5.0
Oxysterol-binding protein-related protein 2 (PF3D7_1131800)	1	22	1577	1598	: .:.:::::::::::. :::	Cleavage	4.0
Conserved Plasmodium protein, unknown function (PF3D7_1140700)	1	22	6329	6350	:.: :::::::...: :::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_1150400)	1	22	6518	6539	::. . ::.:::::.:.::::	Cleavage	5.0
Conserved Plasmodium protein, unknown function (PF3D7_1420100)	1	22	1907	1928	.:::::::::::. :::	Cleavage	5.0
40S ribosomal protein S5 (PF3D7_1447000)	1	22	344	365	:..::.::::::.:::	Cleavage	4.5
Appr-1-p processing domain protein (PF3D7_1448800)	1	22	629	650	:::..:::.::::: . ::::	Cleavage	5.0
26S proteasome regulatory subunit RPN2,putative (PF3D7_1466300)	1	22	2763	2784	: ::::: .::::: .::::	Cleavage	4.5
Conserved Plasmodium protein, unknown function (PF3D7_1467600)	1	22	3575	3596	..::::::::::.:::	Cleavage	5.0
Diacylglycerol kinase, putative (PF3D7_1471400)	1	22	545	566	.:..::: ::::::.: :::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0712800)	1	22	2633	2654	::::.::::::::::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0808600)	1	22	212	233	::::.: ::.::::..::: :	Cleavage	4.5
DNA repair and recombination protein RAD54 (PF3D7_0803400)	1	22	2504	2525	.:::::::::::.:::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0800100)	1	22	332	353	:: .::::::::.::: ::	Cleavage	4.5
Serine/threonine protein kinase, putative (PF3D7_0214600)	1	22	557	578	::::::::::.: :::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0223500)	1	22	3866	3887	.:: . :::.::::.:.:::::	Cleavage	4.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_1373500)	1	22	3842	3863	.:: . :::.::::.:.:::::	Cleavage	4.0
Perforin-like protein 2 (PF3D7_1216700)	1	22	2593	2615	.: ::: :::.:::::::.:: ::	Cleavage	4.5
ATP-dependent protease subunit ClpQ (PF3D7_1230400)	1	22	426	446	::: :.:::::::::.:.: :::	Cleavage	5.0

Table 1 *(continued)*

Table 1 *(continued)*

Target_Desc	miRNA_start	miRNA_end	Target_start	Target_end	Alignment	Inhibition	Expectation
ATP-dependent protease subunit ClpQ (PF3D7_1230400)	1	22	152	172	::.::.::::: ::.:::::	Cleavage	4.5
Protein TOC75, putative (PF3D7_1234600)	1	22	2030	2051	.:::::::::::.: ::	Cleavage	4.5
Apicoplast dimethyladenosine synthase, putative (PF3D7_1249900)	1	22	1151	1172	:: : :.:::::::::::: :	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_1255200)	1	22	1043	1064	::. . :::.:::::::.:: ::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0100100)	1	22	1640	1661	::.:. ::::::::::.::::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0300100)	1	22	3680	3701	.:. .: :.::::: .::::::::	Cleavage	5.0
DNA polymerase delta small subunit, putative (PF3D7_0308000)	1	22	1322	1343	:.:.: ::::::::: :::	Cleavage	4.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0400100)	1	22	1709	1730	:..:. ::::::::::.::::	Cleavage	4.5
Protein phosphatase PPM1, putative (PF3D7_0410300)	1	22	1988	2009	: .:.: ::::: ::.:::::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0412400)	1	22	2756	2777	:::: ::.::::::::: :::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0420700)	1	22	218	239	:::::.: ::::::::..::::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0420900)	1	22	218	239	:::::.: ::::::::..::::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0421100)	1	22	1016	1037	.:. . :::.:::::::.:: ::	Cleavage	5.0
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0421300)	1	22	3800	3821	::.::::::.:.:::::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0426000)	1	22	2519	2540	:::: ::.::::::::: :::	Cleavage	5.0
Merozoite surface protein 8 (PF3D7_0502400)	1	22	293	314	.: ::::::::::.:: ::	Cleavage	4.5
Single-stranded DNA-binding protein (PF3D7_0508800)	1	22	674	695	:.: :::::::::.: :::	Cleavage	3.5
Conserved Plasmodium protein, unknown function (PF3D7_0529700)	1	22	2366	2387	::.: ::: :::::. ::::	Cleavage	5.0
Falstatin (PF3D7_0911900)	1	22	853	875	.:.::::::: ::: ::.:::::	Cleavage	5.0
Alkaline phosphatase, putative (PF3D7_0912400)	1	22	648	669	:: : :: ::::: ::.:::::	Cleavage	5.0
SUMO-conjugating enzyme UBC9 (PF3D7_0915100)	1	22	86	107	: :::.::::: :: :::::	Cleavage	4.5

Table 1 (*continued*)

Table 1 (*continued*)

Target_Desc	miRNA_start	miRNA_end	Target_start	Target_end	Alignment	Inhibition	Expectation
Conserved Plasmodium protein, unknown function (PF3D7_0924600)	1	22	4271	4292	: : :: ::::::::...: :::	Cleavage	5.0
Delta tubulin, putative (PF3D7_0933800)	1	22	995	1016	.: :.::::::.::::::	Cleavage	4.5
Conserved Plasmodium protein, unknown function (PF3D7_0506500)	1	22	7283	7304	:.: ::.::::::.:::	Cleavage	5.0
1-cys peroxiredoxin (PF3D7_0729200)	1	22	497	518	: :::.::::::::. :::	Cleavage	4.5
Erythrocyte membrane protein 1, PfEMP1 (PF3D7_0425800)	1	22	7856	7877	:..:. :::.::::.:.:: ::	Cleavage	4.5
Phosphatidylserine synthase, putative (PF3D7_1366800)	1	22	375	396	.:::: : :::: .:.:::::	Cleavage	4.5
Rifin (PF3D7_1372700)	1	22	668	689	:.:.: ::::::.:.: :::	Cleavage	5.0
Rifin (PF3D7_1373400)	1	22	1021	1042	:: ::.::::::. ::::	Cleavage	4.5
WD repeat-containing protein 16, putative (PF3D7_1348700)	1	22	911	932	.:::.::::.:.::::	Cleavage	5.0
Polyadenylate-binding protein, putative (PF3D7_1360900.1)	1	22	458	479	:::::: ::: :.:::::	Cleavage	4.5
ATP-dependent RNA helicase, putative (PF3D7_0103600)	1	22	3875	3896	.:::::.::::. ::::::	Cleavage	4.0
Conserved Plasmodium protein, unknown function (PF3D7_1238700)	1	22	146	167	.:: ::::::::. ::::	Cleavage	4.5
Rifin (PF3D7_0600700)	1	22	671	692	:. .: :::::::.:.: :::	Cleavage	5.0
Cleavage stimulation factor subunit 1, putative (PF3D7_0620500)	1	22	1667	1688	:.:.: : ::::::.:::::	Cleavage	4.5

hsa-miR-451a-5p was targeted on the *P. falciparum* transcript and crucial gene targets were identified having seed region 2–13 nucleotide consist of 1 or 2 gaps and expect value ≥4.5. hsa-miR-451a downregulates the various genes of *P. falciparum* by cleavage of mRNAs. *P. falciparum, Plasmodium falciparum*; hsa-miR-451a-5p, hsa-microRNA-451a-5p; *PfEMP1, Plasmodium falciparum 3D7 erythrocyte membrane protein; TOC75, Translocon of Outer Chloroplast Membrane; PPM1, protein phosphatase PPM1, putative.*

falciparum (*Table 1*).

miRNA-target genes' interactions

Considering the importance of miRNA-451a during host-parasitic interactions, it is essential to determine if any of the gene is having more than one targets for the mir-451a. As expected, number of targeting events appeared more than one in certain genes (*Figure 1*). These may be due to the length of the genes and having multiple complementary sites within the gene. Out of total targeted genes *PfEMP1* gene contributed 33% participation (*Figure 1*). miR-451a showed multiple targets on the *PfEMP1* gene and suppressed its expression by cleavage of *PfEMP1* mRNA. After *PfEMP1*, uncharacterized genes with unknown functions were reported with maximum targets (*Figure 1*). These uncharacterized genes and their products add to the difficulties of recognizing the parasite biology. *Rifin* gene contributed to 8% with four targets within the gene (*Figure 1*). The remaining genes showed one target site for miR-451a (*Figure 1*).

Gene ontology of targeted P. falciparum genes

In order to characterize the crucial predicted gene products, GO categorization using g:Profiler Software (g:GOSt) (https://biit.cs.ut.ee/gprofiler/gost) was performed to investigate the MF, BP, CC and associated GO terms with hsa-miR-451a-5p targets (*Figure 2*).

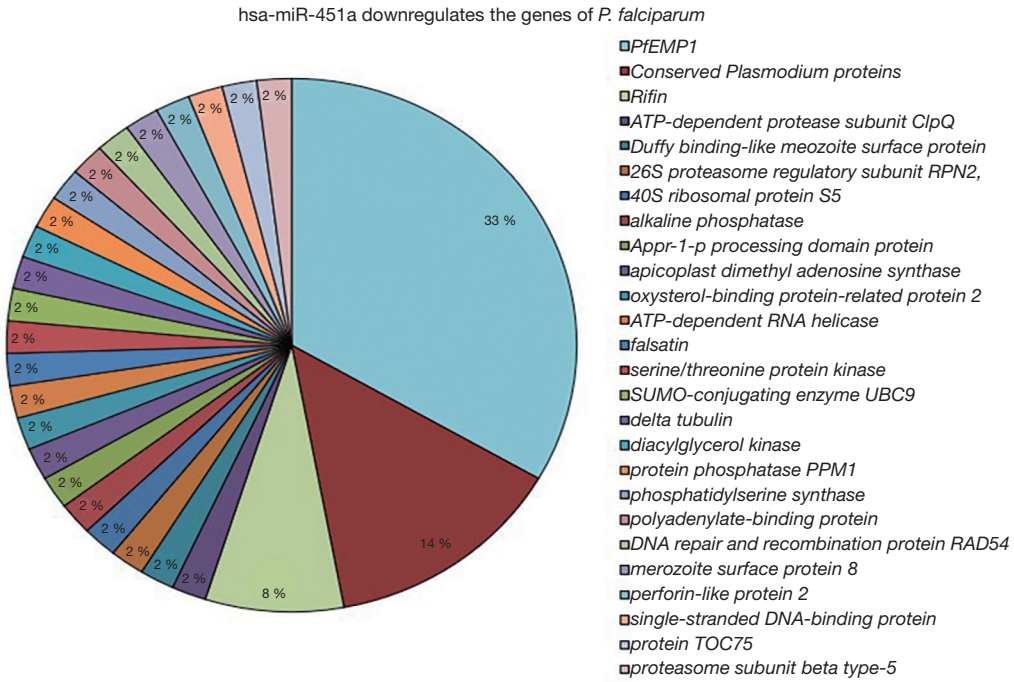


Figure 1 Identification of predicted *P. falciparum* gene targets gaining single or multiple target events within the gene by hsa-miR-451a-5p. *P. falciparum* mRNAs'-miR-451a association confirm single or multiple target sites in the respected genes. *P. falciparum*, *Plasmodium falciparum*; hsa-miR-451a-5p, hsa-microRNA-451a-5p; *PPM1*, *protein phosphatase PPM1*, *putative; TOC75*, *Translocon of Outer Chloroplast Membrane*.

Figure 2 shows the annotation studies of targeted genes mainly contribute to molecular activities like cell adhesion molecule binding, host cell surface and its receptor binding. Whereas, biological processes include cell-cell adhesion, modulation of the host cell processes, response to stimuli/ host's immune response, antigenic variation, etc. Moreover, cellular components include intracellular components' synthesis of the *Plasmodium*.

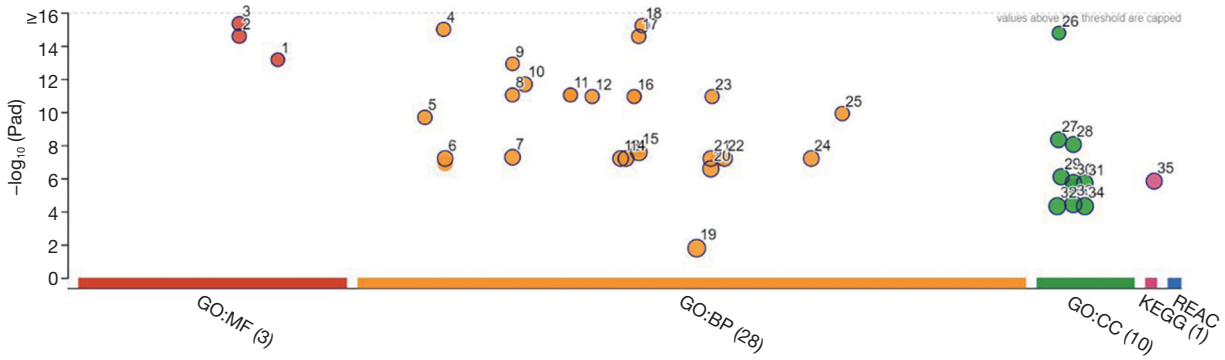
Further detailed analysis uncovered regarding the genes involved in each function mentioned below (*Figure 3*).

Functional studies determined the role of each targeted genes at the molecular, cellular and biological level. As per the list of genes mentioned in the MFs (*Figure 3*), it can be easily projected that *PfEMP 1* having gene IDs: PF3D7_1150400, PF3D7_0223500, PF3D7_1255200) promotes all the three MFs of host cell surface receptor binding (GO ID: 0046789), host cell surface binding (GO ID: 0046812) and cell adhesion molecule binding (GO ID: 0050839) (*Figure 3*). These genes can efficiently rearrange the host cell membrane as well as developing strong intracellular network between PV and cytoplasm of erythrocytes to accomplish the erythrocytic life stages. Majority of miR-451a targeted genes code for the proteins which can efficiently promote BPs such as cell-cell adhesion (GO:0098609), cytoadherence (GO:0020035), antigenic variation (GO:0020033), erythrocytic aggregation (GO:0034117), sense and respond the external stimuli (GO:009605; GO:0009605; GO:0050896) including hosts' immune response (GO:0052200; GO:0052572), etc. (*Figure 3*). miR-451a targeted genes mostly actively synthesize knob on the host cell surface (GO:0020030), cell membrane (GO:0020002), cytoplasmic content (GO:0030430; GO:0033643) and intracellular components (GO:0043656) as a part of cellular component (*Figure 3*).

Analysis of hsa-miR-451a targeted genes to obtain their protein class details using PANTHER classification system

The PANTHER database is designed for browsing ontology terms to retrieve associated protein families, subfamilies as well as individual protein. Targeted genes encoding proteins can be classified to a family or subfamily, and most of these are associated with significant MF or BP or CC classifications (*Figure 4*).

The total proteins class details retrieved from the targeted genes, cytoskeletal protein covers 65% (*Figure 4*),



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0050839	cell adhesion molecule binding	6.880×10^{-14}
2	GO:MF	GO:0046812	host cell surface binding	2.632×10^{-15}
3	GO:MF	GO:0046789	host cell surface receptor binding	4.574×10^{-16}
4	GO:BP	GO:0009405	pathogenesis	1.008×10^{-15}
5	GO:BP	GO:0007155	cell adhesion	2.078×10^{-10}
6	GO:BP	GO:0009607	response to biotic stimulus	6.336×10^{-8}
7	GO:BP	GO:0020033	antigenic variation	5.285×10^{-8}
8	GO:BP	GO:0020013	modulation by symbiont of host erythrocyte aggre...	9.248×10^{-12}
9	GO:BP	GO:0020035	cytoadherence to microvasculature, mediated by s...	1.218×10^{-13}
10	GO:BP	GO:0022610	biological adhesion	2.092×10^{-12}
11	GO:BP	GO:0034109	homotypic cell-cell adhesion	9.248×10^{-12}
12	GO:BP	GO:0035821	modulation of process of other organism	1.149×10^{-11}
13	GO:BP	GO:0042783	evasion of host immune response	6.336×10^{-8}
14	GO:BP	GO:0043207	response to external biotic stimulus	6.336×10^{-8}
15	GO:BP	GO:0044419	biological process involved in interspecies interact...	2.681×10^{-8}
16	GO:BP	GO:0044003	modulation by symbiont of host process	1.149×10^{-11}
17	GO:BP	GO:0044406	adhesion of symbiont to host	2.717×10^{-15}
18	GO:BP	GO:0044650	adhesion of symbiont to host cell	5.997×10^{-16}
19	GO:BP	GO:0050896	response to stimulus	1.636×10^{-2}
20	GO:BP	GO:0051701	biological process involved in interaction with host	2.664×10^{-7}
21	GO:BP	GO:0051707	response to other organism	6.336×10^{-8}
22	GO:BP	GO:0052572	response to host immune response	6.336×10^{-8}
23	GO:BP	GO:0051817	modulation of process of other organism involved...	1.149×10^{-11}
24	GO:BP	GO:0075136	response to host	6.336×10^{-8}
25	GO:BP	GO:0098609	cell-cell adhesion	1.221×10^{-10}
26	GO:CC	GO:0020030	infected host cell surface knob	1.689×10^{-15}
27	GO:CC	GO:0020002	host cell plasma membrane	4.670×10^{-9}
28	GO:CC	GO:0033644	host cell membrane	9.013×10^{-9}
29	GO:CC	GO:0030430	host cell cytoplasm	8.058×10^{-7}
30	GO:CC	GO:0033646	host intracellular part	1.784×10^{-6}
31	GO:CC	GO:0043656	host intracellular region	1.893×10^{-6}
32	GO:CC	GO:0018995	host cellular component	4.793×10^{-5}
33	GO:CC	GO:0033643	host cell part	3.647×10^{-5}
34	GO:CC	GO:0043657	host cell	4.793×10^{-5}
35	KEGG	KEGG:05144	Malaria	1.462×10^{-6}

version	e103_eg50_p15_68c0e33
date	21/04/2021, 18:35:45
organism	pfalciparum

Figure 2 Graphical representation of GO annotations of hsa-miR-451a-5p targeted genes with g:Profiler Software (g:GOSt). It is a g:GOSt multi query Manhattan plot. X-axis shows the functional terms grouped and colour-coded by data source. P values in the table outputs are color-coded from yellow (insignificant) to blue (highly significant). The P values from other queries are indicated next to the y-axis for easier comparison (1,3,10,28). GO, gene ontology; MF, molecular function; BP, biological process; CC, cellular component; hsa-miR-451a-5p, hsa-microRNA-451a-5p.

which is encoded by vital genes of *P. falciparum* such as *PfEMP1* and *Duffy* binding-like (*DBL*) merozoite surface protein *MSP8* (PF3D7_1035700). Targeted gene products except uncharacterized proteins have been classified under PANTHER protein class of PANTHER database and as mentioned in *Table 2*.

Figure 3 Functional profiling of targeted genes displayed with their respective gene IDs. It is showing detailed result table with numeric targeted Gene IDs with its GO studies. GO, gene ontology; MF, molecular function; BP, biological process; CC, cellular component.

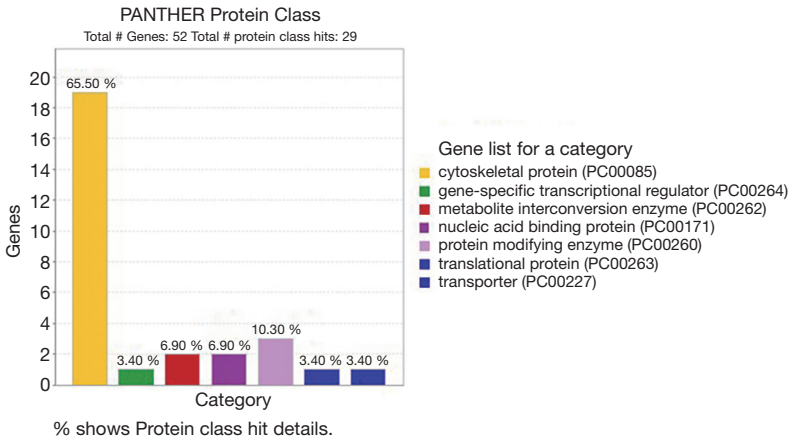


Figure 4 It is PANTER-Protein Class details. Bar-chart for the protein classes of *Plasmodium* genes which were targeted by erythrocytic miRNAs. Protein classes of genes were obtained from Panther. X-axis shows the number of targeted genes with respected protein classes. Y-axis shows the no. of genes involved in the respected category. % Shows the Protein class hit details. miRNAs, microRNAs.

Table 2 Protein class details of targeted gene from PANTHER database

Gene ID	Mapped Ids	Gene name-gene symbol-ortholog	PANTHER family/subfamily	PANTHER protein class
PLAF7|EnsemblGenome=PF3D7_0410300|UniProtKB=Q9U0I5	PF3D7_0410300	*Protein phosphatase PPM1, putative;PF3D7_0410300;ortholog*	PROTEIN PHOSPHATASE 1G (PTHR13832:SF321)	Protein phosphatase (PC00195)
PLAF7|EnsemblGenome=PF3D7_0300100|UniProtKB=O97324	PF3D7_0300100	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0300100;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0420700|UniProtKB=Q8IFQ6	PF3D7_0420700	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0420700;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_1216700|UniProtKB=Q8I5P0	PF3D7_1216700	*Perforin-like protein 2;PF3D7_1216700;ortholog*	PERFORIN-LIKE PROTEIN 1 (PTHR19324:SF33)	
PLAF7|EnsemblGenome=PF3D7_0712800|UniProtKB=Q8IBW8	PF3D7_0712800	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0712800;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0214600|UniProtKB=O96226	PF3D7_0214600	*Serine/threonine protein kinase, putative;PF3D7_0214600;ortholog*	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE KINASE (PTHR43895:SF32)	
PLAF7|EnsemblGenome=PF3D7_1238700|UniProtKB=A0A144A4I8	PF3D7_1238700	*Uncharacterized protein;PF3D7_1238700;ortholog*	RE57120P (PTHR11145:SF8)	–
PLAF7|EnsemblGenome=PF3D7_0924600|UniProtKB=Q8I2R1	PF3D7_0924600	*Uncharacterized protein;PF3D7_0924600;ortholog*	–	–

Table 2 (*continued*)

Table 2 (*continued*)

Gene ID	Mapped Ids	Gene name-gene symbol-ortholog	PANTHER family/subfamily	PANTHER protein class
PLAF7|EnsemblGenome=PF3D7_1467600|UniProtKB=A0A144A2Q6	PF3D7_1467600	*Uncharacterized protein;PF3D7_1467600;ortholog*	YALI0C04136P (PTHR35683:SF7)	–
PLAF7|EnsemblGenome=PF3D7_0426000|UniProtKB=Q8IFK7	PF3D7_0426000	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0426000;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_1249900|UniProtKB=A0A144A0V8	PF3D7_1249900	*rRNA adenine N(6)-methyltransferase;PF3D7_1249900;ortholog*	RRNA ADENINE N(6)-METHYLTRANSFERASE (PTHR11727:SF18)	RNA methyltransferase (PC00033)
PLAF7|EnsemblGenome=PF3D7_0502400|UniProtKB=Q8I476	PF3D7_0502400	*Merozoite surface protein 8;PF3D7_0502400;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_0620500|UniProtKB=C6KT45	PF3D7_0620500	*Cleavage stimulation factor subunit 1, putative;PF3D7_0620500;ortholog*	WD REPEAT-CONTAINING PROTEIN WDR-5.2-RELATED (PTHR42968:SF10)	Scaffold/adaptor protein (PC00226)
PLAF7|EnsemblGenome=PF3D7_1230400|UniProtKB=Q8I5B6	PF3D7_1230400	*ATP-dependent protease subunit ClpQ;PF3D7_1230400;ortholog*	ATP-DEPENDENT PROTEASE SUBUNIT HSLV (PTHR32194:SF0)	Metalloprotease (PC00153)
PLAF7|EnsemblGenome=PF3D7_1234600|UniProtKB=Q8I576	PF3D7_1234600	*Protein TOC75, putative;PF3D7_1234600;ortholog*	TOC75, PUTATIVE-RELATED (PTHR37001:SF10)	
PLAF7|EnsemblGenome=PF3D7_0803400|UniProtKB=Q8IAN4	PF3D7_0803400	*DNA repair and recombination protein RAD54,putative;PF3D7_0803400;ortholog*	DNA EXCISION REPAIR PROTEIN ERCC-6-RELATED (PTHR45629:SF7)	
PLAF7|EnsemblGenome=PF3D7_1255200|UniProtKB=Q8I4N5	PF3D7_1255200	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_1255200;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0100100|UniProtKB=Q9NFB6	PF3D7_0100100	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0100100;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0412400|UniProtKB=Q9U0G6	PF3D7_0412400	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0412400;ortholog*	–	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0911900|UniProtKB=Q8I333	PF3D7_0911900	*Falstatin;PF3D7_0911900;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_0729200|UniProtKB=Q5MYR6	PF3D7_0729200	*Peroxiredoxin;prx;ortholog*	PEROXIREDOXIN-5, MITOCHONDRIAL (PTHR10430:SF16)	
PLAF7|EnsemblGenome=PF3D7_1011400|UniProtKB=Q8IJT1	PF3D7_1011400	*Proteasome subunit beta;PF3D7_1011400;ortholog*	PROTEASOME SUBUNIT BETA (PTHR11599:SF63)	

Table 2 (*continued*)

Table 2 (*continued*)

Gene ID	Mapped Ids	Gene name-gene symbol-ortholog	PANTHER family/subfamily	PANTHER protein class
PLAF7|EnsemblGenome=PF3D7_0506500|UniProtKB=C0H4C9	PF3D7_0506500	*Uncharacterized protein;PF3D7_0506500;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_1420100|UniProtKB=Q8ILP9	PF3D7_1420100	*Uncharacterized protein;PF3D7_1420100;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_1471400|UniProtKB=Q8IKC5	PF3D7_1471400	*Diacylglycerol kinase;PF3D7_1471400;ortholog*	DIACYLGLYCEROL KINASE (PTHR11255:SF84)	Kinase (PC00137)
PLAF7|EnsemblGenome=PF3D7_1373400|UniProtKB=C0H5N9	PF3D7_1373400	*Rifin;PF3D7_1373400;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_1447000|UniProtKB=Q8IL02	PF3D7_1447000	*40S ribosomal protein S5;PF3D7_1447000;ortholog*	RIBOSOMAL PROTEIN S2 (PTHR13718:SF4)	Ribosomal protein (PC00202)
PLAF7|EnsemblGenome=PF3D7_1007700|UniProtKB=Q8IJW6	PF3D7_1007700	*Transcription factor with AP2 domain(S);PF3D7_1007700;ortholog*	COUNTIN-LIKE PROTEIN-RELATED (PTHR23353:SF16)	GTPase-activating protein (PC00257)
PLAF7|EnsemblGenome=PF3D7_0600700|UniProtKB=C6KSL2	PF3D7_0600700	*Rifin;PF3D7_0600700;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_1035700|UniProtKB=Q8IJ52	PF3D7_1035700	*Duffy binding-like merozoite surface protein;PF3D7_1035700;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_1466300|UniProtKB=Q8IKH3	PF3D7_1466300	*26S proteasome regulatory subunit RPN2,putative;PF3D7_1466300;ortholog*	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 1 (PTHR10943:SF2)	Protease (PC00190)
PLAF7|EnsemblGenome=PF3D7_1348700|UniProtKB=Q8IDL2	PF3D7_1348700	*WD repeat-containing protein 16, putative;PF3D7_1348700;ortholog*	CILIA- AND FLAGELLA-ASSOCIATED PROTEIN 52 (PTHR13720:SF14)	
PLAF7|EnsemblGenome=PF3D7_1140700|UniProtKB=Q8IHV8	PF3D7_1140700	*Uncharacterized protein;PF3D7_1140700;ortholog*	DHARMA (PTHR24329:SF337)	Homeodomain transcription factor (PC00119)
PLAF7|EnsemblGenome=PF3D7_1372700|UniProtKB=C0H5N3	PF3D7_1372700	*Rifin;PF3D7_1372700;ortholog*	–	–
PLAF7|EnsemblGenome=PF3D7_0421300|UniProtKB=Q8IFQ2	PF3D7_0421300	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0421300;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_1150400|UniProtKB=Q8IHM0	PF3D7_1150400	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_1150400;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)

Table 2 (*continued*)

Table 2 (*continued*)

Gene ID	Mapped Ids	Gene name-gene symbol-ortholog	PANTHER family/subfamily	PANTHER protein class
PLAF7|EnsemblGenome=PF3D7_1373500|UniProtKB=Q8ID12	PF3D7_1373500	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_1373500;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0420900|UniProtKB=Q8IFQ5	PF3D7_0420900	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0420900;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0912400|UniProtKB=Q8I328	PF3D7_0912400	*Alkaline phosphatase, putative;PF3D7_0912400;ortholog*	CALCINEURIN-LIKE METALLO-PHOSPHOESTERASE SUPERFAMILY PROTEIN (PTHR33987:SF1)	
PLAF7|EnsemblGenome=PF3D7_0400100|UniProtKB=Q8I220	PF3D7_0400100	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0400100;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_1448800|UniProtKB=Q8IKY5	PF3D7_1448800	*Appr-1-p processing domain protein;PF3D7_1448800;ortholog*	POLY [ADP-RIBOSE] POLYMERASE (PTHR11106:SF27)	
PLAF7|EnsemblGenome=PF3D7_0808600|UniProtKB=Q8IAS3	PF3D7_0808600	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0808600;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0421100|UniProtKB=Q8IFQ4	PF3D7_0421100	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0421100;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)
PLAF7|EnsemblGenome=PF3D7_0933800|UniProtKB=Q8I2I0	PF3D7_0933800	*Delta tubulin, putative;PF3D7_0933800;ortholog*	TUBULIN DELTA CHAIN (PTHR11588:SF4)	Tubulin (PC00228)
PLAF7|EnsemblGenome=PF3D7_0529700|UniProtKB=Q8I3H1	PF3D7_0529700	*Uncharacterized protein;PF3D7_0529700;ortholog*	TRANSMEMBRANE PROTEIN 212 (PTHR23320:SF130)	Transporter (PC00227)
PLAF7|EnsemblGenome=PF3D7_0425800|UniProtKB=Q8I098	PF3D7_0425800	*Erythrocyte membrane protein 1, PfEMP1;PF3D7_0425800;ortholog*	TRANSGELIN (PTHR18959:SF23)	Non-motor actin binding protein (PC00165)

Displays the information of *Plasmodium* proteins coded by miR-451a targeted genes. Protein details consist of information regarding protein class, protein name and their functions which may help in understanding the possible protein-protein interactions between erythrocytic and plasmodium proteins. PfEMP1, *Plasmodium falciparum 3D7 erythrocyte membrane protein; TOC75, Translocon of Outer Chloroplast Membrane;* miR-451a, microRNA-451a.

Proteins like *tubulin*, dorsal interacting protein 4, oxysterol-binding protein, ribosomal proteins and enzymes like ATP dependent RNA helicase, proteases are crucial targets to affect the growth and intracellular development of *P. falciparum*. Proteases and *tubulin* may participate in establishing intracellular network for the transport of nutrients and some essential bio molecules between the parasite and its host erythrocytes. These proteins can be key molecules to comprehend the host-parasitic information and help us to discover the targets of various anti-malarial bioactive compounds.

Protein-protein interactions and pathway prediction analysis of the miR-451a targeted genes

Comprehensive pathway prediction analysis was performed of the targeted genes of *P. falciparum*. Among the all genes, mainly genes were found principally associated with RNA

Table 3 Total 07 genes were found in protein-protein intearctions—STRING database

#node	Annotation of Input proteins
MAL13P1.303.1	Polyadenylate-binding protein (414 aa)
MSP8	Merozoite Surface Protein 8 (597 aa)
PF08_0042	Uncharacterized protein; ATP-dependent RNA helicase prh1 (867 aa)
PFA_0180w	Uncharacterized protein; ATP-dependent RNA Helicase (1472 aa)
PFC0915w	DEAD-box ATP-dependent RNA helicase 6; RNA helicase (433 aa)
PFL1170w	Polyadenylate-binding protein; Binds the poly(A) tail of mRNA (875 aa)
PFL2395c	rRNA adenine N(6)-methyltransferase; Apicoplast dimethyladenosine synthase (639 aa)

degradation (map03018) and RNA transport (map03013) KEGG pathways. Whereas around 20 genes demonstrated a correlation with the KEGG:05144 Malaria pathway (https://www.kegg.jp/entry/pathway+map03018).

To identify the groups of interacting proteins and hub genes STRING database and Cytoscape software has been used. Out of total genes, 07 genes were found in protein-protein interactions. The input gene list has been mentioned below (*Table 3*) which involved in the protein-protein interactions (PPI) with the other genes and code for the protein domains belong to Helicase conserved superfamily C terminal domain region, Middle domain of eukaryotic initiation factor 4 G (eIFG4), epidermal growth factors, DEAD/DEAH box superfamily. While predicted functional protein partners in the PPI involved uncharacterized protein; DEAD box helicase 19 (PF14_0185), *MSP* 4, *MSP* 5 and uncharacterized protein; ATP-dependent RNA helicase, MIF4G domain containing protein—(PF11_0086), Aspargine rich protein—(MAL13P1.63), uncharacterized protein, ATP dependent RNA Helicase (PFL0100c), DnaJ/SEC63 protein (PF13_0102), RNA helicase (PF08_0111), uncharacterized protein eIF4, belong to the eukaryotic initiation factor 4E family (PFC0635c), uncharacterized protein DEAD/DEAH box Helicase (PF14_0370), merozoites surface protein 8—*MSP8* (*Figure 5*).

The hsa-miR-451a targeted crucial gene *PfEMP1* showed PPI with the ten genes (*Figure 6*). Among these genes most of them are the variants of *PfEMP1* (*Figure 6*). *Rifin* and gene belong to *Var* gene family are showed interactions and coexpression with *PfEMP1*.

Protein domains description involve PFEMP *DBL* domain (PF03011), N-terminal segments of *PfEMP1* (PF15447), acidic terminal segments, variant surface antigen of *PfEMP1* (PF15445), Duffy binding domain (PF05424). These genes were associated with KEGG malaria pathway (map05144).

Discussion

To uncover the role of erythrocytic miRNA (miR-451a) mediated mRNA modulation in *Plasmodium* and to predict the cross-kingdom dynamics between different species, target identification and possible interactions between erythrocytic miRNA-*Plasmodium* mRNAs' study carried out. Among the several erythrocytic miRNAs, miR-451 is extensively studied to understand the erythrocytic physiology under various pathophysiological conditions (19,20,23,29,36). Many studies have been conducted to determine its significance and to establish as a biomarker or therapeutic agents in various erythrocytes related disorders or diseased conditions including malaria (19,20,30,31,37). Translocation of ~100 erythrocytic miRNAs have been found in the PV formed by the parasite inside the erythrocytes. After parasite invasion, expression of miR-451 peaked after 32 hrs, indicating uptake of this miRNA during intra-erythrocytic cycle (38). It has been observed that miR-451 is located in the parasitophorous vacuole membrane (PVM) in infected erythrocytes (19,20), which suggests that translocation of these miRNAs to transcriptome of parasite takes place as a part of host protective mechanism. As per the miR-451a target prediction study, *PfEMP1* and *rifin* genes contributed with multiple binding sites for miR-451a and their downregulation may affect the occurrence like cell-cell adhesion, antigenic variation, erythrocytic aggregation and host cell surface knob formation. Protein families associated with these genes are *DBL* adhesive domains, present on the surface of the iEs and mainly involve in erythrocyte invasion and cytoadherence of iEs. The genes (*PfEMP1*, *rifin*, *MSP8*, *TOC75*, *oxysterol binding proteins*, *tubulin*, *DBL MSP)* belonged to the formation and alteration of host's intracellular, cytoplasmic and membrane components because of these protein details retrieved from the total

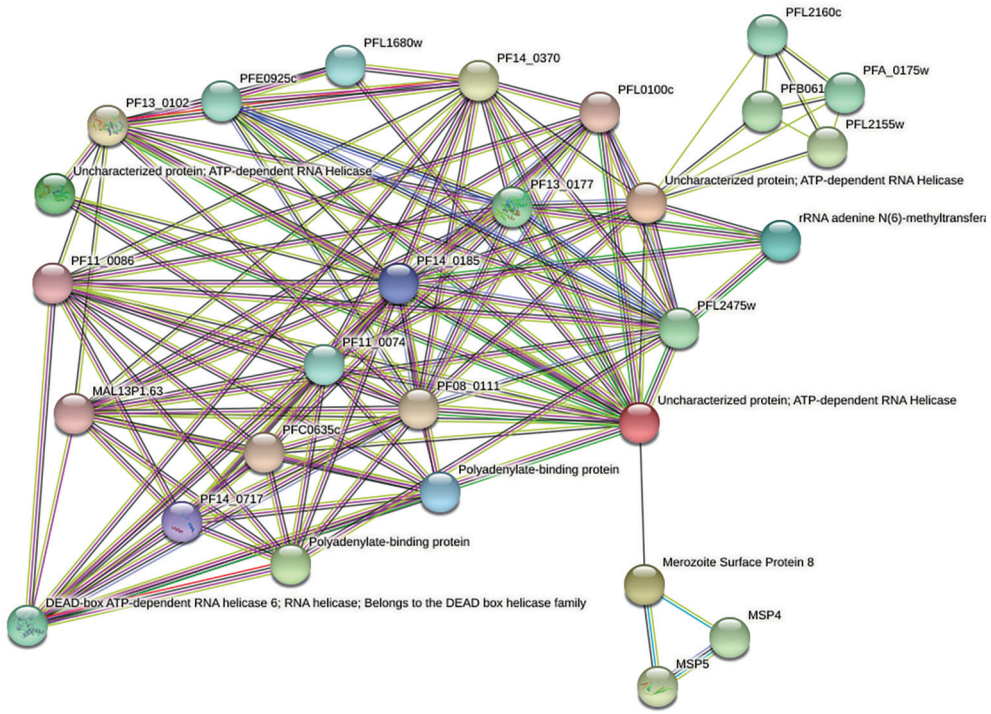


Figure 5 Identification of PPIs and hub proteins. The top 07 hub genes were found in PPI network of predicted target genes in STRING database. Figure shows number of nodes: 28, number of edges: 130, average node degree: 9.29, avg. local clustering coefficient: 0.794, expected number of edges: 46, PPI enrichment P value: <1.0e-16. PPI, protein-protein interaction.

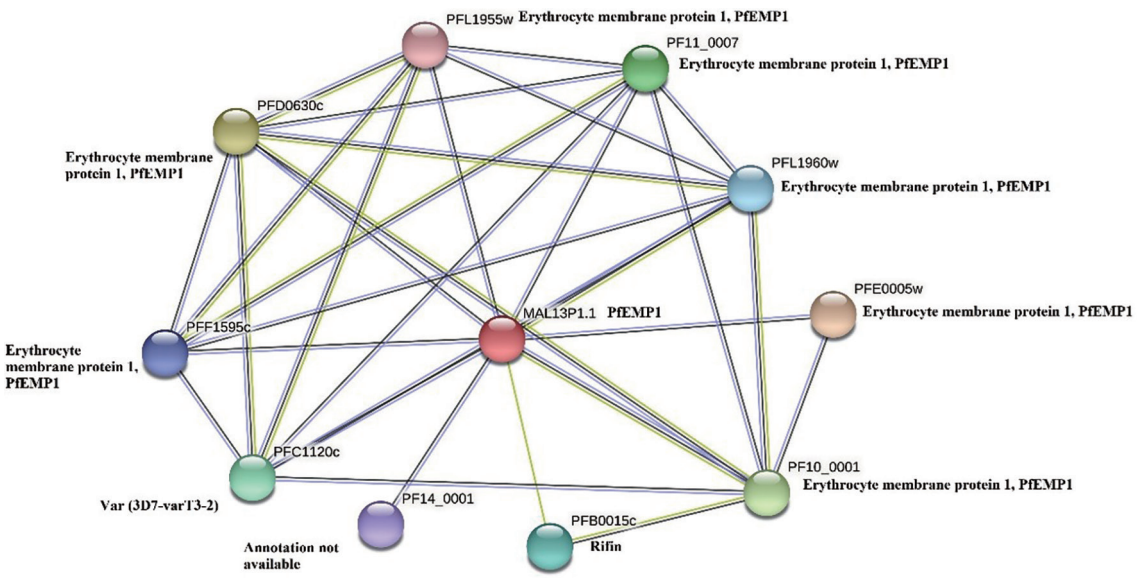


Figure 6 shows that *PfEMP1* gene was found in PPI network of ten predicted target genes in STRING database. Number of nodes: 11, number of edges: 31, average node degree: 5.64, avg. local clustering coefficient: 0.878, expected number of edges: 10, PPI enrichment P value: 1.1e-07. *PfEMP1*, *Plasmodium falciparum 3D7 erythrocyte membrane protein*; PPI, protein-protein interaction.

targeted genes belong to the cytoskeletal proteins. Gene annotation and protein-protein interaction studies indicated mainly uncharacterized proteins. Beside the uncharacterized genes, chiefly genes (PF3D7_0620500, PF3D7_1007700, PF3D7_1447000, PF3D7_1448800, PF3D7_0915100, PF3D7_0103600, PF3D7_1360900.1, PF3D7_1249900) engaged in the RNA metabolism chiefly in RNA processing and degardation pathways. The active participation of these genes showed the importance of RNA metabolism due to the various intraerythrocytic life stages (trophozoites, schizonts, ring stages) specific protein expressions and its strict regulation. Erythrocytic miR-451a targeted *P. falciparum* genes that have a major role in governing various pathways include (I) vesicular trafficking pathway mediated by *Perforin Like Protein 2*, *TOC75*; (II) intracellular lipid/sterol pathway mediated by *oxysterol binding protein* which serve as a key molecule with varied functional relevance. More than one noteworthy genes belong to the key enzymes, those were found to be down regulated by miR-451 are (I) peroxidase (*2-Cys peroxiredoxin)*; (II) phosphatases [*alkaline phosphatase*, *protein phosphatase PPM1*, *putative (PPM1)*]; (III) proteases *ATP-dependent protease subunit ClpQ*; (IV) proteases inhibitor *(falstatin)*; (V) kinases *(diacylglycerol kinase*, *serine threonine protein kinase*); (VI) synthase (*apicoplast dimethyl adenosine synthase*); (VII) helicases (*ATP-dependent RNA helicase)*; (VIII) polymerases (*DNA polymerase delta small subunit*). These key enzymes are thought to be essential regulators of numerous cells signaling pathways.

Detailed analysis of target genes and their products confirm their role in pathogenesis mentioned in *Table 4*.

Several reports indicated that, miR-451 is found to be elevated in sickle cell erythrocytes (19,76), and is associated with significantly reduced parasitaemia (19,20,77). miRNAs profile of HbAS and HbSS erythrocytes may significantly affect the parasite growth and survival (20,30,76,77). Thus, this may be the one of the protective mechanisms mediated by the host erythrocytes, which affects the intracellular parasite growth and survival (20). While the average levels of plasma miR-451 and miR-16 were significantly found lower in malaria patients (28). Moreover, transfection of miR-451 and miR-223 into *P. falciparum* iEs resulted into 50% reduction in parasitemia (20). This means miR-451a has strong relationship with the intracellular parasite growth and its survival (*Figure 7*) but, the expression of this miRNA can be crucial factor during the pathogensis and it will decide the fate of the diseased condition in the body.

Now a days many bioinformatic analysis have been performed to understand the relationship between the dysregulation of miRNA and many infectious diseases (23,78,79). Based on this study it can be said that, erythrocytic miRNAs can modulate the gene expression via complementary binding on the target gene. This analysis predicts the significant outcome of a hsa-miR-451a (*Figure 7*). Based on this study, the mechanism behind altered miR 451a profile of sickle erythrocytes and their contribution in providing protection against plasmodial attack can also be easily understood miR-451.

Conclusions

Conclusively, the result indicated that miR-451a targeted maximally to the uncharacterized genes comprising unknown functions, which means no. of key protein molecules and its role in pathogenesis is yet to be discovered. Rest of the targeted genes and their products are involved mainly in the (I) modifications of erythrocyte membrane, cytoskeletal proteins to establish strong interactions with the host cell, as well as in the build-up of a strong intracellular network for the transportation of nutrients and release of waste materials; (II) RNA metabolism to regulate the synthesis of stage specific required proteins etc. Very few proteins are usually get involved in synthesis of transporters, their proliferation, response to external stimuli, DNA repair, etc. This shows the significance of miR-451a during the erythrocytic stages of *P. falciparum*. Enrichment of miR-451a in sickle cell anaemia can be one of the important initiatives of host defence mechanism to combat the parasite *Plasmodium* infection.

Additional *in vitro* as well as *in vivo* studies will be required to elucidate the mechanism of trans kingdom interactions related to erythrocytic miR-451a and *P. falciparum* transcript. Moreover, level of miR-451a inside the erythrocytes in normal as well as during various pathophysiological conditions and in the blood as form of extracellular vesicles (EVs) may provide key information about host-parasite complexity and the influence of its interaction with *Plasmodium* can be proved this molecule as a disease specific and accurate potential biomarker in malaria. Additionally, miRNA based novel therapeutic

Table 4 miR-451a downregulated targeted genes of P. falciparum and its association with the malaria

Target gene	Mode of action by miR-451a	Role in pathogenesis of malaria	References
PfEMP1 (PF3D7_1373500) (PF3D7_0223500) (PF3D7_0100100) (PF3D7_0808600) (PF3D7_0400100) (PF3D7_0425800) (PF3D7_0800100 (PF3D7_0421300) (PF3D7_0420900) (PF3D7_0420700) (PF3D7_0412400) (PF3D7_0426000 (PF3D7_1150400) (PF3D7_0712800) (PF3D7_1255200)	Down regulation	Responsible for cerebral and placental malaria. Key player in antigenic variation	(39-41)
Rifin (PF3D7_0701800) (PF3D7_1373400) (PF3D7_0600700) (PF3D7_1372700)	Down regulation	Belongs to surface antigen family and exports proteins into host cell	(42-44)
ClpQ (PF3D7_1230400)	Down regulation	ATP dependent threonine dependent protease-active mediators of the heat shock response network and essential for Plasmodium's growth and development	(45-48)
Peroxiredoxin (Prx)	Down regulation	Principal enzyme to reduce peroxides	(49,50)
26S proteasome regulatory subunit RPN2 (PF3D7_1466300)	Down regulation	Involved in the ubiquitin proteasome pathway (26 s proteosome)	(51)
40S ribosomal protein S5 (PF3D7_1447000)	Down regulation	Structural component of apicoplast ribosome and forms ribonucleoprotein complex	(52)
Alkaline phosphatase (PF3D7_0912400)	Down regulation	Noticed on the surface of merozoites. Interacts with GTP cyclohydrolase I and 6-pyruvoyl tetrahydropterin synthase	(52,53)
Appr-1-pase (PF3D7_1448800)	Down regulation	Ubiquitous enzyme involved in the tRNA splicing pathway. It catalyses the conversion of ADP-ribose-1" monophosphate (Appr-1"-p) into ADP-ribose	(54)
Apicoplast dimethyl adenosine synthase (PF3D7_1249900)	Down regulation	Belongs to the class I like SAM-binding methyltransferase superfamily. It interacts non covalently with RNA molecule	(55)
Oxysterol-binding protein-related protein 2 (PF3D7_1131800)	Down regulation	Intracellular lipid transport/sensor between various compartments inside the cell, increases plasma membrane cholesterol levels on the contrary, decreases phosphatidylinositol-4, and 5-bisphosphate levels	(56,57)

Table 4 (*continued*)

Table 4 (*continued*)

Target Gene	Mode of action by miR-451a	Role in pathogenesis of malaria	References
ATP-dependent RNA helicase (PF3D7_0103600)	Down regulation	Associated with RNA processing and synthesis of more mature rRNAs	(58)
TOC75 (PF3D7_1234600)	Down regulation	Role in transportation of apicoplast proteins to the innermost PV Depletion of TOC75 leads to rapid defect in transportation of apicoplast proteins to the innermost PV membrane	(59)
Perforin-like protein 2 (PF3D7_1216700)	Down regulation	Expressed at schizont stage and mature gametocytes. Helps in erythrocyte burst for coming out of Plasmodium due to its membranolytic capabilities	(60)
PfMSP 8 (PF3D7_0502400)	Down regulation	It is GPI anchored protein MSP8 alone or combination of MSP8/MSP1 can be a potential vaccine candidate, as it shares structural similarity with MSP1	(61)
Falstatin (PF3D7_0911900)	Down regulation	Endogenous protease inhibitor, role in hepatocyte invasion by sporozoites. Regulate the erythrocyte rupture	(62-64)
SUMO-conjugating enzyme UBC9 (PF3D7_0915100)	Down regulation	Post-translationally regulators, lowest expression during ring stages, peaking up during trophozoite stages and continues increases during late erythrocytic stages. It promotes cell survival during certain adverse conditions like oxidative stress, hypoxia, heat, etc.	(65,66)
Serine/threonine protein kinase (PF3D7_0214600)	Down regulation	Associated with membranous structure specifically parasite-induced knobs	(67)
Diacyl glycerol kinase-1 (DGKs) (PF3D7_1471400)	Down regulation	Supports PA generation for microneme secretion. PA facilitates signal transduction, membrane dynamics and exocytosis, etc. Depletion of DGK leads to impairs egress of apicomplexan parasite and apoptosis	(68)
Delta tubulin (PF3D7_0933800)	Down regulation	Microtubules are vital in maintaining shape, motility, and infectivity of sporozoites of Plasmodium spp	(69,70)
PPM1 (PF3D7_0410300)	Down regulation	cGMP dependent protein kinase - plays crucial role in exflaggelation.	(71,72)
Polyadenylate-binding protein (PF3D7_1360900.1)	Down regulation	Role in translational repression to regulate protein synthesis during life cycle progression in Plasmodium	(73,74)
DNA repair and recombination protein RAD54 (PF3D7_0803400)	Down regulation	Involves in DNA repair	(75)

P. falciparum, Plasmodium falciparum; *PfEMP1, Plasmodium falciparum 3D7 erythrocyte membrane protein 1*; miR-451a, microRNA-451a; Prx, Peroxiredoxin; *TOC75, Translocon of Outer Chloroplast Membrane*; PV, parasitophorous vacuole; *PfMSP 8, Plasmodium falciparum merozoite surface protein 8; PPM1, protein phosphatase PPM1, putative*; DGKs, diacylglycerol kinase-1; PA, phosphatidic acid.

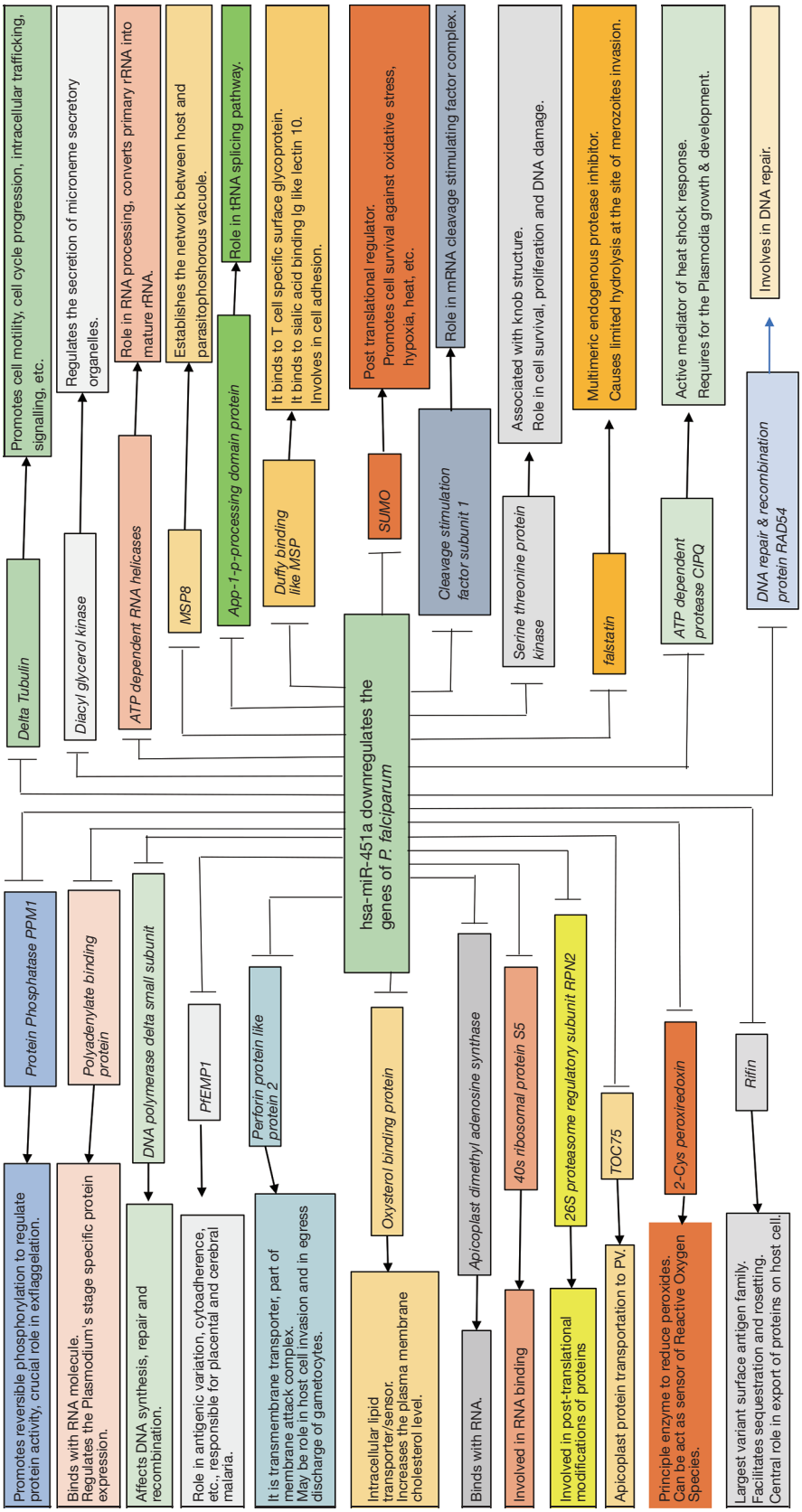


Figure 7 Diagramatic representation shows miR-451a mediated down regulation of *Plasmodium* genes and its possible significant outcome. miR-451a, microRNA-451a; *PPM1, protein phosphatase PPM1, putative; PfEMP1, Plasmodium falciparum 3D7 erythrocyte membrane protein; TOC75, Translocon of Outer Chloroplast Membrane; MSP8, merozoite surface protein 8.*

approach can also be designed in near future using the miRNA mimics or anti-miRs for the treatment of a variety of disease including malaria.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/exrna-21-5). All authors report laboratory and administrative assistance from Gujarat University. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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