CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

01. BHARTI (Hina)
Characterization of *Plasmodium Falciparum* Deubiquitinase, PfUCHL3 and Identification of Its Inhibitors with Implication in Malaria Therapy.
Supervisor : Dr.Alo Nag
Th 25602

Abstract
(Not Verified)

Deubiquitinases (DUBs) are highly sophisticated class of enzymes that are specifically primed for removal or editing of the ubiquitin codes and control a variety of critical cellular activities. Extensive involvement and regulation of key biological processes portray DUBs as a prominent therapeutic target for a variety of diseases including malaria, however, parasite DUBs remain poorly explored. PfUCHL3, a member of UCH family of DUBs is identified in P. falciparum with strong implications in parasite survival and emerges as an attractive therapeutic target. Considering its importance, it becomes imperative not only to functionally characterize this enzyme but also to develop specific PfUCHL3 antagonists as novel antimalarials. To this end, a comprehensive structure-function characterization of heterologously expressed PfUCHL3 was performed by utilizing various biochemical and biophysical tools. These assays revealed a constitutive cytoplasmic expression of PfUCHL3 in all asexual stages of the parasite life cycle along with remarkable functional stability in varied stress conditions. Pull-down assay was employed to identify the interactome of this cardinal enzyme. In this series, mutational studies furnish insight into the structure-function importance of non-active site residues in preserving the integrity of PfUCHL3. A combinatorial adventure of in-silico and in-vitro investigations has yielded two novel promising, selective and non-toxic antagonists of PfUCHL3 with potent antimalarial properties. Subsequent biochemical investigations unravelled the critical connection of PfUCHL3 in ART-mediated antimalarial action. In summation, this study characterized the structure-function framework of PfUCHL3, identified potent antimalarials as colorable PfUCHL3 antagonists and presented a plausible mechanism by which ART might pursue its antimalarial endeavor.

Contents

02. CHEEMA (Pradeep Singh)

Identification and Validation of Anti Cancer Ability of Small Molecule Inhibitors against FoxM1.

Supervisor : Dr.Alo Nag

Th 24968

Abstract

(Verified)

Considering the dearth of efficient anti-cancer agents and the high failure rate of candidate molecules in clinical trials, addition of more potent candidate compounds to the drug discovery pipeline seems a vital approach. Forkhead Box M1 is a pleiotropic oncoprotein with crucial impact on various cellular functions by virtue of its transcriptional activity. Upregulation of FoxM1 has established it as a critical therapeutic target, however none of the strategies developed against it have reached the clinics and hence necessitates further attention. In order to develop specific inhibitors against FoxM1, we have performed in silico screening of the diversity set library of small molecules from NCI against the crystal structure of the DNA binding domain of FoxM1 utilizing the Autodock 4.2 software. Preliminary screening of the compounds by MTT assay and subsequent investigation for their impact on the transcriptional activity of FoxM1 provided us 4 compounds with promising anti-FoxM1 activity. These potential hits minimally affect the viability of non-cancerous HEK293T cells. Electrophoretic mobility shift assay revealed that these molecules can hamper the binding of purified FoxM1 DNA binding domain to its consensus DNA. Binding kinetics, mode of interaction and number of binding sites were explored by tryptophan specific fluorescence quenching studies. Our top hits demonstrated a significant curtailing of the malignant behaviour of the cancer cells. In our search for effective therapeutic agents targeting FoxM1, we explored the possible connection between FoxM1 and the anti-cancer drug Camptothecin (CPT). We identified FoxM1 as a novel target of CPT and showed that CPT interacts with FoxM1, obstructs its promoter occupancy and its subsequent transcriptional property. We also demonstrated the influence of CPT treatment on enhancing the sensitivity of cancer cells to the existing FoxM1 inhibitor, Thiostrepton. In a nutshell, this study unveils novel candidates as credible FoxM1 inhibitors with promising anti-cancer properties.

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03. GAURAV KUMAR

Characterization of Cytochrome b5 Educates 3 As A Target to Combat Hypertension by Modulating Nitric Oxide Diffusion.

Supervisor : Prof. Suman Kundu

Th 24969

Abstract

(Verified)

Hypertension is one of the most misunderstood and mishandled medical conditions. Endothelial dysfunction, a pathological state where normal functions of vascular endothelial lining become deleterious, is the major reason of hypertension. Endothelial nitric oxide (NO) acts as a vasodilator and its vascular bioavailability is controlled by cytochrome b5 reductase 3 (CYB5R3). For the sufficiently available NO, inhibition of CYB5R3 is pre-requisite. With the aim of inhibition of CYB5R3, this study initiated as a course of biophysical and biochemical characterization of CYB5R3. Thereafter, putative amino acid residues involved in binding of substrate (alpha globin, α-Hb) with CYB5R3 were identified by in silico approaches and mutated through molecular approach. Further, wild type and mutant counterparts of CYB5R3 were structurally and functionally compared with one another. Thereafter, virtual screening of small molecules was performed against the crystal structure of the CYB5R3 enzyme (PDB ID: 1 UMK). The hit molecules identified by in silico means were screened against the activity of the enzyme in vitro and their inhibitory efficacies were determined. Potential inhibitory leads were analyzed for their kinetic and binding parameters with CYB5R3 using UV-visible and fluorescence spectroscopy, respectively. The effect of leads was checked on the conformation of the enzyme using CD spectroscopy. Additionally, before moving to further in vivo studies executed in spontaneously hypertensive rats (SHRs) for anti-hypertensive effects of lead molecules, they were screened for their possible cytotoxic and hemolytic effects by ex vivo analysis performed with human embryonic kidney 293 (HEK 293) cells and erythrocytes, respectively. Once the molecules were found to be non-cytotoxic and non-hemolytic ex vivo, they were declared “fit” for further in vivo studies. We also screened some natural or plant-derived anti-CYB5R3 extracts for structural and functional parameters. The anti-hypertensive effect of some of small molecules was evaluated in SHRs, while some of small molecules and plant-derived extracts are ready to be evaluated for similar anti-hypertensive potential.

Contents

1. Introduction, review of literature, rationale of the study, hypothesis aims and objectives 2. Expression and purification of CYB5R3 from recombinant sources and biophysical and biochemical analysis of recombinantly purified wild type (WT) and mutant CYB5R3 as anti hypertensive drug target for inhibitor identification 3. In silico and in vitro screening of small molecules and investigation of their interaction with CYB5R3 to identify putative inhibitors 4. Ex vivo evaluation of lead molecules for hemolytic and cytotoxic and anti hypertensive effects and hemodynamic parameters in spontaneously hypertensive rat (SHR) model 5. Evaluation of the effect of natural/plant extracts on structural and functional aspects of CYB5R3. Summary and future perspectives. Appendix.

04. NANDI (Deeptashree)

Deciphering the Role of FOXM1 in Development and Progression of Hepatocellular Carcinoma.

Supervisor : Dr.Alo Nag

Th 25601

Abstract
(Not Verified)

Hepatocellular carcinoma presents high mortality despite existing diagnostic and treatment modules. For comprehensive understanding of the molecular mechanisms triggering HCC to design improved therapies, we explored the implications of the Forkhead box M1 oncogene during hepatocarcinogenesis mediated by HBV oncoprotein, HBx. Our results showed that loss of FoxM1 in HBx-expressing HCC cells led to diminished neoplastic transformations. Interestingly, HBx interacted with FoxM1, stimulating FoxM1 transcriptional activity, causing augmented expression of its downstream targets that are

primarily involved in pro-proliferative functions. Mutational analysis confirmed that HBx mutants, defective in their ability to overexpress FoxM1, failed to instigate tumorigenesis, corroborating the crucial involvement of FoxM1 in HBx-related oncogenesis. Combined knockdown of HBx and FoxM1 also mitigated HCC, affirming the HBx-FoxM1 axis a promising target for intervention regimes. Moreover, our findings unearthed that HBx associates with and promotes enhanced proteolysis of Cdh1, an essential negative regulator of FoxM1. The subsequent rescue in FoxM1 level may facilitate its perturbed expression in presence of HBx. Finally, in our search for potent anticancer intervention strategies targeted at FoxM1, we investigated the credible potential of Artemisinin, recently documented to exhibit promising anti-tumor activity. Our results exhibited Artemisinin to appreciably reduce tumorigenic index of HCC cells. We observed, for the first time, that Artemisinin transcriptionally inhibits FoxM1 and disrupts its transactivation function. Intriguingly, FoxM1 knockdown in resistant HCC cells sensitized them to Artemisinin treatment. Finally, Artemisinin, when used in combination with Thiostrepton, a FoxM1 inhibitor, displayed more pronounced cytotoxicity in HCC cells. Altogether, our study revealed FoxM1 as a novel partner in HBx-induced HCC and illuminated on the abrogation of Cdh1-mediated degradation of FoxM1 by HBx during HCC development. We established FoxM1 as a key target of Artemisinin and demonstrated the potential application of Artemisinin, in conjunction with FoxM1 suppression, for superior therapeutic response in resistant HCC patients.

Contents

1. Background of the problem 2. Review of literature 3. Identifying the physical and fictional correlation between HBx and FoxM1 4. Studying the effect of HBx on Cdh1, the negative regulator of FoXM1 5. Identification of inhibitors FoxM1 for more efficient therapy in HCC. Future perspective. Bibliography and appendix.