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Title: Artemisinin resistance in P. falciparum: Probing the interacting partners of Kelch13 protein in parasite

Abstract:

Malaria remains a global health issue affecting half of the world's population. The current treatment regimen which includes artemisinin and other combination therapies is being threatened with the rapid emergence of resistance. P. falciparum under drug pressure has revealed insights into mechanisms of resistance most commonly used antimalarials, such as Chloroquine, Amodiaquine, Piperaquine, DHFR inhibitors etc. There are currently no alternative drugs available to replace Artemisinin. ART resistance has been shown to be mediated by the Plasmodium Kelch13 (PfK13) protein. Pf kelch 13 gene is situated in chromosome 13 and associated with ART resistance, owing to the association of majority of mutations at the kelch BTB/POZ & propeller domain. The present study recombinantly expr essed the PfK13-p (BTB/POZ & propeller domain) and generated anti-PfK13-p anti bodies for cellular localization and co-immunoprecipitation (co-IP) assays and mass spect roscopy was performed to identify the PfK13 interacting partners. Unique coimmunoprecipitated proteins were identified barring few proteins overlapping with previous studies- Protein disulfide isomerase, heat shock proteins, merozoite surface protei n 1 (MSP1), L-lactate dehydrogenase, elongation factor 1-alpha. The unique hits of the s tudy were- falcilysin, enolase, phosphoethanolamine N-methyltransferase, glideosome-asso ciated protein 50, fructose-bisphosphate aldolase, adenylate kinase, peptidyl-prolyl cis-trans isomerase, thioredoxin-related protein, putative, 20 kDa chaperonin, ornithine aminotra nsferase, rhoptry-associated protein 1. The identified proteins were categorized into protein folding, protein binding/invasion, cellular metabolism and mobility functions. Further, bioinformatics proteins identified by the STRING database represent the PfK13 protein and the respective potential interactors or performing shared functions are shown in network. The minimum interaction score was set to medium confidence level (0.400) and no more than 10 interactors were selected. PGK (Phosphoglycerate kinase) and Q7KQL9 (Fructose- biphosphate aldolase) are the two predicted proteins, which have been identified via co-IP assays. In other experiment, strong binding affinities of PfK13-p and two coimmunoprecipitated proteins- Heat Shock Protein 70 and PfFBAP (6.6 and 7.6 µM, respectively) were observed using surface plasmon resonance (SPR). Additionally, PfMSP1 formed a complex with PfK13-p, as evidenced by pull-down assays. Interestingly, PfKelch13 forms a stable hexamer in N-termini BTB-POZ domain. Further, Using anti-PfK13-p antibodies, the endogenous PfK13 protein was observed to colocalization with a cytosolic marker- PfAspRS (aspartyl transfer-RNA synthetase). Together, this work identified unique interacting partners of endogenous PfK13 protein, which might have crucial implications in the PfK13 protein network and its role in mediating ART resistance.

Biography

Preeti Chaudhary studied biotechnology at the Manav Rachna International University, India graduated in 2015 and then joined as lab assistant in clinical laboratory for 1 year at Asian Institute of Medical Sciences and joined lab as PhD scholar at the Host-Parasite Interaction Biology Group, ICMR-National Institute of Malaria Research, New Delhi, India under the supervision of Dr. Kailash C. Pandey (Scientist-F). I have published 2 research articles.