INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Kinome of *Plasmodium sp.* underwent unprecedented evolutionary divergence from other eukaryotic kinases. Some of these kinases do not come under the known kinase family due to their extreme divergence from their ancestors. All these kinases are clustered as orphan kinases, FIKK kinases are one among them. *Plasmodium falciparum* FIKK's are the only kinase family among eukaryotic kinases that diverged extraordinarily and expanded from their ancestor. These are trafficked throughout the parasite-infected erythrocytes and pursue important tasks for the survival of plasmodium. The study explored detailed structural characteristics of FIKK9.1 and recommends possible interacting partners in host RBC. FIKK9.1 is essential for parasite survival, but its structural and biochemical characterization will enable us to understand its role in the parasite life cycle. The recombinant FIKK9.1 kinase is monomeric with a native molecular weight of 60 ± 1.6 kDa. Structural characterization of FIKK9.1 kinase reveals that it consists of N-terminal FHA like domain and C-terminal kinase domain. The C-terminal domain has a well-defined pocket, but it displayed an RMSD deviation of 1.38 - 3.2 Å from host kinases. ITC analysis indicates that ATP binds to the protein with a Kd of 45.6±2.4 µM. Co-localization studies revealed FIKK9.1 in the parasite cytosol with a component trafficked to the apicoplast and IRBC. FIKK9.1 has 23 pockets to serve as potential docking sites for substrates. Correlation analysis of peptides from the combinatorial library concluded that peptide P277 (MFDFHYTLGPMWGTL) fitted nicely into the binding pocket. The peptide P277 picked up candidates from parasites and key players from the RBC cytoskeleton. Interestingly, FIKK9.1 is phosphorylating spectrin, CD44, and band-3 from the RBC cytoskeleton. After that, our study explored potential antimalarial from synthetic molecules and natural sources. The rapid emergence of drug-resistant malaria parasites to all frontline antimalarial drugs urges a continuous search for new antimalarial drugs that are beneficial for chemotherapy and prophylaxis. Virtual screening of diverse organic structural scaffolds from the chemical library has identified seven molecules, which could arrest the growth of parasites by inhibiting FIKK9.1 kinase. Evaluation of top hit

compounds in antimalarial activity assay indicates that the highly substituted 1,3-selenazolidin-2-imine 1 and thiophene 2 inhibit parasite growth. The functionalized heterocyclic compounds 1 and 2 are killing the malaria parasite with an IC50 of 2.68 ± 0.02 μg/ml and 3.08 ± 0.14 μg/ml, respectively. Isothermal titration calorimetry analysis indicates heterocyclic scaffolds 1 and 2 abolish the binding of ATP into the FIKK9.1 binding pocket. They in-turn reduces the ability of FIKK9.1 kinase to phosphorylate its substrate, and both compounds are potent inhibitor of FIKK9.1 kinase. Inhibition of FIKK9.1 kinase is disturbing the parasite life cycle and resulting in the parasite's death. Further as a natural source, we exploit potentials of Triphala and shukramatrika as antimalarial and efforts to find its mechanism and mode of action as antimalarial. The water extract of Triphala shows promising effects with schizonticidal and parasiticidal in-vitro plasmodium falciparum 3d7 cultures. The antimalarial activity reveals that inhibition of parasites follows parasiticidal nature. The cytotoxicity on HEK293 and hemolysis analysis suggests Triphala and Shukramatrika is safe to use as antimalarial. The underlying mechanism of parasitic death is evaluated through apoptotic biomarkers, including ROS generation, mitochondrial dysfunction, and in situ DNA fragmentation in Triphala, and Shukramatrika treated and untreated parasites. Our results showed that Triphala and Shukramatrika induce oxidative stress by increasing ROS levels, destabilizing the mitochondrial membrane, and increasing the population of fragmented DNA parasites. Certainly, all those major factors, such as the importance of FIKK's in parasite survival, stable expression in all stages of malaria lifecycle, stable gene transfer, and regions specific to *P. falciparum* paves a new insight through the field of antimalarial drug discovery and development of a new diagnostic marker for malaria. Each FIKK has its unique N-terminal sequences with non-homologs to eukaryotic kinases and kinases in *Plasmodium sp*. These exclusive regions are highly potent to be antigenic which helps in producing specific antibodies against the FIKK kinase(s). Therefore we raised antibodies against FIKK9.1 kinase and identified the anti-FIKK9.1 antibody present in serum specifically detects purified FIKK9.1 as well as *pf*FIKK9.1 present in parasite lysate. These are found to have no cross-reactivity to proteins present in RBC lysate. The antibodies from serum are purified and analyzed for their sensitivity and selectivity. The sensitivity of purified anti-FIKK9.1 polyclonal antibody is validated by estimating the limit of detection using rFIKK9.1 protein. The purified anti-FIKK9.1 detects antigens even at 3 nmole. Further, the cross-reactivity is analyzed using human serum and complete media devoid of parasites. Both the samples show no cross-reactivity with anti-FIKK9.1. The anti-FIKK9.1 antibody detects FIKK9.1 antigens at least to 0.1% parasitemia grown under in-vitro conditions. Surprisingly, purified antibody detects FIKK9.1 in parasite culture media, and further, the detection increases with an increased level of parasitemia. The data suggests FIKK9.1 may export outside the infected parasite. The Semi-quantitative measurements of Parasite load in the infected RBC is studied by performing concentration curve analysis for both rFIKK9.1 and parasite lysate. Further, the anti-FIKK9.1 antibody is validated by mimicking the patient sample and mocking coinfection with other infectious agents. In conclusion, our study highlights the structural and biochemical features of FIKK9.1 to exploit it as a drug target. It provides new insight into the target FIKK9.1 kinase in malaria parasites to develop potent antimalarials. The study finds Triphala and Shukramatrika kill malaria parasites irreversibly through the apoptotic pathway. Further, the research finds that FIKK9.1 is a better biomarker for malaria diagnosis.