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CUL4A silencing attenuates cervical carcinogenesis and improves Cisplatin sensitivity

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Abstract

CUL4A is an ubiquitin ligase deregulated in numerous pathologies including cancer and even hijacked by viruses for facilitating their survival and propagation. However, its role in Human papilloma virus (HPV)-mediated cervical carcinogenesis remains elusive. The UALCAN and GEPIA datasets were analyzed to ascertain the transcript levels of CUL4A in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patients. Subsequently, various biochemical assays were employed to explore the functional contribution of CUL4A in cervical carcinogenesis and to shed some light on its involvement in Cisplatin resistance in cervical cancer. Our UALCAN and GEPIA datasets analyses reveal elevated CUL4A transcript levels in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patients that correlate with adverse clinicopathological parameters such as tumor stage and lymph node metastasis. Kaplan–Meier plot and GEPIA assessment depict poor prognosis of CESC patients having high CUL4A expression. Varied biochemical assays illustrate that CUL4A inhibition severely curtails hallmark malignant properties such as cellular proliferation, migration, and invasion of cervical cancer cells. We also show that CUL4A knockdown in HeLa cells causes increased susceptibility and better apoptotic induction toward Cisplatin, a mainstay drug used in cervical cancer treatment. More interestingly, we find reversion of Cisplatin-resistant phenotype of HeLa cells and an augmented cytotoxicity towards the platinum compound upon CUL4A downregulation. Taken together, our study underscores CUL4A as a cervical cancer oncogene and illustrates its potential as a prognosis indicator. Our investigation provides a novel avenue in improving current anti-cervical cancer therapy and overcoming the bottle-neck of Cisplatin resistance.

Keywords CUL4A · Cervical cancer · Cisplatin · Cisplatin resistance · Carcinogenesis · Ubiquitin ligase

Abbreviations

TCGA	The Cancer Genome Atlas
GEPIA	Gene Expression Profiling Interactive Analysis
HPV	Human papillomavirus
DNA	Deoxyribonucleic acid
CUL4A	Cullin4A
UV	Ultraviolet
HIV	Human immunodeficiency virus
HBV	Hepatitis B virus
EBV	Epstein–barr virus
SV5	Simian virus 5
HPVI2	Human para influenza virus type 2
EMT	Epithelial-to-mesenchymal transition
PARP	Poly (ADP-ribose) polymerase
pATM	Phosphorylated Ataxia-telangiectasia mutated
pChk1	Phosphorylated Checkpoint kinase 1
DSB	Double-strand break
CDK	Cyclin-dependent kinase
CDDP	Cis-diamminedichloroplatinum(II)
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
DMSO	Dimethyl sulfoxide
HCT-116	Human colorectal carcinoma cells
NER	Nucleotide excision repair
HeLa-CR	HeLa-Cisplatin-resistant cells
IAP	Inhibitors of apoptosis proteins
O.D.	Optical density
FACS	Fluorescence activated cell sorting
EDTA	Ethylenediamine tetraacetic acid
PBS	Phosphate buffer saline
γ-H2AX	Phosphorylated Histone2A

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Mechanistic insights into the oncogenic partnership of h paving ways for improved cervical cancer

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High risk Human Papillomavirus (HPV) is considered the primary causative agent of cervi with significant morbidity. In India, cervical cancer is one of the major reasons of cance treatment outcomes of this disease is a matter of grave concern and hence demands agg discovery of more effective therapies. Understanding the intricacies of HPV oncogenesis ar the discovery of promising anti-viral drugs. Our research aims at catering to the need of th key molecular mechanisms that contributes to HPV oncogenesis that can be utilized tc molecules. We delineated the oncogenic connections between hADA3 and HPVE6 and ilh transformation. Our work also shows how HPV oncoproteins exploits the cellular SU hADA3 to induce malignancy. This intrigued us to identify the hot spots of hADA3-E6 i peptides against HPV induced cervical cancer. Present review is an attempt to outline our HPV pathogenesis and its implication on development of improved cervical cancer therapie

Keywords: Cervical cancer, E6, hADA3, HPV, Peptide, SUMOylation

Introduction

Cervical cancer is a significant gynaecological health concern that has evolved into an obliterating plague with a high death toll of 18.7% women deaths in India and 7.7% worldwide for year 2020. Alarmingly, in India, this statistic is predicted to rise with an estimated 47,329 annual deaths by 2040[1]. Prolonged high-risk Human Papilloma Viruses (HPV) infection and lack of comprehensive prognosis testing are the two major factors for increasing mortality rate[2]. Besides this, smoking, multiple pregnancies, oral contraceptive usage, and poor hygiene conditions are the other common risk factors. Epidemiological investigations identified sexual activity as one of the

Discovery of prophyl Gardasil-4 and Ga breakthrough for co cancers. However, th types and prevent the only in women who vaccine-associated HI mortality rates have success of such va unavailability of thes to HPV infection prio more due to incorpor host genome[5]. For t early stages, radiothe-

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Cloning, expression and *in vitro* validation of chimeric multi epitope vaccine candidate against visceral leishmaniasis infection

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Keywords:
Leishmaniasis
Multiepitope vaccine
Immune response
Cytokines

ABSTRACT

Visceral Leishmaniasis or Kala-Azar is one of the most severe and deadly neglected tropical disease caused by the *Leishmania* parasite. A few number of vaccines are going through different phases in clinical trial but failing of these vaccines in successive phase trial or less efficacy, urge to develop highly immunogenic and cost-effective treatment to get rid of deadly VL. This study focuses on the development of more potent vaccine candidate against VL. The recombinant vaccine candidate LeiSp was expressed in *Pichia pastoris*, followed by purification and characterization. The purified protein was also tested for any post-translation modification, which favors being a potent immunogenic candidate. Further, the expression modulation of different pro-inflammatory and anti-inflammatory cytokines was evaluated in THP1 cell lines. A significant upregulation in the expression of pro-inflammatory cytokines while no significant changes were observed in the expression of anti-inflammatory cytokines. The impact of recombinant vaccine protein candidates in infected conditions were determined. Here, upon treatment with chimeric vaccine protein candidate, we observed a considerable recovery in the expression level of pro-inflammatory cytokines, which were downregulated upon infection alone. In addition to this, we found a significant decrease in the expression of anti-inflammatory cytokines, which were upregulated during infection alone. We further validated our findings in infected hPBMCs and observed similar expression modulation of pro-inflammatory and anti-inflammatory cytokines with and without treatment. Thus, the present study indicates that the chimeric LeiSp protein which was designed using bioinformatics approaches shows a potential inductive efficacy for pro-inflammatory cytokines in *Leishmania*-infected cells.

1. Introduction

The tropical neglected disease Leishmaniasis, caused by the *Leishmania* parasite and pass on by infected sand-fly Phlebotomine. Several other risk factors include malnutrition, poor sanitation, urbanization, and irrigation problems, leading to increased exposure to sand flies and, ultimately, to *Leishmania* parasite infection [1]. Presently, there is no effective treatment available for the complete cure of the disease. Various anti-leishmanial drugs are available but associated with various side effects, and none of the vaccines has been reported, and treatment only relies upon chemotherapeutics. These challenges remain to be overcome; therefore, the research world continues to scrutinize immunization for the prevention of VL. Vaccination is the only preventive way to cure the disease and control the outbreaks completely. The involvement of microbial components or proteins from pathogens, which helps them enhance their pathogenesis, is mainly targeted to generate potential cell-mediated and humoral immunity. The antigen-presenting cells, dendritic cells (DC's), and macrophages play a significant role and work as a link between the antigens and adaptive branch of the immune system [2].The immune cell epitopes prediction through immuno-informatics tools made the reverse vaccinology approach remarkable due to the epitope-specific generation of immune responses [3].

In *Leishmania* infection conditions, Th1 cell-mediated immune response protects against the disease by producing pro-inflammatory cytokines (IFN-γ, TNF-α, IL-12) and (gG2a isotype antibodies. On the contrary, Th2 cells are known to have a role in disense development by producing anti-inflammatory cytokines (IL-10, TNF-β, IL-4) and IgG2

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From the journal:
Molecular Systems Design & Engineering

Multifaceted mutational immunotherapeutic approach to design therapeutic mAbs to combat monkeypox disease via integrated screening algorithms and antibody engineering

Satyendra Singh, Abhishek Rao, Anshuman Mishra, Amit Mishra and Vijay Kumar Prajapati

Abstract

After a multi-country outbreak, the monkeypox (MPX) disease was designated a global public health emergency on July 23, 2022. Some antiviral medications tailored to the smallpox virus are currently being used to treat the disease. There is no specific treatment for MPX disease with minimal negligible side effects. The engineering of antibodies has increased dramatically since the US Food and Drug Administration (US FDA) approved the first therapeutic monoclonal antibody (mAb) in 1986. mAbs have revolutionized biomedical research and have been used with remarkable precision for undesirable consequences. So, in this study, mAbs from Thera-SAbDab (Therapeutic Structural Antibody Database) were screened using the ClusPro protein-protein docking server against the critical enzymes of monkeypox virus (thymidine kinase, methyltransferase, D9 decapping enzyme, and RNA polymerase). Based on the predicted ClusPro docking score, binding affinity (ΔG), dissociation constant (Kd), and physiochemical properties, the best two mAbs (Eculizumab and Vofatamab) were designated for further investigation. Furthermore, the CUPSAT server and PyMol mutagenesis wizard were employed to generate a mutant pool (up to triple mutant through permutation combinations) and investigate the binding affinity of candidate mAbs following point mutation. Eventually, the were identified as the most effective and promising inhibitors targeting all four MPXV enzymes, based on molecular dynamics (MD) simulations and MD trajectory assessment. In the future, in vitro and in vivo experiments on promising mAbs identified and developed by us could aid virus neutralization in MPXV-infected patients.

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Review

A review of chromium (Cr) epigenetic toxicity and health hazards

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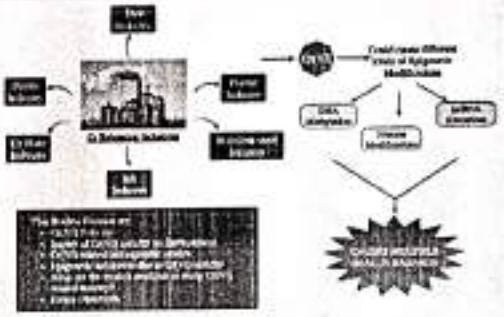
HIGHLIGHTS

- Cr (VI) toxicity induced detrimental health effects
- Epigenetic alterations due to Cr (VI) toxicity
- A bibliographic output of papers published about Cr (VI) toxicity
- Signaling pathways allied in Cr poisoning
- Impact of Cr (VI) exposure in causing oxidative stress

GRAPHICAL ABSTRACT



Abbreviations: 8-OH-dG, 8-hydroxy-deoxyguanosine;; Bcl-2, B-cell lymphoma-2; CpG, cytosine-guanine; Cr, Chromium; Cr(VI), Hexavalent Chromium; Cyt c, Cytochrome c; DNA, Deoxyribonucleic acid; GR, glutathione reductase; GSH, Glutathione; H3K27, histone 3 lysine 27; H3K4, histone 3 lysine 4; H3K9, histone 3 lysine 9; H3R2, histone 3 arginine 2; IARC, International Agency for Research on Cancer; MMP, matrix metalloproteinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRCC1/2, mitochondrial respiratory chain complexes; ROS, Reactive Oxygen Species.

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RESEARCH ARTICLE

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Oncogenic role of an uncharacterized cold-induced zinc finger protein 726 in breast cancer

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Abstract

The unobtrusive cold environmental temperature can be linked to the development of cancer. This study, for the first time, envisaged cold stress-mediated induction of a zinc finger protein 726 (ZNF726) in breast cancer. However, the role of ZNF726 in tumorigenesis has not been defined. This study investigated the putative role of ZNF726 in breast cancer tumorigenic potency. Gene expression analysis using multifactorial cancer databases predicted overexpression of ZNF726 in various cancers, including breast cancer. Experimental observations found that malignant breast tissues and highly aggressive MDA-MB-231 cells showed an elevated ZNF726 expression as compared to benign and luminal A type (MCF-7), respectively. Furthermore, ZNF726 silencing decreased breast cancer cell proliferation, epithelial–mesenchymal transition, and invasion accompanied by the inhibition of colony-forming ability. Concordantly, ZNF726 overexpression significantly demonstrated opposite outcomes than ZNF726 knockdown. Taken together, our findings propose cold-inducible ZNF726 as a functional oncogene demonstrating its prominent role in facilitating breast tumorigenesis. An inverse correlation between environmental temperature and total serum cholesterol was observed in the previous study. Furthermore, experimental outcomes illustrate that cold stress elevated cholesterol content hinting at the involvement of the cholesterol regulatory pathway in cold-induced ZNF726 gene regulation. This observation was bolstered by a positive correlation between the expression of cholesterol-regulatory genes and ZNF726. Exogenous cholesterol treatment elevated ZNF726 transcript levels while knockdown of ZNF726 decreased the cholesterol content via down-regulating various cholesterol regulatory gene expressions (e.g., SREBF1/2, HMGCoR, LDLR). Moreover, an underlying mechanism supporting cold-driven tumorigenesis is proposed through interdependent regulation of cholesterol regulatory pathway and cold-inducible ZNF726 expression.

KEYWORDS

breast cancer, cholesterol regulatory pathway, cold stress, epithelial-to-mesenchymal transition, oncogene, zinc finger protein 726 (ZNF726)

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A novel single sensor hemoglobin domain from the thermophilic cyanobacteria *Thermosynechococcus elongatus BP-1* exhibits higher pH but lower thermal stability compared to globins from mesophilic organisms☆

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Keywords:
Hemoglobin sensor domain
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ABSTRACT

Thermosynechococcus elongatus-BP1 belongs to the class of photoautotrophic cyanobacterial organisms. The presence of chlorophyll *a*, carotenoids, and phycocyanobilin are the characteristics that categorize *T. elongatus* as a photosynthetic organism. Here, we report the structural and spectroscopic characteristics of a novel hemoglobin (Hb) Synel Hb from *T.elongatus*, synonymous with *Thermosynechococcus vestitus BP-1*. The X-ray crystal structure (2.15 Å) of Synel Hb suggests the presence of a globin domain with a pre-A helix similar to the sensor domain (S) family of Hbs. The rich hydrophobic core accommodates heme in a penta-coordinated state and readily binds an extraneous ligand (imidazole). The absorption and circular dichroic spectral analysis of Synel Hb reiterated that the heme is in Fe^{III} state with a predominantly α-helical structure similar to myoglobin. Synel Hb displays higher resistance to structural perturbations induced via external stresses like pH and guanidium hydrochloride, which is comparable to *Synechocystis* Hb. However, Synel Hb exhibited lower thermal stability compared to mesophilic hemoglobins. Overall, the data is suggestive of the structural sturdiness of Synel Hb, which probably corroborates its origin in extreme thermophilic conditions. The stable globin provides scope for further investigation and may lead to new insights with possibilities for engineering stability in hemoglobin-based oxygen carriers.

1. Introduction

The rampant population explosion worldwide enforces innovative thinking to meet the requirements of food, drugs, diagnostics, and environmental crisis management strategies. A new trendline for biotechnology and pharmaceutical sciences has been to cope with such formidable challenges. It has led to the exploration of protein targets from natural resources, (e.g plants, microbes, algae, photocyanin) to develop diverse applications by protein engineering approaches. In this niche, hemoglobins (Hbs) have emerged as tractable targets and their omnipresence has been a key discovery [1].

The globin superfamily of heme proteins offers a large, diverse, and thoroughly studied set of proteins [2,3]. Their ubiquitous nature is indicative of their immensely important physiological role [4,5]. Their function is tightly related to small gaseous non-polar ligands (mainly O_2, but also NO and CO) and slightly larger molecules such as azide, cyanide, and imidazole with varied affinity and reactivity [2,6,7]. They can be classified based on their capacity to bind O_2. Those with high O_2 affinity, such as mycobacterial Hbs, usually function as O_2 (and other reactive oxygen and nitrogen -RNOS- species) redox-related enzymes. Moderate O_2 affinity globins, like the mammalian monomeric sperm whale myoglobin (Mb) and tetrameric hemoglobin, usually act as oxygen carrier or storage proteins; while low O_2 affinity globins, such as soluble guanylate cyclase or the globin coupled sensors (GCS), are NO, CO or redox sensors. It is now established that Hbs may possess functions other than oxygen storage and transport, including enzymatic and sensing functions. Such Hbs remain yet illusive to the research community at large [8,9].

☆ Database: Structural data are available in RCSB-PDB database(s) under the accession number: 8H17

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Research Article

An analysis of direct and indirect effects in *Drosophila melanogaster* undergoing a few cycles of experimental evolution for stress-related traits

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Experimental evolution
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D. melanogaster

ABSTRACT

The physiological mechanisms underpinning adaptations to starvation and cold stresses have been extensively studied in *Drosophila*, yet the understanding of correlated changes in stress-related and life-history traits, as well as the energetics of stress tolerance, still remains elusive. To answer the questions empirically in this context, we allowed *D. melanogaster* to evolve for either increased starvation or cold tolerance (24-generations / regime) in an experimental evolution system, and examined whether selection of either trait affects un-selected stress trait, as well as the impacts potential changes in life-history and mating success-related traits. Our results revealed remarkable changes in starvation/cold tolerance (up to 1.5-fold) as a direct effect of selection, while cold tolerance had been dramatically reduced (1.26-fold) in the starvation tolerant (ST) lines compared to control counterparts, although no such changes were evident in cold-tolerant (CT) lines. ST lines exhibited a higher level of body lipids and a reduced level of trehalose content, while CT lines accumulated a greater levels of body lipid and trehalose contents. Noticeably, we found that selection for starvation or cold tolerance positively correlates with larval development time, longevity, and copulation duration, indicating that these traits are among the most common targets of selection trajectories shaping stress tolerance. Altogether, this study highlights the complexity of mechanisms evolved in ST lines that contribute to enhanced starvation tolerance, but also negatively impact cold tolerance. Nevertheless, mechanisms foraging enhanced cold tolerance in CT lines appear not to target starvation tolerance. Moreover, the parallel changes in life history/mating success traits across stress regimes could indicate some generic pathways evolved in stressful environments, targeting life-history and mating success characteristics to optimize fitness.

1. Introduction

As all living organisms experience scarcity of food at some point in their life-cycles, starvation is categorized as an omnipresent stressor as well as a prominent natural selection agent (Wang et al., 2006; Rion and Kawecki, 2007; McCue, 2010; Aggarwal, 2014). Similarly, thermal stresses affect fitness, survival, and potentially determine the physiological limits of almost all living organisms (Angilletta, 2009; Kellermann et al., 2009; Hoffmann, 2010; Hoffmann and Sgrò, 2011). In essence, these notions describe the existence of some generic pathways that prevail in organisms in their natural environments and enable them to adapt to a wide range of stresses, as well as provide the basis for trait correlations and trade-offs. In ectotherms, *Drosophila* represents a unique model for unraveling the physiological and genetic basis of abiotic stress adaptations (Hoffmann and Parsons, 1991; Hoffmann and Harshman, 1999), and for examining their evolutionary correlations and trade-offs between traits (Hoffmann, 2010; Bubliy and Loeschcke, 2005). Yet, it remains puzzlingly unclear whether starvation- and cold tolerance evolved as co-evolving traits in *Drosophila*, despite reports which have claimed that these stress-related traits evolved together. Eastern Australian *D. melanogaster* populations, for instance, showed high levels of cold tolerance at higher altitudes, despite no evidence of such variations in starvation tolerance (see Hoffmann, 2010). In contrast, *D. melanogaster* populations from the Western Himalaya (India) inhabiting highland localities displayed higher levels of cold tolerance, though less starvation tolerance than those from lowland areas (Parkash et al., 2011a; Parkash and Aggarwal, 2012). Similarly, *D. simulans* seasonal populations also exhibited a trade-off between starvation and cold tolerance (Kenny et al., 2006). Thus, the evolutionary relationship between starvation and cold tolerance in *Drosophila* species occurring in

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Novel Variant Identified in the Enhancer Region of Host Transcription Factor, BRN3A, is a Significant Risk Factor for HPV-Induced Uterine Cervix Cancer

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ABSTRACT

Among the HPV-mediated cervical cancers, cellular factor BRN3A has gained considerable attention due to its role in promoting an anti-apoptotic cellular environment and in facilitating epitheliotropic transformations of the host. The majority of previous studies looked at BRN3A's molecular characteristics; however, the possibility of genetic variations in BRN3A's auto-regulatory region in relation to cervical cancer risk has been underestimated until now. In a retrospective study in the Eastern UP population, India, we detected genetic variations in the cis-regulatory proximal enhancer region located around 5.6 kb upstream of transcription start site of *BRN3A*. Our analysis of PCR and DNA sequencing confirmed this novel SNP (*BRN3A* g.60163379A>G) within the auto-regulatory region of *BRN3A*. As compared to control subjects, cancer cases exhibited a 1.32-fold higher allele frequency ($\chi 2 = 6.315$, $p = 0.012$). In homozygous (GG) but not in heterozygous conditions, odds ratio (OR) analysis suggests a significant association of cancer risk with the SNP (OR = 2.60, $p \leq 0.004$). We further confirmed using the functional analysis that this SNP increased the luciferase gene activity in HPV-positive cervical cancer SiHa cells that were exposed to progesterone. As a result of the association of polymorphisms in a non-coding region of an oncogene with increased cancer risks, we are suggesting that this genetic variation in non-coding region can be used in prediction, diagnosis, or predicting the progression of the disease.

Keywords: Cervical cancer, HPV, BRN3A, *BRN3A* proximal enhancer region, India, g. 60163379A >G

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Identification of a peptide that disrupts hADA3-E6 interaction with implications in HPV induced cancer therapy

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ABSTRACT

Aim: High risk Human Papillomavirus (HPV) is an infectious pathogen implicated in a variety of cancers with poor clinical outcome. The mechanism of HPV induced cellular transformation and its intervention remains to be elucidated. Human ADA3 (hADA3), a cellular target of HPV16 E6, is an essential and conserved component of the ADA transcriptional coactivator complex. High risk HPV-E6 binds and functionally inactivates hADA3 to initiate oncogenesis. The aim of this study was to identify the interaction interface between hADA3 and HPV16E6 for designing inhibitory peptides that can potentially disrupt the hADA3-E6 interaction.

Material methods: The present investigation employed structure-based in silico tools supported by biochemical validation, in vivo interaction studies and analysis of posttranslational modifications.

Key findings: First 3D-model of hADA3 was proposed and domains involved in the oncogenic interaction between hADA3 and HPV16E6 were delineated. Rationally designed peptide disrupted hADA3-E6 interaction and impeded malignant properties of cervical cancer cells.

Significance: Intervention of hADA3-E6 interaction thus promises to be a potential strategy to combat HPV induced oncogenic conditions like cervical cancer. The investigation provides mechanistic insights into HPV pathogenesis and shows promise in developing novel therapeutics to treat HPV induced cancers.

1. Introduction

Cervical cancer continues to be a global health menace for women [1]. More than 80% of the cervical cancer deaths occur in developing countries. HPV is documented to be the major causative agent of cervical cancer, a devastating disease with significant morbidity [2–4]. Current treatment strategies include invasive surgical removal of the lesions, use of general cytotoxic compounds or modulation of the immune response [5,6]. Viable anti-HPV specific therapies, which are desirable and a preferred approach for an immunosuppressed population do not exist at present. Targeted therapies also have an advantage against diseases with multifocal lesions and/or asymptomatic patients. Previous investigations on host-pathogen interaction led to the discovery of several lead compounds (small molecule HPV antagonists) for the inactivation of E1 and E2 and the disruption of E6-E6AP interactions; however, their biological efficacies and feasibility are still being investigated [7,8]. Hence, there is a pressing need for developing less invasive and more effective anti-HPV therapies.

A significant body of research in anti-HPV therapies is yet to identify intervention strategies that are robust. Thus, while some vaccines are available, these are meant to provide protection against new HPV infections, if at all, but unable to treat established HPV infection or disease caused by HPV [9,10]. No effective drug treatment for HPV has been reported yet. Therefore, development of novel inhibitors with powerful anti-HPV activities is of high importance.

Exploring promising HPV specific therapies for cervical cancer is dependent on the knowledge of how the pathogen induces oncogenesis and the key players associated in the process. Research initiatives are essential to understand the complexities of the malignancy and the molecular details of the host and viral protein interactions. HPV

Abbreviations: HPV, Human Papillomavirus; hADA3, human alteration/deficiency in activation3; HEK293T, human embryonic kidney 293T cells; SUMO, small ubiquitin-like modifier; aa, amino acid; NLS, nuclear localization signal; MDS, molecular dynamics simulations; 3D, three-dimensional.

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Direct Correlation between the Secondary Structure of an Amphiphilic Polymer and Its Prominent Antiviral Activity

Atish Nag, Kumarjeet Banerjee, Ranajit Barman, Joy Kar, Debi P Sarkar, Siddhartha Sankar Jana,* and Subrit Ghosh*

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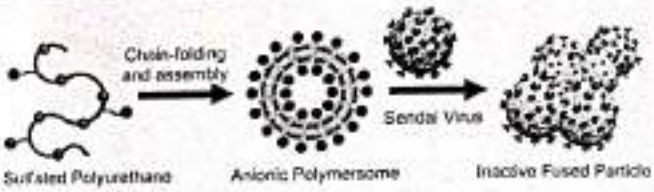
ABSTRACT: An amphiphilic segmented polyurethane (F-PU-S), with pendant sulfate groups and a flexible hydrocarbon backbone, exhibits intrachain H-bonding-reinforced folding and hierarchical assembly, producing an anionic polymersome with efficient display of sulfate groups at the surface. It shows an excellent antiviral activity against Sendai virus (SV) by inhibiting its entry to the cells. Mechanistic investigation suggests fusion of the SV and the polymersome to produce larger particles in which neither the folded structure of the polymer nor the fusogenic property of the SV exists anymore. In sharp contrast, a structurally similar polymer R-PU-S, in which the chain folding pathway is blocked by replacing the flexible C6 chain with a rigid cyclohexane chain in the backbone, cannot form a similar polymersome structure and hence does not exhibit any antiviral activity. On the other hand, the third polymer (F-PU-C), which is similar to F-PU-S except for the pendant anionic groups (carboxylate instead of sulfate), also fails to exhibit any antiviral activity against SV, confirming the essential role of the chain folding as well as the pendant sulfate groups for the fusion-induced antiviral activity of F-PU-S, which provides an important structural guideline for developing new antiviral polymers.



INTRODUCTION

Virus, a submicroscopic agent that can only replicate in living cells, has been the cause of different infectious diseases and made havoc on humanity in numerous occasions.[1] Despite significant advances in vaccines and drug development, antiviral therapy continues to remain a challenging task due to the frequent appearance of new variants and drug resistance.[2] A successful strategy to combat viral infections is to inhibit the entry of the virus into the host cells at an early stage, which would cure infection and limit its spread in different organisms. In this context, antiviral polymers[3,4] have been studied with great interest as they are suitable candidates for programming effective multivalent interactions[5,6] by simultaneous binding with multiple complementary receptors in biological surfaces. Unlike small-molecule drugs, polymers also offer distinct advantages in tuning the biological activity by adjustment of different structural parameters such as the degree of polymerization, attached functional groups, and the hydrophobic/hydrophilic balance or architecture, all of which may strongly influence their bioavailability, pharmacokinetics, and other properties, which are relevant in the context of different biological applications.[7−11] Although the antiviral activity of polymers was known since 1947,[12] the field gained momentum in 1960s with the realization of the antiviral activity of various sulfated polysaccharides[13,14] against HIV and other viruses. It is believed that these natural sulfated polysaccharides or their unnatural synthetic analogues[15−20] exhibit an antiviral activity by interacting with the envelope spike composed of gp120, thus making the virus ineffective for invading host cells. Although many such systems showed promising results *in vitro*, most of them could not reach the benchmark of clinical trials due to several problems including blood coagulation, poor bioavailability, inhibition of platelet production, cytotoxicity, and others.[3,4] Hence, it is imperative to explore structural diversity with new polymeric scaffolds for tunable physiochemical and biological properties to tackle the escalating global health concern arising out of highly infectious viral diseases. Most of the reported antiviral polymers are amphiphilic in nature in which the sialic acid motif or sulfated glycans are attached to a hydrophobic macromolecular (linear or branched) scaffold.[3,4,15−20] Such polymers are expected not to remain in the unimer state in water but rather exhibit immiscibility-driven aggregation with little control over their aggregation properties. In the recent past, we have introduced amphiphilic polymers based on the biocompatible and biodegradable polyurethane (PU) backbone, which adopt a pleated structure in water by intrachain H-bonding-reinforced chain folding and exhibit hierarchical assembly producing polymersome structures with excellent surface functional group

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Optimization and Identification of Single Mutation in Hemoglobin Variants with 2,2,2 Trifluoroethanol Modified Digestion Method and Nano−LC Coupled MALDI MS/MS

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Abstract: Background: Hemoglobin (Hb) variants arise due to point mutations in globin chains and their pathological treatments rely heavily on the identification of the nature and location of the mutation in the globin chains. Traditional methods for diagnosis such as HPLC and electrophoresis have their own limitations. Therefore, the present study aims to develop and optimize a specific method of sample processing that could lead to improved sequence coverage and analysis of Hb variants by nano LC−MALDI MS/MS. **Methods:** In our study, we primarily standardized various sample processing methods such as conventional digestion with trypsin followed by 10% acetonitrile treatment, digestion with multiple proteases like trypsin, Glu−C, Lys−C, and trypsin digestion subsequent to 2,2,2 trifluoroethanol (TFE) treatment. Finally, the peptides were identified by LC−MALDI MS/MS. All of these sample processing steps were primarily tested with recombinant Hb samples. After initial optimization, we found that the TFE method was the most suitable one and the efficiency of this method was applied in Hb variant identification based on high sequence coverage. **Results:** We developed and optimized a method using an organic solvent TFE and heat denaturation prior to digestion, resulting in 100% sequence coverage in the β−chains and 95% sequence coverage in the α−chains, which further helped in the identification of Hb mutations. A Hb variant protein sequence database was created to specify the search and reduce the search time. **Conclusion:** All of the mutations were identified using a bottom−up non−target approach. Therefore, a sensitive, robust and reproducible method was developed to identify single substitution mutations in the Hb variants from the sequence of the entire globin chains. **Biological Significance:** Over 330,000 infants are born annually with hemoglobinopathies and it is the major cause of morbidity and mortality in early childhood. Hb variants generally arise due to point mutation in the globin chains. There is high sequence homology between normal Hb and Hb variant chains. Due to this high homology between the two forms, identification of variants by mass spectrometry is very difficult and requires the full sequence coverage of α− and β−chains. As such, there is a need for a suitable method that provides 100% sequence coverage of globin chains for variant analysis by mass spectrometry. Our study provides a simple, robust, and reproducible method that is suitable for LC−MALDI and provides nearly complete sequence coverage in the globin chains. This method may be used in the near future in routine diagnosis for Hb variant analysis.

Keywords: hemoglobin disorders; mass spectrometry; hemoglobin variants; liquid chromatography; MALDI TOF/TOF; nano LC−MALDI MS/MS; Hb variant identification

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JBC RESEARCH ARTICLE

Lipoate protein ligase B primarily recognizes the C_8-phosphopantetheine arm of its donor substrate and weakly binds the acyl carrier protein

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Lipoic acid is a sulfur-containing cofactor indispensable for the function of several metabolic enzymes. In microorganisms, lipoic acid can be salvaged from the surroundings by lipoate protein ligase A (LplA), an ATP-dependent enzyme. Alternatively, it can be synthesized by the sequential actions of lipoate protein ligase B (LipB) and lipoyl synthase (LipA). LipB takes up the octanoyl chain from C_8-acyl carrier protein (C_8-ACP), a byproduct of the type II fatty acid synthesis pathway, and transfers it to a conserved lysine of the lipoyl domain of a dehydrogenase. However, the molecular basis of its substrate recognition is still not fully understood. Using *Escherichia coli* LipB as a model enzyme, we show here that the octanoyltransferase mainly recognizes the 4′-phosphopantetheine-tethered acyl-chain of its donor substrate and weakly binds the apo-acyl carrier protein. We demonstrate LipB can accept octanoate from its own ACP and noncognate ACPs, as well as C_8-CoA. Furthermore, our ^{1}H saturation transfer difference and ^{31}P NMR studies demonstrate the binding of adenosine, as well as the phosphopantetheine arm of CoA to LipB, akin to binding to LplA. Finally, we show a conserved ^{71}RGG^{73} loop, analogous to the lipoate-binding loop of LplA, is required for full LipB activity. Collectively, our studies highlight commonalities between LipB and LplA in their mechanism of substrate recognition. This knowledge could be of significance in the treatment of mitochondrial fatty acid synthesis related disorders.

Lipoic acid is a hydrophobic cofactor, essential for the function of several metabolic enzyme complexes, *viz.* pyruvate dehydrogenase complex (PDC) required for pyruvate oxidation, 2-oxoglutarate dehydrogenase as a component of Krebs cycle (OGDHc), glycine cleavage system (GCS) involved in glycine degradation, branched-chain keto acid dehydrogenases (BCKDHc) necessary for the metabolism of branched chain amino acids, and 2-oxoadipate dehydrogenase in lysine metabolism (1–5). These are multimeric enzymes with a conserved lipoyl domain that serves as a substrate for lipoic acid modification. Lipoyl lysine acts as a "swinging arm," helping to shuttle intermediates between the active site of multienzyme complexes (1, 4, 6–9). The flexibility of lipoic acid is crucial for substrate channeling and electron transfer during oxidation–reduction reactions (10).

Most of the knowledge with regard to lipoic acid metabolism comes from studies conducted on *Escherichia coli*, *Listeria monocytogenes*, *Bacillus subtilis*, and *Staphylococcus aureus* (11). In the presence of free lipoate or octanoate, lipoic acid is salvaged from the environment by lipoate protein ligase A (LplA) (12–14). An activated lipoyl-5′-AMP intermediate is formed from ATP and lipoic acid on the surface of LplA. Thereafter, the ε-amino group of a lipoyl lysine attacks the noncovalently bound lipoyl-AMP, forming an amide linkage with lipoyl-group and releasing AMP (3, 8, 9, 14, 15). All LplA molecules comprise a large N-terminal catalytic domain (lipoic acid binding) and a small C-terminal domain (16). In lipoic acid–deficient environments, lipoate protein ligase B (LipB) also known as Lipoyl-octanoyl transferase catalyzes the first biosynthetic step of lipoic acid synthesis (4). Studies on *Mycobacterium tuberculosis* (Protein Data Bank [PDB] 1W66) and *Thermus thermophilus* (PDB 2QHS, 2QHT, 2QHU, and 2QHV) LipB suggest conservation of a structural scaffold, similar to the N-terminal catalytic domain of LplA. LipB functions as a cysteine/lysine dyad acyltransferase, cysteine 176 and Lys 142 of *M. tuberculosis* LipB (Cys169 and Lys 135 of *E. coli* LipB) functioning as acid/base catalysts (17–19). A covalent octanoyl-LipB thioester intermediate is formed by the transfer of octanoyl-chain from C_8-ACP (acyl carrier protein) to Cys176 of LipB (20). Subsequently, the thioester bond is attacked by the ε-amino group of the lipoyl lysine, resulting in C_8- modification of the latter. In the following step, octanoyl group covalently tethered to the lipoyl domain is converted to lipoic acid in the presence of LipA (lipoyl synthase) by insertion of two sulfur atoms.

In metazoans, three different enzymes participate in lipoic acid synthesis: (a) lipoyl transferase 2 (LipT2) that transfers octanoyl chain from C_8-ACP to the lipoyl subunit of GCS, (b) lipoic acid synthase (LIAS), a sulfur insertion enzyme that adds two sulfur atoms to the C_8- chain to form lipoic acid, and (c)

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Therapeutic enzymes as non-conventional targets in cardiovascular impairments: A comprehensive review

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Affiliations + expand

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Abstract

Over the last few decades, substantial progress has been made towards the understanding of cardiovascular diseases. In-depth mechanistic insights have also provided opportunities to explore novel therapeutic targets and to discover new treatment regimens. Therapeutic enzymes are examples of such opportunities. The enzymes protect against a variety of cardiovascular diseases, however, even minor malfunctioning of these enzymes may lead to deleterious outcomes. Owing to their great versatility, the inhibition and activation of these enzymes are key regulatory approaches to counter the onset and progression of several cardiovascular impairments. While cardiovascular remedies are already available in excess and are efficacious, a comprehensive description of novel therapeutic enzymes to combat cardiovascular diseases would still be of great benefit. In the light of this, the regulation of functional activities of these enzymes also opens a new avenue for the treatment approaches to be employed. This review describes the importance of non-conventional enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), phosphodiesterase (PDE), arginase, superoxide dismutase (SOD), thioredoxin reductase (TXNRD) and selenoprotein T (SELENOT), cytochrome b5 reductase 3 (CYB5R3), epoxide hydrolase (EHs), xanthine oxidoreductase (XOR), matrix metalloprotease (MMPs), and dopamine beta hydroxylase (DBH), as potential candidates in several cardiovascular disorders while highlighting some of the recently targeted therapeutic enzymes in cardiovascular diseases. We also discuss the role of intrinsic antioxidant defense system involved in cardioprotection followed by addressing some of the clinical investigations considering the use of antioxidant as a preferred therapy of cardiovascular complications.

Keywords: cardiovascular diseases; cardiovascular impairments; inhibiteurs; inhibitors; maladies cardiovasculaires; therapeutic enzymes; traitements enzymatiques; troubles cardiovasculaires.

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Original Article

Original Article

The anaphase-promoting complex/cyclosome co-activator, Cdh1, is a novel target of human papillomavirus 16 E7 oncoprotein in cervical oncogenesis

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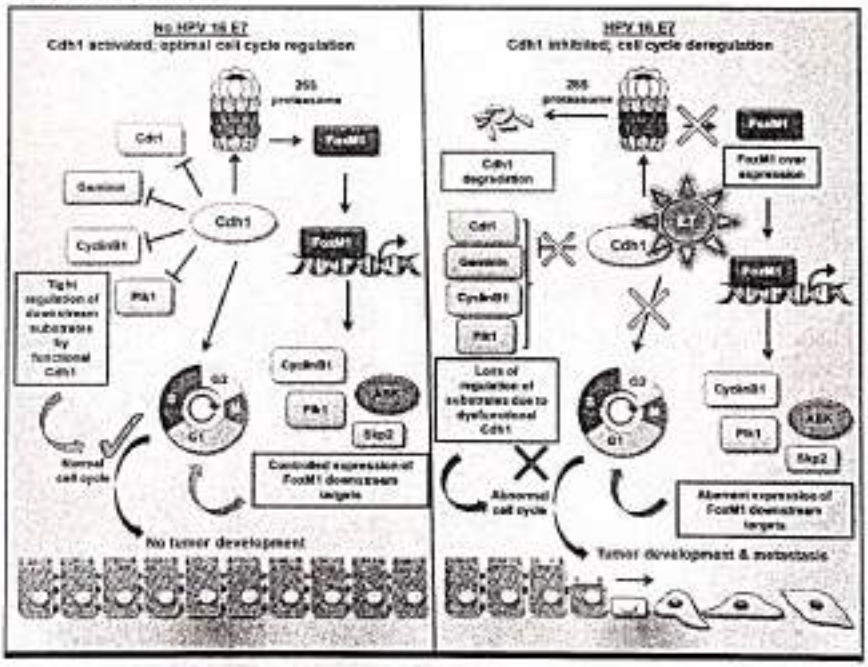
Abstract

The transforming properties of the high-risk human papillomavirus (HPV) E7 oncoprotein are indispensable for driving the virus life cycle and pathogenesis. Besides inactivation of the retinoblastoma family of tumor suppressors as part of its oncogenic endeavors, E7-mediated perturbations of eminent cell cycle regulators, checkpoint proteins and proto-oncogenes are considered to be the tricks of its transformative traits. However, many such critical interactions are still unknown. In the present study, we have identified the anaphase-promoting complex/cyclosome (APC) co-activator, Cdh1, as a novel interacting partner and a degradation target of E7. We found that HPV16 E7-induced inactivation of Cdh1 promoted abnormal accumulation of multiple Cdh1 substrates. Such a mode of deregulation possibly contributes to HPV-mediated cervical oncogenesis. Our mapping studies recognized the C-terminal zinc-finger motif of E7 to associate with Cdh1 and interfere with the timely degradation of FoxM1, a bona fide Cdh1 substrate and a potent oncogene. Importantly, the E7 mutant with impaired interaction with Cdh1 exhibited defects in its ability for overriding typical cell cycle transition and oncogenic transformation, thereby validating the functional and pathological significance of the E7-Cdh1 axis during cervical carcinoma progression. Altogether, the findings from our study discover a unique nexus between E7 and APC/C-Cdh1, thereby adding to our understanding of the mechanism of E7-induced carcinogenesis and provide a promising target for the management of cervical carcinoma.

Graphical Abstract



Abbreviations: APC, anaphase-promoting complex/cyclosome; CHX, cycloheximide; FBS, fetal bovine serum; HPV, human papillomavirus; IP, immunoprecipitation; UPS, ubiquitin proteasomal system.

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scientific reports

OPEN

Repurposing the Pathogen Box compounds for identification of potent anti-malarials against blood stages of *Plasmodium falciparum* with PfUCHL3 inhibitory activity

Hina Bharti, Aakriti Singal, Manisha Saini, Pradeep Singh Cheema, Mohsin Raza, Suman Kundu & Alo Nag✉

Malaria has endured as a global epidemic since ages and its eradication poses an immense challenge due to the complex life cycle of the causative pathogen and its tolerance to a myriad of therapeutics. PfUCHL3, a member of the ubiquitin C-terminal hydrolase (UCH) family of deubiquitinases (DUBs) is cardinal for parasite survival and emerges as a promising therapeutic target. In this quest, we employed a combination of computational and experimental approaches to identify PfUCHL3 inhibitors as novel anti-malarials. The Pathogen Box library was screened against the crystal structure of PfUCHL3 (PDB ID: 2WE6) and its human ortholog (PDB ID: 1XD3). Fifty molecules with better comparative score, bioavailability and druglikeliness were subjected to in-vitro enzyme inhibition assay and among them only two compounds effectively inhibited PfUCHL3 activity at micro molar concentrations. Both MMV676603 and MMV688704 exhibited anti-plasmodial activity by altering the parasite phenotype at late stages of the asexual life cycle and inducing the accumulation of polyubiquitinated substrates. In addition, both the compounds were non-toxic and portrayed high selectivity window for the parasite over mammalian cells. This is the first comprehensive study to demonstrate the anti-malarial efficacy of PfUCHL3 inhibitors and opens new avenues to exploit UCH family of DUBs as a promising target for the development of next generation anti-malaria therapy.

Abbreviations

PfUCHL3	*Plasmodium falciparum* ubiquitin C-terminal hydrolase 3
DUB	Deubiquitinase
MMV	Medicine for malaria venture
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
HepG2	Human hepatocellular carcinoma
HEK-293T	Human embryonic kidney
Ub-AMC	Ubiquitin C-terminal 7-amido-4-methylcoumarin
IPTG	Isopropyl β-D-1-thiogalactopyranoside
PTM	Post translational modifications
IC_{50}	Half maximal inhibitory concentration
S.I.	Selective index

Malaria is a globally acknowledged parasitic disease that causes an enormous socio-economic burden worldwide. Despite endless preventive measures, it continues to impact millions of people across the regions of sub-Saharan Africa and Asia, impelling thousands of deaths annually[1]. It is caused by six species of the unicellular eukaryotic

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The Anaphase Promoting Complex/ cyclosome co-activator, Cdh1, is a novel target of human Papillomavirus 16 E7 oncoprotein in cervical oncogenesis

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ABSTRACT

The transforming properties of the high risk human papillomavirus E7 oncoprotein are indispensable for driving the virus life cycle and pathogenesis. Besides inactivation of retinoblastoma (Rb) family of tumor suppressors as part of its oncogenic endeavors, E7-mediated perturbations of eminent cell cycle regulators, checkpoint proteins and proto-oncogenes are considered to be the tricks of its transformative traits. However, many such critical interactions are still unknown. In the present study, we have identified the anaphase promoting complex/ cyclosome (APC/C) co-activator, Cdh1, as a novel interacting partner and a degradation target of E7. We found that HPV16 E7-induced inactivation of Cdh1 promoted abnormal accumulation of multiple Cdh1 substrates. Such a mode of deregulation possibly contributes to HPV-mediated cervical oncogenesis. Our mapping studies recognized the carboxyl-terminal zinc finger motif of E7 to associate with Cdh1 and interfere with the timely degradation of FoxM1, a *bona fide* Cdh1 substrate and a potent oncogene. Importantly, the E7 mutant with impaired interaction with Cdh1 exhibited defects in its ability for overriding typical cell cycle transition and oncogenic

frontiers in Oncology

ORIGINAL RESEARCH

Artemisinin Mediates Its Tumor-Suppressive Activity in Hepatocellular Carcinoma Through Targeted Inhibition of FoxM1

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The aberrant up-regulation of the oncogenic transcription factor Forkhead box M1 (FoxM1) is associated with tumor development, progression and metastasis in a myriad of carcinomas, thus establishing it as an attractive target for anticancer drug development. FoxM1 overexpression in hepatocellular carcinoma is reflective of tumor aggressiveness and recurrence, poor prognosis and low survival in patients. In our study, we have identified the antimalarial natural product, Artemisinin, to efficiently curb FoxM1 expression and activity in hepatic cancer cells, thereby exhibiting potential anticancer efficacy. Here, we demonstrated that Artemisinin considerably mitigates FoxM1 transcriptional activity by disrupting its interaction with the promoter region of its downstream targets, thereby suppressing the expression of numerous oncogenic drivers. Augmented level of FoxM1 is implicated in drug resistance of cancer cells, including hepatic tumor cells. Notably, FoxM1 overexpression rendered HCC cells poorly responsive to Artemisinin-mediated cytotoxicity while FoxM1 depletion in resistant liver cancer cells sensitized them to Artemisinin treatment, manifested in lower proliferative and growth index, drop in invasive potential and repressed expression of EMT markers with a concomitantly increased apoptosis. Moreover, Artemisinin, when used in combination with Thiostrepton, an established FoxM1 inhibitor, markedly reduced anchorage-independent growth and displayed more pronounced death in liver cancer cells. We found this effect to be evident even in the resistant HCC cells, thereby putting forth a novel combination therapy for resistant cancer patients. Altogether, our findings provide insight into the pivotal involvement of FoxM1 in the tumor suppressive activities of Artemisinin and shed light on the potential application of Artemisinin for improved therapeutic response, especially in resistant hepatic malignancies. Considering that Artemisinin compounds are in current clinical use with favorable safety profiles, the results from our study will potentiate its utility in juxtaposition with established FoxM1 inhibitors, promoting maximal therapeutic efficacy with minimal adverse effects in liver cancer patients.

Keywords: artemisinin (ART), FOXM1 (Forkhead box M1), hepatocellular carcinoma (HCC), anticancer, anticarcinogenic agent, drug resistance

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Suramin, penciclovir, and anidulafungin exhibit potential in the treatment of COVID-19 via binding to nsp12 of SARS-CoV-2

Sanjay Kumar Dey[a,b,c], Manisha Saini[b], Chetna Dhembla[b], Shruti Bhatt[b], A. Sai Rajesh[d], Vamita Anand[b], Hirendra Kumar Das[e] and Suman Kundu[b]

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ABSTRACT

COVID-19, for which no confirmed therapeutic agents are available, has claimed over 48,14,000 lives globally. A feasible and quicker method to resolve this problem may be 'drug repositioning'. We investigated selected FDA and WHO-EML approved drugs based on their previously promising potential as antivirals, antibacterials or antifungals. These drugs were docked onto the nsp12 protein, which reigns the RNA-dependent RNA polymerase activity of SARS-CoV-2, a key therapeutic target for coronaviruses. Docked complexes were reevaluated using MM-GBSA analysis and the top three inhibitor-protein complexes were subjected to 100 ns long molecular dynamics simulation followed by another round of MM-GBSA analysis. The RMSF plots, binding energies and the mode of physicochemical interaction of the active site of the protein with the drugs were evaluated. Suramin, Penciclovir, and Anidulafungin were found to bind to nsp12 with similar binding energies as that of Remdesivir, which has been used as a therapy for COVID-19. In addition, recent experimental evidences indicate that these drugs exhibit antiviral efficacy against SARS-CoV-2. Such evidence, along with the significant and varied physical interactions of these drugs with the key viral enzyme outlined in this investigation, indicates that they might have a prospective therapeutic potential in the treatment of COVID-19 as monotherapy or combination therapy with Remdesivir.

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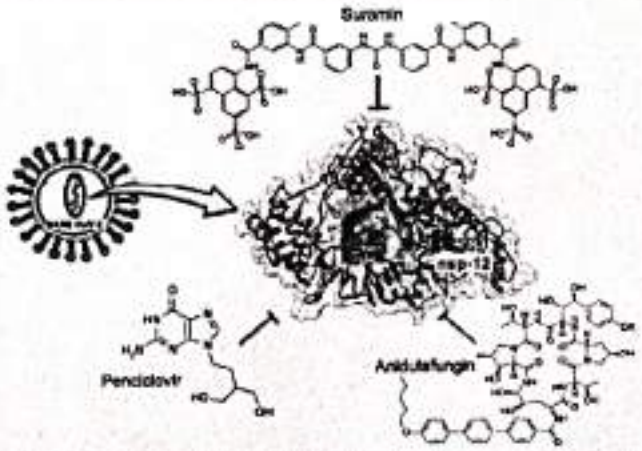

1. Introduction

The novel coronavirus disease 2019 (COVID-19) was declared a global pandemic on the 11th of March 2020 by WHO, barely within three months of the emergence of its first case (Bogoch et al., 2020; Hui et al., 2020). Since that initial instance, it has kept shifting its epicenter through various continents and has now spread to 215 countries and

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Review

An overview of the fatty acid biosynthesis in the protozoan parasite *Leishmania* and its relevance as a drug target against leishmaniasis

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ABSTRACT

Leishmaniasis is one of the fast-growing parasitic diseases worldwide. The treatment of this fatal disease presents a daunting challenge because of its adverse effects, necessity for long-term treatment regime, unavailability of functional drugs, emergence of drug resistance and the related expenditure. This calls for an urgent need for novel drugs and the evaluation of new targets. Proteins of the fatty acid biosynthetic pathway are validated as drug targets in pathogenic bacteria and certain viruses. Likewise, this pathway has been speculated as a suitable target against parasite infections. Fatty acid synthesis in parasites seems to be very complex and distinct from the counterpart mammalian host due to the presence of unique mechanisms for fatty acid biosynthesis and acquisition. In recent times, there have been few evidences of the existence of this pathway in the bloodstream form of some pathogens. The fatty acid biosynthesis thus presents a viable and attractive target for emerging therapeutics. Understanding the mechanisms underlying fatty acid metabolism is key to identifying a potential drug target. However, investigations in this direction are still limited and this article attempts to outline the existing knowledge, while highlighting the scope and relevance of the fatty acid biosynthetic pathway as a drug target. This review highlights the advances in the treatment of leishmaniasis, the importance of lipids in the pathogen, known facts about the fatty acid biosynthesis in *Leishmania* and how this pathway can be manipulated to combat leishmaniasis, suggesting novel drug targets.

1. Introduction

Leishmaniasis, a vector borne protozoan disease spread by female sand flies of the genus *Phlebotomus* [1] and *Lutzomyia* [2], is a prevalent parasitic disease second in significance to malaria. It is one of the six endemic diseases considered as high priorities worldwide [3] with plethora of clinical manifestations.

This disease is characterized by both diversity and complexity. It is transmitted by ~98 different species including 42 phlebotomine sandflies and 56 *Lutzomyia* species [2]. Approximately 70 animal species comprising humans serve as natural reservoirs (hosts) of the parasite [4]. The taxonomic classification of the genera *Leishmania* is summarized in Fig. 1.

There are several forms of leishmaniasis that occur in humans, among which some have tranquil infection i.e., without any evident symptoms [7]. For others, as described below, the clinical manifestations are deadly and of major concern.

Cutaneous leishmaniasis (CL), the most common form of the disease affecting humans, is characterized by the self-limiting skin ulcer at the site of the bite [7]. Alexander Russel in 1756 first described a lesion observed in a Turkish patient resembling cutaneous leishmaniasis as the "Aleppo evil" [4]. Skin lesions are caused on exposed parts of the body

Abbreviations: CL, cutaneous leishmaniasis; VL, visceral leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; WHO, world health organization; LPG, lipophosphoglycan; FAE, fatty acid elongase; FAS, fatty acid synthase; ELO, elongase; GPI, glycosylphosphatidylinositol; BSF, blood stream form; PCF, procyclic form; VSG, variant surface glycoprotein; CoA, coenzyme-A; ACP, acyl carrier protein; PPT, phosphopantetheinyl transferase; AcpS, holo-ACP synthase; Sfp, surfactin phosphopantetheinyl transferase; PUFA, polyunsaturated fatty acids; LGT, lateral gene transfer.

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Herbs and their bioactive ingredients in cardio-protection: Underlying molecular mechanisms and evidences from clinical studies

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ABSTRACT

Background: Medicinal plants or herbs produce a bounty of bioactive phytochemicals. These phytochemicals can influence a variety of physiological events related to cardiovascular health through multiple underlying mechanisms, such as their role as antioxidative, anti-ischemic, anti-proliferative, hypotensive, anti-thrombotic, and anti-hypercholesterolemic agents.

Purpose: The purpose of this review is to summarize and connect evidences supporting the use of phytotherapy in the management of some of the most common cardiovascular impairments, molecular mechanisms underlying cardio-protection mediated by herbs, and clinical studies which are positively linked with the use of herbs in cardiovascular biology. Additionally, we also describe several adverse effects associated with some of the herbal plants and their products to provide a balanced set of studies in favor or against phytotherapy in cardiovascular health that may help global discourses on this matter.

Methods: Studies relating to the use of medicinal plants were mined by strategically searching scientific databases including Google Scholar, PubMed and Science Direct. Investigations involving approximately 175 articles including reviews, research articles, meta-analyses, and cross-sectional and observational studies were retrieved and analyzed in line with the stated purpose of this study.

Results: A positive correlation between the use of medicinal plants and cardiovascular health was observed. While maintaining cardiovascular physiology, medicinal plants and their derivatives seem to govern a variety of cellular mechanisms involved in vasoconstriction and vasorelaxation, which in turn, are important aspects of cardiovascular homeostasis. Furthermore, a variety of studies including clinical trials, cross-sectional studies, and meta-analyses have also supported the anti-hypertensive and thus, cardio-protective effects, of medicinal plants. Apart from this, evidence is also available for the potential drawbacks of several herbs and their products indicating that the unsupervised use of many herbs may lead to severe health issues.

Conclusions: The cardio-protective outcomes of medicinal plants and their derivatives are supported by ever-increasing studies, while evidences exist for the potential drawbacks of some of the herbs. A balanced view about the use of medicinal plants and their derivative in cardiovascular biology thus needs to be outlined by researchers and the medical community. The novelty and exhaustiveness of the present manuscript is reflected by the detailed outline of the molecular basis of "herbal cardio-protection", active involvement of several herbs in ameliorating the cardiovascular status, adverse effects of medicinal plants, and the clinical studies considering the use of phytotherapy, all on a single platform.

Abbreviations: ABCA1, ATP-binding cassette transporter 1; ACE, angiotensin-converting enzyme; CVDs, cardiovascular diseases; CYP450, Cytochrome P450; DBP, diastolic blood pressure; ECM, extracellular matrix; ENOS, endothelial nitric oxide (NO) synthase; LDL, low-density lipoprotein; LXR, liver X receptor; NO, nitric oxide; PGI 2, prostaglandin I 2; PPAR, peroxisome proliferator-activated receptor; RAS, renin-angiotensin system; ROS, reactive oxygen species; SBP, systolic blood pressure; VSMC, vascular smooth muscle cell; WHO, World Health Organization.

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The association of ABO blood group with the asymptomatic COVID-19 cases in India

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ABSTRACT

The COVID-19 pandemic resulted in multiple waves of infection worldwide. The large variations in case fatality rate among different geographical regions suggest that the human susceptibility against this virus varies substantially. Several studies from different parts of the world showed a significant association of ABO blood group and COVID-19 susceptibility. It was demonstrated that individuals with blood group O are at the lower risk of coronavirus infection. To establish the association of ABO blood group in SARS-CoV-2 susceptibility, we for the first time analysed SARS-CoV-2 neutralising antibodies among 509 individuals, collected from three major districts of Eastern Uttar Pradesh region of India. Interestingly, we found neutralising antibodies in a significantly higher percentage of people with blood group AB (0.36) followed by B (0.31), A (0.22) and lowest in people with blood group O (0.11). We further estimated that people with blood group AB are at comparatively higher risk of infection than other blood groups. Thus, among the asymptomatic SARS-CoV-2 recovered people blood group AB has highest, whilst individuals with blood group O has lowest risk of infection.

1. Introduction

COVID-19 has impacted life of billions because of its virulence. The extensive ongoing research revealed the complex nature of this novel SARS-CoV-2 virus transmitted to the humans [1–6]. With the growing knowledge about this disease, it is clear that there are certain risk factors associated with morbidity and mortality [7–9]. More importantly, many of the studies have found strong association of the ABO blood group and COVID-19 with morbidity and mortality [6,10–14], whilst, a few studies have also found no association of COVID-19 with the ABO blood group [10,15,16]. In the past, there have been several studies suggesting the association of ABO blood group with diseases. For example, individual with the blood group O were reported to be more susceptible to the Cholera in Gangetic plain populations [17] and *Helicobacter pylori* infection [18]. However, blood group O was found to be less susceptible for Dengue [19,20] and SARS (Severe Acute Respiratory Syndrome) viruses [14,21].

The ABO blood type is administered by the gene *ABO*, located at chromosome 9 [22]. Studies have found that the this gene modulates the COVID-19 susceptibility directly or indirectly [23–25]. Several genetic variants of this gene affect morbidity and mortality in COVID-19 and many other diseases. For example, it affects red blood related physiology [26,27], venous thromboembolism [28], type 2 diabetes [29], ischemic stroke [30], heart related functions [31] and coronary artery disease [31–33]. Thus, studying the association of ABO blood type with SARS-CoV-2 infection, it is feasible to ascertain the factors influencing

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Advances in mass spectrometric methods for detection of hemoglobin disorders

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ABSTRACT

Hemoglobin disorders are caused due to alterations in the hemoglobin molecules. These disorders are categorized in two broad classes - hemoglobin variants and thalassemias. The hemoglobin variants arise due to point mutations in the alpha (α), beta (β), gamma (γ), delta (δ), or epsilon (ε) globin chains of these proteins, while thalassemias are caused due to the under-production of α or β globin chain. Hemoglobin disorders account for 7 % of the major health issues globally. Mass Spectrometry is an extensively used analytical tool in the field of protein identification, protein-protein interaction, biomarker discovery and diagnosis of several impairments including hemoglobin related disorders. The remarkable advancements in the technology and method development have enormously augmented the clinical significance of mass spectrometry in these fields. The present review describes hemoglobin disorders and the recent advancements in mass spectrometry in the detection of such disorders, including its advantages, lacunae, and future directions. The literature evidence concludes that mass spectrometry can be potentially used as a 'First Line Screening Assay' for the detection of hemoglobin disorders in the near future.

1. Introduction

Hemoglobin (Hb) disorders are among the most common monogenic diseases in the world [1]. Hb disorders are generally classified into two categories comprising of qualitative and quantitative defects. Qualitative defects arise due to single point mutations such as addition, deletion or substitution of amino acids in globin chains; for instance, sickle cell disease (SCD) causing Hemoglobin S (HbS) and Hemoglobin E (HbE), which are called as Hb variants [2,3]. Quantitative defects arise due to decreased production of α or β globin chains and include impairments such as α-thalassemia and β-thalassemia [4,5]. Such abnormalities alter the oxygen-carrying capacity of Hb rendering them unstable.

Hb, a tetrameric protein consisting of two α and two β chains, is confined to circulating erythrocytes in usual circumstances and supplies oxygen to the cells [6]. Each constituent monomer of Hb contains one heme molecule in its hydrophobic pocket [7]. In normal adults, human Hb contains 95–98 % HbA ($\alpha_2\beta_2$), 1.5–3.5 % HbA_2 ($\alpha_2\delta_2$) and <1.0 % HbF ($\alpha_2\gamma_2$) [8–10]. In humans, α-globin gene is located on the 16th chromosome whereas β-globin gene is located on the 11th chromosome, and comprises of 141 and 146 amino acids, respectively [10]. Since each of these types of Hbs contains two α chains in tetramer, hence α chain represents half of the Hb. Therefore, the amount of β, δ, or γ chains will be a specific representative of HbA, HbA_2 and HbF, respectively. As a consequence, the relative concentration of HbA_2 will be proportional to the measurement of the δ chain against α chain and the same is true for HbF (containing γ chain). This property of Hb is widely used for the detection of Hb related disorders [11].

Hb disorders are genetic disorders and hence, they are inherited to offsprings in an autosomal recessive manner [12]. Autosomal recessive disorders are caused due to defects in both the copies of the autosomal genes leading to the "loss of function". These mutations may be homozygous or heterozygous. Homozygous mutations may have deleterious effect due to defects in both the copies of the genes, while defect in only one of the copy of the gene is considered heterozygous resulting in individuals who are carriers of the defect but are asymptomatic [13]. Males and females are equally affected in the population.

Hb disorders are primarily found in tropical regions, in Africa, Asia, the Mediterranean basin and the Middle East [1]. However, Hb disorders have now spread throughout the globe due to migration and hence, they pose a significant global health burden [14]. More than 270 million people are carrier for hemoglobinopathies and at least 300,000 affected

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Analysis of the dark proteome of Chandipura virus reveals maximum propensity for intrinsic disorder in phosphoprotein

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Chandipura virus (CHPV, a member of the *Rhabdoviridae* family) is an emerging pathogen that causes rapidly progressing influenza-like illness and acute encephalitis often leading to coma and death of the human host. Given several CHPV outbreaks in Indian sub-continent, recurring sporadic cases, neurological manifestation, and high mortality rate of this infection, CHPV is gaining global attention. The 'dark proteome' includes the whole proteome with special emphasis on intrinsically disordered proteins (IDP) and IDP regions (IDPR), which are proteins or protein regions that lack unique (or ordered) three-dimensional structures within the cellular milieu. These proteins/regions, however, play a number of vital roles in various biological processes, such as cell cycle regulation, control of signaling pathways, etc. and, therefore, are implicated in many human diseases. IDPs and IPPRs are also abundantly found in many viral proteins enabling their multifunctional roles in the viral life cycles and their capability to highjack various host systems. The unknown abundance of IDP and IDPR in CHPV, therefore, prompted us to analyze the dark proteome of this virus. Our analysis revealed a varying degree of disorder in all five CHPV proteins, with the maximum level of intrinsic disorder propensity being found in Phosphoprotein (P). We have also shown the flexibility of P protein using extensive molecular dynamics simulations up to 500 ns (ns). Furthermore, our analysis also showed the abundant presence of the disorder-based binding regions (also known as molecular recognition features, MoRFs) in CHPV proteins. The identification of IDPs/IDPRs in CHPV proteins suggests that their disordered regions may function as potential interacting domains and may also serve as novel targets for disorder-based drug designs.

Chandipura virus (CHPV) was first isolated in 1965 in the Indian state of Maharashtra, from a patient suffering from the febrile illness, with the ability to produce cytopathic effect on cell culture[1]. CHPV is a member of the Genus *Vesiculovirus* in the family *Rhabdoviridae*. Later it was also isolated from the encephalopathy patients in 1980[2]. However, the first evidence for the CHPV association with human epidemics was obtained in 2003, when this virus was identified in patient samples during an outbreak in India as a determinant of the acute encephalitis with a high fatality rate claiming 183 lives, mostly children below the age of 12[3]. The medical examination of patients recorded high-grade fever, occasional vomiting, rigours, sensorium, drowsiness leading to coma and death within 48 h. Subsequently, another outbreak of CHPV infection with more than 75% fatality rate was reported in the eastern region of Gujarat, India, in 2004[4]. These recurrent occurrences indicated possible emergence of CHPV as a deadly human pathogen in the Indian subcontinent causing acute encephalitis syndrome and involving severe human pathology, which progresses rapidly from an influenza-like illness to coma and death[3]. The female sandflies (*Phlebotomine sandfly*)[3], ticks[5], and mosquitoes[2] are proposed to be the

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ARTICLE

Seasonal changes in recombination characteristics in a natural population of *Drosophila melanogaster*

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Environmental seasonality is a potent evolutionary force, capable of maintaining polymorphism, promoting phenotypic plasticity and causing bet-hedging. In *Drosophila*, environmental seasonality has been reported to affect life-history traits, tolerance to abiotic stressors and immunity. Oscillations in frequencies of alleles underlying fitness-related traits were also documented alongside SNPs across the genome. Here, we test for seasonal changes in two recombination characteristics, crossover rate and crossover interference, in a natural *D. melanogaster* population from India using morphological markers of the three major chromosomes. We show that winter flies, collected after the dry season, have significantly higher desiccation tolerance than their autumn counterparts. This difference proved to hold also for hybrids with three independent marker stocks, suggesting its genetic rather than plastic nature. Significant between-season changes are documented for crossover rate (in 9 of 13 studied intervals) and crossover interference (in four of eight studied pairs of intervals); both single and double crossovers were usually more frequent in the winter cohort. The winter flies also display weaker plasticity of both recombination characteristics to desiccation. We ascribe the observed differences to indirect selection on recombination caused by directional selection on desiccation tolerance. Our findings suggest that changes in recombination characteristics can arise even after a short period of seasonal adaptation (~8–10 generations).

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INTRODUCTION

Environmental seasonality plays an important role as an ecological factor, and its significance as a potent evolutionary force is becoming increasingly evident. The evolutionary consequences of within-year oscillations in selection directions and intensities considerably depend on the generation time. In perennials, exposure to environmental seasonality as lifespan-long regular background may select for pleiotropy and phenotypic plasticity. In annuals, whose developmental stages are distributed throughout a year, it may additionally select for fine-tuning of life-history traits and bet-hedging (Williams et al. 2017). Yet, seasonality effects in multivoltine species, having several generations per year, can be even more complex, leading to far-reaching population-level effects, including maintenance of balanced polymorphism (Haldane and Jayakar 1963; Korol et al. 1996; Wittmann et al. 2017), complex dynamics of allele frequencies (Kirzhner et al. 1995, 1996) and evolving dominance (Otto and Bourguet 1999; Connallon and Chenoweth 2019), in addition to those mentioned above. Moreover, multivoltine species seem to be the most appropriate models for addressing the intriguing interplay between different adaptations to seasonality, including the interaction between plastic and heritable responses to periodical environmental stressors.

Fruit flies are particularly informative models in seasonality studies. The population size of various *Drosophila* species has long been known to fluctuate during a year (Goldschmidt et al. 1955; Prakash and Reddy 1979). Later studies have also shown seasonal oscillation in several important fitness-related phenotypic traits, including desiccation tolerance (McKenzie and Parsons 1974; Parkash et al. 2011; Aggarwal et al. 2013), the activity of metabolic enzymes (Knibb 1986), life-history traits, resistance to heat, cold and starvation (Behrman et al. 2015) and innate immunity (Behrman et al. 2018). In a recent extensive genome-wide analysis, Bergland et al. (2014) identified hundreds of SNPs whose frequency oscillates among seasons; the authors related them to variation in adaptive phenotypic traits, first of all cold- and starvation tolerance.

In contrast to stress tolerance and other fitness-related traits considered in the above-mentioned studies, changes in recombination have never been studied in the context of seasonal adaptation, to the best of our knowledge. Typically, recombination does not directly affect the survival of the individual. However, it does affect the diversity of its progeny and, thereby, the genetic structure of the whole population in the next generation. This suggests that variation in recombination can be adaptive (Korol

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Review

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Exploring the therapeutic potential of forkhead box O for outfoxing COVID-19

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The COVID-19 pandemic has wreaked unprecedented societal havoc worldwide. The infected individuals may present mild to severe symptoms, with nearly 20% of the confirmed patients impaired with significant complications, including multi-organ failure. Acute respiratory distress imposed by SARS-CoV-2 largely results from an aggravated cytokine storm and deregulated immune response. The forkhead box O (FoxO) transcription factors are reported to play a significant role in maintaining normal cell physiology by regulating survival, apoptosis, oxidative stress, development and maturation of T and B lymphocytes, secretion of inflammatory cytokines, etc. We propose a potent anti-inflammatory approach based on activation of the FoxO as an attractive strategy against the novel coronavirus. This regime will be focused on restoring redox and inflammatory homeostasis along with repair of the damaged tissue, activation of lymphocyte effector and memory cells. Repurposing FoxO activators as a means to alleviate the inflammatory burst following SARS-CoV-2 infection can prove immensely valuable in the ongoing pandemic and provide a reliable groundwork for enriching our repertoire of antiviral modalities for any such complication in the future. Altogether, our review highlights the possible efficacy of FoxO activation as a novel arsenal for clinical management of COVID-19.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has wreaked havoc across the globe since its emergence in late 2019 [1]. The spread of the coronavirus disease pushed the World Health Organization (WHO) to declare COVID-19 a worldwide pandemic. Proved to be exceptionally contagious with a current basic reproduction number (R_0) of approximately 3, this global catastrophe demands immediate and adequate attention [2]. Estimates clearly state that at least 60–80% of a given population is falling prey to the grasp of this pandemic, with high fatalities. We have already witnessed a rapid downward spiralling of health sectors across all nations with multiple countries failing to accommodate the soaring number of cases. However, the far-reaching impacts of this calamity on other aspects of civilization, such as economy, are beyond our comprehension. The clinical manifestations of COVID-19 appear mild in most of those infected; however, nearly 20% of the patients suffer from more severe symptoms, including acute respiratory distress syndrome, septic shock and systemic failure. This often culminates in the death of the patient. Although physicians have been trying out various modes of treatment, clinical management of this disease is primarily symptomatic.

The Forkhead Box O (FoxO) subfamily of transcription factors has been elucidated to play critical functions in pulmonary homeostasis apart from their involvement in various cellular biochemical functions [3]. Their importance in maintaining normal lung physiology is highlighted by the appearance of

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L. major apo-acyl carrier protein forms ordered aggregates due to an exposed phenylalanine, while phosphopantetheine inhibits aggregation in the holo-form

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ABSTRACT

L. major acyl carrier protein (ACP) is a mitochondrial protein, involved in fatty acid biosynthesis. The protein is expressed as an apo-protein, and post-translationally modified at Ser 37 by a 4'-Phosphopantetheinyl transferase. Crystal structure of the apo-form of the protein at pH 5.5 suggests a four helix bundle fold, typical of ACP's. However, upon lowering the pH to 5.0, it undergoes a conformational transition from α-helix to β-sheet, and displays amyloid like properties. When left for a few days at room temperature at this pH, the protein forms fibrils, visible under Transmission electron microscopy (TEM). Using an approach combining NMR, biophysical techniques, and mutagenesis, we have identified a Phe residue present on helix II of ACP, liable for this change. Phosphopantetheinylation of *Lm*ACP, or mutation of Phe 45 to the corresponding residue in *E. coli* ACP (methionine), slows down the conformational change. Conversely, substitution of methionine 44 of *E. coli* ACP with a phenylalanine, causes enhanced ThT binding. Thus, we demonstrate the unique property of an exposed Phe in inducing, and phophopantetheine in inhibiting amyloidogenesis. Taken together, our study adds *L. major* acyl carrier protein to the list of ACPs that act as pH sensors.

1. Introduction

Acyl carrier protein (ACP) is a small acidic protein, that plays a central role in the biosynthesis of fatty acids [1], polyketides [2,3], oligosaccharides [4], toxins [5], lipoic acid [6], non-ribosomal peptides, signaling factors [7,8] *etc.* ACP's involved in fatty acid biosynthesis (FAS) have been well studied and characterized. In the type II fatty acid synthesis pathway, *e.g. Plasmodium falciparum*, *E. coli*, spinach, *L. major* and eukaryotic mitochondria, multiple enzymes catalyze different reactions, and ACP exists as an independent protein. In the type I pathway, found in the cytosol of eukaryotes, ACP exists as an integral domain of one large, single, multidomain, multifunctional fatty acid synthase (FAS), a 540 -kDa complex, each domain catalyzing a specific reaction. Yeast and CMN group of bacteria (Corynebacteria, Mycobacteria and Nocardia) also have a Type I FAS, and their ACP comprises two subdomains, an ACP domain, and a structural domain, both participating in the formation of a large barrel shaped 2.6 MDa complex [9]. Despite the structural and organizational differences, ACP from different organisms can be swapped, and are recognized as a substrate *in vitro* by noncognate FAS enzymes [10]. These observations highlight the commonality in the structures of ACP, and the high level of sequence conservation in helix II, crucial for ACP interaction.

ACP is expressed as an apo-protein, and post-translationally modified to the holo-form by the covalent attachment of a Coenzyme A derived phosphopantetheine moiety, catalysed by 4'-Phosphopantetheinyl transferase [11]. The acyl chain covalently attaches to the free SH-group of the phosphopantetheine arm, by a thioester linkage. A hydrophobic cavity in the ACP protects the lengthening acyl chains from the hydrophilic environment, and shuttles it to the catalytic site of fatty acid synthesis enzymes during biosynthesis. The cavity opens at the top, near the amino- terminus of helix II, through which the acyl chain enters. An Ile residue at the bottom of the cavity is crucial for guiding the acyl chain to the base [12]. Upon interaction with FAS enzymes, the 4'-phosphopantetheine moiety, along with the acyl chain is transferred from the hydrophobic cavity of ACP to the active site cleft of the partner enzyme, by a 'chain flipping mechanism' [13]. Despite a very similar biosynthetic mechanism, the final products of fatty acid synthesis vary; in *E. coli* $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, in *P. falciparum*, $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$, in plants $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ are formed [14,15].

Structurally, ACP is a four helix bundle fold, comprising three major helices; helix I antiparallel to helices II and IV, and a 3_{10} helix III, which connects helix II and IV. The helices enclose a flexible hydrophobic

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Research paper

Inhibition of ABCG2 efflux pumps renders the *Mycobacterium tuberculosis* hiding in mesenchymal stem cells responsive to antibiotic treatment

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ABSTRACT

The lengthy TB chemotherapeutic regimen, resulting in the emergence of drug resistance strains, poses a serious problem in the cure of the disease. Further, one-quarter of the world's population is infected with dormant *M.tb*, which creates a lifetime risk of reactivation. *M.tb* has a remarkable tendency to escape the host immune responses by hiding in unconventional niches. Recent studies have shown that bone-marrow mesenchymal stem cells (BM-MSCs) can serve as a reservoir of the pathogen and have been suggested to keep them beyond the reach of anti-TB drugs. In this study, we have shown that *M.tb* infects and grows inside BM-MSCs and were unresponsive to the anti-TB drugs rifampicin and isoniazid when compared to the pathogen residing inside THP-1 macrophages. It was further shown that the ABCG2 efflux pumps of the BM-MSCs were upregulated upon exposure to rifampicin, which may be the contributing factor for the antibiotic unresponsiveness of the bacteria inside these cells. Subsequently, it was shown that inhibition of ABCG2 efflux pumps along with administration of anti-TB drugs led to an increased susceptibility and consequently an enhanced killing of the *M.tb* inside BM-MSCs. These findings for the first time show that the MIC_{99} values of anti-TB drugs increase many folds for the *M. tb* residing in BM-MSCs as compared to *M.tb* residing inside macrophages and the involvement of ABCG2 efflux pumps in this phenomenon. Our study substantiates that these BM-MSCs acts as a useful niche for *M.tb* wherein they can survive by escaping the antibiotic assault that can be attributed to the host ABCG2 efflux pumps. Inhibiting these efflux pumps can be an attractive adjunctive chemotherapy to eliminate the bacteria from this protective niche.

1. Introduction

The current chemotherapeutic regimen is extremely effective against the susceptible *Mycobacterium tuberculosis (M.tb)*, however, it still requires a lengthy duration of administration to completely eliminate the bacterial burden. One of the reason for a longer duration of anti-TB drugs administration is to reach various intracellular niches where bacteria may survive/latently present or may hide in the cells impervious to the action of drugs. Recent studies conducted by Das et al. have shown that *M.tb* may escape the host immune responses by hiding inside the bone marrow mesenchymal stem cells (BM-MSCs) (Das et al., 2013). The evidence of the presence of mesenchymal stem cells at the site of infection by Raghuvanshi et al. primarily showed the involvement of these stem cells in TB (Raghuvanshi et al., 2010). These cells help *M.tb* evade the host immunity and establish a successful infection. *M.tb* has been shown to recruit MSCs and suppress the T-lymphocyte responses at the site of infection (Raghuvanshi et al., 2010). It was demonstrated that *M.tb* can survive in BM-MSCs, in a non-replicating, dormant but viable state (Das et al., 2013). Das et al. could recover viable bacteria from $CD271^+$ BM-MSCs from infected mice by employing mouse model of dormant TB infection as well as from TB treated individuals that were successively diagnosed as TB-free (Das et al., 2013). Additionally, Benner et al. demonstrated that antibiotic treatment could eliminate the bacterial burden from lungs and spleen of infected animals but could not clear the bacteria from bone marrow, which were present majorly in the mesenchymal stem cell population suggesting them to be an antibiotic protective niche, however, the underlying mechanism was not understood (Benner et al., 2014). Subsequently, Garhyan et al. have looked into this phenomenon in clinical and preclinical evaluations and validated that TB treated patients have viable bacteria residing in the

Abbreviations: *M.tb*, *Mycobacterium tuberculosis*; BM-MSC, bone marrow derived mesenchymal stem cells; MSC, mesenchymal stem cells.

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Multiple putative methemoglobin reductases in *C. reinhardtii* may support enzymatic functions for its multiple hemoglobins

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ABSTRACT

The ubiquitous nature of hemoglobins, their presence in multiple forms and low cellular expression in organisms suggests alternative physiological functions of hemoglobins in addition to oxygen transport and storage. Previous research has proposed enzymatic function of hemoglobins such as nitric oxide dioxygenase, nitrite reductase and hydroxylamine reductase. In all these enzymatic functions, active ferrous form of hemoglobin is converted to ferric form and reconversion of ferric to ferrous through reduction partners is under active investigation. The model alga *C. reinhardtii* contains multiple globins and is thus expected to have multiple putative methemoglobin reductases to augment the physiological functions of the novel hemoglobins. In this regard, three putative methemoglobin reductases and three algal hemoglobins were characterized. Our results signify that the identified putative methemoglobin reductases can reduce algal methemoglobins in a nonspecific manner under *in vitro* conditions. Enzyme kinetics of two putative methemoglobin reductases with methemoglobins as substrates and *in silico* analysis support interaction between the hemoglobins and the two reduction partners as also observed *in vitro*. Our investigation on algal methemoglobin reductases underpins the valuable chemistry of nitric oxide with the newly discovered hemoglobins to ensure their physiological relevance, with multiple hemoglobins probably necessitating the presence of multiple reductases.

1. Introduction

Globins are heme-containing proteins with a characteristic three-dimensional structure containing alpha-helical globin fold [1]. Hemoglobins are known to be attached covalently to the prosthetic group by a conserved histidine residue and have the ability to bind oxygen and other gaseous ligands reversibly. The oxygen transport function is considered as one of the most distinctly understood physiological role of hemoglobins. Increased genome sequence information has revealed that hemoglobins are present in all forms of life [2]. The ubiquitous nature of hemoglobins implies that these proteins must have some other significant physiological function that needs further investigation.

Most of the newly discovered globins do not function as oxygen transporters [3,4]. Several studies on diverse animal globins have proposed roles of cytoglobins as tumor suppressors and neuroglobins as neuroprotective agent in neurodegenerative diseases [5,6]. One of the most widely accepted roles of hemoglobins in plants and bacterial cells is nitric oxide (NO) scavenging [3,7–11]. Nitric oxide is an important signaling free radical that can act as a potential toxin to cells beyond a certain concentration [3]. Many organisms have evolved various strategies for NO detoxification, including enzymes like NO reductases and NO dioxygenases [7,12]. Oxidative enzymatic functions for low abundance hemoglobin and oxidative reactions of nitrogen-containing diatomic radicals with hemoglobins have been reported earlier by many researchers [13,14]. It was hypothesized that the blood hemoglobin, myoglobin might have functioned as an enzyme utilizing bound activated oxygen to dioxygenate NO or other substrates in microbes. Flavo hemoglobins reported in *E coli* and yeast are known to have a hybrid protein with a globin domain performing the NO dioxygenase function, while the reductase domain helps in the reduction of ferric to ferrous form to repeat the reaction cycle [7,15].

Although the novel hemoglobins lack the reductase domain, they showed NO dioxygenase function *in-vitro* [3,15]. However, for these globins to act as NO dioxygenase *in vivo*, they would need association

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The pint- sized powerhouse: Illuminating the mighty role of the gut microbiome in improving the outcome of anti- cancer therapy

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ABSTRACT

Cancer persists as a major health catastrophe and a leading cause of widespread mortality across every nation. Research of several decades has increased our understanding of the pivotal pathways and key players of the host during tumor development and progression, which has enabled generation of precision therapeutics with improved efficacy. Despite such tremendous advancements in our combat against this fatal disease, a majority of the cancer patients suffer from poor tumor- free survival owing to the increased incidence of recurrent tumor. This is primarily due to the development of resistance against contemporary anti- cancer strategies. Recent studies have pointed towards the involvement of the human symbiotic gut microbiota in regulating the outcome of chemotherapy and immunotherapy. It does so primarily by modulating the metabolism of the drugs and host immune response, thereby enhancing the efficacy and ameliorating the toxicity. The interactions between the therapeutic agents, microbial community and host immunity may provide a new avenue for the clinical management of cancer. In addition, consumption of dietary pro-, pre- and synbiotics has been recognized to confer protection against tumor genesis and also promote improved response to traditional tumor suppressive strategies. Naturally, the use of various combinatorial regimes containing dietary supplements that improve the gut microbiome in amalgamation with conventional cancer treatment methods may significantly augment the therapeutic outcome of cancer patients and circumnavigate the resistance mechanisms that confound traditional therapies. In this review, we have summarized the role of the gut microbiome, which is the largest assembly of commensals within the human body, in regulating the efficacy and toxicity of various existing anti- cancer therapies including chemotherapy, immunotherapy and surgery. Furthermore, we have discussed how novel strategies integrating the application of probiotics, prebiotics, synbiotics and antibiotics in combination with the aforementioned anti- cancer modules manipulate the gut microbiota and, therefore, augment their therapeutic outcome. Together, such innovative anti- tumorigenic approaches may prove highly effective in improving the prognosis of cancer patients.

1. Introduction

The World Health Organization (WHO) has established cancer as a global menace that claims a mammoth number of lives each year. It was responsible for 9.6 million deaths in the year of 2018 alone, thus representing the second leading cause of mortality worldwide [1]. Over the past decade, persistent and hefty efforts towards the advancements of treatment modalities against cancer, such as surgery, chemotherapy, radiotherapy, immunotherapy and hormonal therapy have succeeded in improving the clinical outcome in most individuals suffering from this disease. Nevertheless, the inadvertent drawbacks associated with such strategies such as increased frequency of tumor recurrence and metastasis and emerging cancer drug resistance outweigh their merits. This adversely affects the quality of life and still keeps the death toll of cancer patients high. The development of resistance against chemotherapy, which is popularly employed to inhibit the spread of tumors, stands as a major impediment to cancer treatment and management. Furthermore, most anti- cancer drugs are found to exert non- specific cytotoxicity to normal cells whereas the tumor cells eventually become resistant to them, thus posing a serious health concern for the modern world [2].

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Significance of human microbiome in breast cancer: Tale of an invisible and an invincible

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ABSTRACT

The human microbiome is a mysterious treasure of the body playing endless important roles in the well-being of the host metabolism, digestion, and immunity. On the other hand, it actively participates in the development of a variety of pathological conditions including cancer. With the Human Microbiome Project initiative, metagenomics, and next-generation sequencing technologies in place, the last decade has witnessed immense explorations and investigations on the enigmatic association of breast cancer with the human microbiome. However, the connection between the human microbiome and breast cancer remains to be explored in greater detail. In fact, there are several emerging questions such as whether the host microbiota contributes to disease initiation, or is it a consequence of the disease is an irrevocably important question that demands a valid answer. Since the microbiome is an extremely complex community, gaps still remain on how this vital microbial organ plays a role in orchestrating breast cancer development. Nevertheless, undeniable evidence from studies has pinpointed the presence of specific microbial elements of the breast and gut to play a role in governing breast cancer. It is still unclear if an alteration in microbiome/dysbiosis leads to breast cancer or is it *vice versa*. Though specific microbial signatures have been detected to be associated with various breast cancer subtypes, the structure and composition of a core "healthy" microbiome is yet to be established. Probiotics seem to be a promising antidote for targeted prevention and treatment of breast cancer. Interestingly, these microbial communities can serve as potential biomarkers for prognosis, diagnosis, and treatment of breast cancer, thereby leading to the rise of a completely new era of personalized medicine. This review is a humble attempt to summarize the research findings on the human microbiome and its relation to breast cancer.

1. Chronicles of the human microbiome

The human body is one such super-creature harbouring about 10 folds as many microbial cells as its own body cells [1]. It may come as a surprise to many that only a small proportion of our genetic material is inherited from our parents whereas a gargantuan portion of our genetic blueprint is actually shared with the microbial communities! In other words, the human body successfully functions due to the combined efforts from our visible organs and our invisible microbial dwellers. The Nobel laureate Joshua Lederberg in 2001 coined the term "Microbiome" for this consortium of symbiotic microflora [2]. Accordingly, for humans, it came to be known as the "Human Microbiome". This conglomerate, or more specifically, the "microbiota", constitutes of the microbial taxa associated with complex organisms like human beings. The human microbiota is as unique as one's fingerprint and undergoes dynamic changes over the course of life. Speaking of the human microbiota, the first thing that sparks in our minds is the gut microflora. A multiplex of bacteria, viruses, fungi, archaea, and small protozoa resides within our gut. Notably, there exists a deep interplay between this community and the host mucosal epithelial cells and immune cells in a reciprocal fashion. Microbiota of the gut plays a cardinal role in multiple cellular and metabolic functions, a few of which include digestion, metabolism of bile acids, synthesis of essential growth factors and vitamins B and K, protection against systemic infiltration and expulsion of intestinal pathogens, and boosting of the host immune system through activation of immune cells [3]. In this manner, the microbiota maintains homeostasis in the gut. However, disruption in this equilibrium facilitated by repeated courses of antibiotics, unhealthy diet, stress, and countless other factors results in a state of "dysbiosis", leading to an impaired microbiota. Consequently, a perturbed microbial ecosystem

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frontiers

Molecular Attributes Associated With Refolding of Inclusion Body Proteins Using the Freeze–Thaw Method

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Understanding the structure–function of inclusion bodies (IBs) in the last two decades has led to the development of several mild solubilization buffers for the improved recovery of bioactive proteins. The recently developed freeze–thaw-based inclusion body protein solubilization method has received a great deal of attention due to its simplicity and cost-effectiveness. The present report investigates the reproducibility, efficiency, and plausible mechanism of the freeze–thaw-based IB solubilization. The percentage recovery of functionally active protein species of human growth hormone (hGH) and L-asparaginase from their IBs in *Escherichia coli* and the quality attributes associated with the freeze–thaw-based solubilization method were analyzed in detail. The overall yield of the purified hGH and L-asparaginase protein was found to be around 14 and 25%, respectively. Both purified proteins had functionally active species lower than that observed with commercial proteins. Biophysical and biochemical analyses revealed that the formation of soluble aggregates was a major limitation in the case of tough IB protein like hGH. On the other hand, the destabilization of soft IB protein like L-asparaginase led to the poor recovery of functionally active protein species. Our study provides insight into the advantages, disadvantages, and molecular-structural information associated with the freeze–thaw-based solubilization method.

Keywords: inclusion bodies, mild solubilization method, freeze–thaw method, protein refolding, human growth hormone, protein aggregates, L-asparaginase

INTRODUCTION

Since the inception of recombinant DNA technology, *Escherichia coli* has been most widely used as a host to produce recombinant proteins whose bioactivity is not dependent on posttranslational modifications. Almost 80% of overexpressed recombinant proteins in *E. coli* result in the formation of protein aggregates known as inclusion bodies (IBs) [ ]. IBs, in general, are highly dense particles having low aqueous solubility. Solubilization of the inclusion body proteins and

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HigB1 Toxin in *Mycobacterium tuberculosis* Is Upregulated During Stress and Required to Establish Infection in Guinea Pigs

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The extraordinary expansion of Toxin Antitoxin (TA) modules in the genome of *Mycobacterium tuberculosis* has received significant attention over the last few decades. The cumulative evidence suggests that TA systems are activated in response to stress conditions and are essential for *M. tuberculosis* pathogenesis. In *M. tuberculosis*, Rv1955-Rv1956-Rv1957 constitutes the only tripartite TAC (Toxin Antitoxin Chaperone) module. In this locus, Rv1955 (HigB1) encodes for the toxin and Rv1956 (HigA1) encodes for antitoxin. Rv1957 encodes for a SecB-like chaperone that regulates HigBA1 toxin antitoxin system by preventing HigA1 degradation. Here, we have investigated the physiological role of HigB1 toxin in stress adaptation and pathogenesis of *Mycobacterium tuberculosis*. qPCR studies revealed that *higBA*1 is upregulated in nutrient limiting conditions and upon exposure to levofloxacin. We also show that the promoter activity of *higBA*1 locus in *M. tuberculosis* is (p)ppGpp dependent. We observed that HigB1 locus is non-essential for *M. tuberculosis* growth under different stress conditions *in vitro*. However, guinea pigs infected with *higB*1 deletion strain exhibited significantly reduced bacterial loads and pathological damage in comparison to the animals infected with the parental strain. Transcriptome analysis suggested that deletion of *higB*1 reduced the expression of genes involved in virulence, detoxification and adaptation. The present study describes the role of *higB*1 toxin in *M. tuberculosis* physiology and highlights the importance of *higBA*1 locus during infection in host tissues.

Keywords: *Mycobacterium tuberculosis*, HigBA1, toxin antitoxin loci, virulence, stringent response

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is a major health concern and infects nearly one-third of the world population. The failure of BCG vaccine to impart protection in adult population and HIV co-infection has negative impact over the control of global TB cases. There is a significant increase in the number of patients infected with the *M. tuberculosis*

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Computational insight into the three-dimensional structure of ADP ribosylation factor like protein 15, a novel susceptibility gene for rheumatoid arthritis

Aditya Sharma , Manisha Saini , Suman Kundu & B. K. Thelma

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ORIGINAL ARTICLE

Coping with stress: role of Arabidopsis phytoglobins in defence against *Sclerotinia sclerotiorum*

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Abstract
Phytoglobins (Pgbs) are multifaceted stress-responsive proteins implicated in regulating various physiological and stress-responsive pathways in plants. Previous work has demonstrated NO dioxygenase and peroxidase-like activity of *Arabidopsis* phytoglobin 3 (AHb3) and its potential role in defense against *Sclerotinia sclerotiorum*. The work reported here highlights the significance of the other two classes of Arabidopsis phytoglobins (AHb1 and AHb2) in response to *S. sclerotiorum*. Constitutive expression of AHb1 (OEAHb1) and AHb2 (OEAHb2) conferred marginal tolerance towards *S. sclerotiorum* whereas respective knockdown (RNAi) lines displayed enhanced susceptibility, with AHb1 RNAi (RNAi-1) lines being more susceptible in comparison to AHb2 RNAi (RNAi-2) lines. Interestingly, transgenic lines with a simultaneous reduction in the transcripts of AHb1 and AHb2 (RNAi-F) displayed greater disease spread in comparison to individual knockdown lines indicative of their additive effect. The enhanced susceptibility upon pathogen challenge correlated with the elevated NO and H_2O_2 levels in these lines. Furthermore, detailed structural analysis hints towards an alternate mechanism of NO dioxyegnation by AHbs. Taken together, the current investigation illustrates the NO dioxygenase and peroxidase-like activity of AHbs and highlights their role in defense against stem rot pathogen *S. sclerotiorum*.

Keywords *Arabidopsis* phytoglobins · *Sclerotinia sclerotiorum* · Nitric oxide dioxygenase · peroxidase

Abbreviations

Pgbs	Phytoglobins
AHb	Arabidopsis phytoglobin
NO	Nitric oxide
ROS	Reactive oxygen species
OE	Overexpression
H_2O_2	Hydrogen peroxide.

Introduction

Three classes of phytoglobin genes (Pgbs) can be distinguished in plant genomes. Each class displays unique kinetic and structural fingerprints (Trevaskis et al. 1997; Watts et al. 2001). Depending upon the plant species, Pgbs are expressed across diverse plant organs throughout all developmental stages and display huge diversity in their expression profiles (Hunt et al. 2001; Garrocho-Villegas et al. 2007; Hebelstrup et al.2007; Bacana et al. 2020). The presence of phytoglobin genes in metabolically active and stressed tissue implicates their role in plant development as well as in mediating various biotic and abiotic stress responses possibly by binding to a wide variety of gaseous ligand including O_2, NO, H_2O_2, etc. (Hebelstrup et al. 2007; Smagghe et al. 2007; Dordas 2009). Attributed to their high oxygen affinities (esp. class I Pgbs), phytoglobins have been implicated to function as O_2 scavengers or in O_2 signaling and maintain cellular redox balance by modulating the levels of Nitric oxide (NO) produced under various biotic/abiotic stress conditions (Seregelyes and Dudits 2003).

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Review article

Functional implications of vascular endothelium in regulation of endothelial nitric oxide synthesis to control blood pressure and cardiac functions

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ABSTRACT

The endothelium is the innermost vascular lining performing significant roles all over the human body while maintaining the blood pressure at physiological levels. Malfunction of endothelium is thus recognized as a biomarker linked with many vascular diseases including but not limited to atherosclerosis, hypertension and thrombosis. Alternatively, prevention of endothelial malfunctioning or regulating the functions of its associated physiological partners like endothelial nitric oxide synthase can prevent the associated vascular disorders which account for the highest death toll worldwide. While many anti-hypertensive drugs are available commercially, a comprehensive description of the key physiological roles of the endothelium and its regulation by endothelial nitric oxide synthase or vice versa is the need of the hour to understand its contribution in vascular homeostasis. This, in turn, will help in designing new therapeutics targeting endothelial nitric oxide synthase or its interacting partners present in the cellular pool. This review describes the central role of vascular endothelium in the regulation of endothelial nitric oxide synthase while outlining the emerging drug targets present in the vasculature with potential to treat vascular disorders including hypertension.

1. Introduction

Vascular disorders, especially hypertension and related heart diseases, have been the key death burden throughout the world [1–3]. Blood vessels and its inner layer, known as the endothelium, play diverse but key roles in nitric oxide (NO) synthesis, which in turn regulates the blood pressure and vascular functions in the human body. However, decreased synthesis of NO can lead to destructive pathophysiological conditions including oxidative stress, hypertension and other cardiovascular impairments [4,5]. Therefore, the prevention of endothelial dysfunction, an adverse pathophysiological condition, has been a major area of research to counter these diseases. Proper functioning of the endothelium may depend on the prevention of multiple pathophysiological or disease conditions including oxidative stress, homocystinemia, hypertension and malfunction of endothelial nitric oxide synthase (eNOS), which regulates the synthesis and maintenance of NO in the endothelium [,]. While eNOS is present in vascular endothelium, its proper functioning can be regulated by multiple factors spread across smooth muscles or myoendothelial junctions (MEJs), as well as by various post-translational modifications [7,8]. The importance of both endothelium and eNOS is well studied. However, their interaction and mechanism of functional regulation in preventing the vascular disorders have not yet been described adequately, thereby, limiting the investigation of these two physiological pathways as targets to treat vascular diseases. This review summarizes the functional role of the endothelium in synchronizing various physiological functions in addition to the regulated production of NO. In addition, it focuses on several biomarkers that signify the onset of dysregulation in normal endothelial functions and several strategies to deal with this in order to combat cardiovascular diseases including hypertension.

2. Vascular endothelium

Maintenance of blood pressure is one of the most coordinated and controlled events. This is in part regulated by a network of arterioles known as resistance arteries. This elaborate network functions to maintain cellular homeostasis consistently by endocrine, paracrine, and autocrine signaling cascades [9]. In resistance arteries, the anatomical location involved in cellular communication is MEJ, where vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) join each other to work in a coordinated and fashionable manner as shown in [ ]. Instead of being just an inactive lining of cells, the endothelium is a highly dynamic structure actively involved in the production of a variety of factors engaged in cell adhesion, thrombosis and

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REVIEW

Natural products and polymeric nanocarriers for cancer treatment: a review

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Abstract
Cancer is one of the most lethal disease that affects humans. Many anticancer agents have been developed but have downsides such as toxicity, affordability and limits of the administered dose. Alternatively, natural anticancer products are promising due to their non-toxic nature. Indeed, natural anticancer products have doubled the human life span in twentieth century. Moreover, over 60% of approved drugs and drug candidates against cancer are of natural origin. Yet, natural drugs have limitations such as non-specific action, low aqueous solubility and reduced bioavailability. These limitations can be circumvented by delivering drugs using polymeric nanocarriers. For instance, polylactic acid and polylactic-co-glycolic acid have been used as carriers in the medical field for a very long time. About 20 products based on these polymers have been approved by the Food and Drug Administration and European Medicine. Polymeric nanocarriers are versatile and have potential to meet actual challenges in the delivery of natural products for cancer treatment. Here we review natural products in treating cancer with focus on polymeric nanocarriers.

Keywords Natural product · Biodegradable polymer · Nanoparticle · Nanocarrier · Cancer · Poly (lactic acid)-poly (lactide-co-glycolic acid) · Chemotherapy

Introduction

Cancer has been the one of the major fatal disease claiming about 1 in every 6 deaths occurring globally. In the year 2018 an estimated 9.6 million lives were lost due to cancer. About 70% of these deaths occurred in low and middle income countries (WHO n.d). It is marked by deregulated cell growth forming a tumoral mass. The capability of new blood vessel formation not only provides them with continued oxygen and nutrient supply but also grant cancer its dreaded metastastic potential leading it to further spread in other sites of the body ultimately culminating in death. Chemotherapy has been the most successful choice for treatment of cancers. It is based on targeting abnormal cancer cells but unfortunately also damages healthy cells of the body (Danhier et al. 2012; Perez-Herrero and Fernandez-Medarde 2015). In the past few years, the use of natural products with anticancer properties has gained much appreciation. Their natural origin, relatively non-toxic nature and pleiotropic effects have been largely responsible for this attention. A wide variety of natural products like epigallocatechin gallate, curcumin, resveratrol and many others have been investigated for their anticancer action. About 6 in 10 drugs approved and pre-new drug application candidates against cancer are from a natural product or derived from them (Demain and Vaishnav 2011). However, most of them face challenges due to low solubility, stability, poor permeability and with approximately 40% of the new chemical entity emerging against cancer being lipophilic (Arora and Jaglan 2016; Siddiqui and Sanna 2016; Behera and Padhi 2020).

The application of synthetic polymeric nanocarriers for cancer chemotherapy has drawn a lot of attention in the scientific community. Polymers like poly lactic acid or poly lactide-co-glycolide have a long been used in biomedical applications (Frazza and Schmitt 1971; O'Hagan and Singh 2003). They are approved by Food and Drug Administration as well as European Medical Agency (Danhier et al. 2012).

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viruses

MDPI

Communication

The Sialoside-Binding Pocket of SARS-CoV-2 Spike Glycoprotein Structurally Resembles MERS-CoV

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Abstract: COVID-19 novel coronavirus (CoV) disease caused by severe acquired respiratory syndrome (SARS)-CoV-2 manifests severe lethal respiratory illness in humans and has recently developed into a worldwide pandemic. The lack of effective treatment strategy and vaccines against the SARS-CoV-2 poses a threat to human health. An extremely high infection rate and multi-organ secondary infection within a short period of time makes this virus more deadly and challenging for therapeutic interventions. Despite high sequence similarity and utilization of common host-cell receptor, human angiotensin-converting enzyme-2 (ACE2) for virus entry, SARS-CoV-2 is much more infectious than SARS-CoV. Structure-based sequence comparison of the N-terminal domain (NTD) of the spike protein of Middle East respiratory syndrome (MERS)-CoV, SARS-CoV, and SARS-CoV-2 illustrate three divergent loop regions in SARS-CoV-2, which is reminiscent of MERS-CoV sialoside binding pockets. Comparative binding analysis with host sialosides revealed conformational flexibility of SARS-CoV-2 divergent loop regions to accommodate diverse glycan-rich sialosides. These key differences with SARS-CoV and similarity with MERS-CoV suggest an evolutionary adaptation of SARS-CoV-2 spike glycoprotein reciprocal interaction with host surface sialosides to infect host cells with wide tissue tropism.

Keywords: SARS-CoV-2; N-terminal domain; spike glycoprotein; MERS-CoV

1. Introduction

Multiple coronaviruses (CoV) are known to cause infection in humans of which β-coronavirus family members, namely severe acquired respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and recently SARS-CoV-2 outbreak is a serious threat to the public health and has infected over 7 million people including over 400,000 deaths worldwide with a latency period of 3–14 days [1]. Recently, the World Health Organization (WHO) officially declared the COVID-19 novel coronavirus disease (caused by SARS-CoV-2) a global pandemic. SARS-CoV-2 is a positive-strand RNA virus and like SARS-CoV and MERS-CoV, attacks the lower respiratory system, causing acute respiratory distress in the lungs [2]. Recent reports suggest that SARS-CoV-2 also targets multiple organ systems like the heart, liver, kidney, gastrointestinal system, and central nervous system [3–5]. The rapid

OPINION

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Dopamine β hydroxylase as a potential drug target to combat hypertension

Sanjay Kumar Dey , Manisha Saini , Pankaj Prabhakar & Suman Kundu

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Identification and characterization of a recombinant cognate hemoglobin reductase from *Synechocystis* sp. PCC 6803

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ABSTRACT

One popular and relevant proposed function for cyanobacterial hemoglobin (*Synechocystis* Hb) is anaerobic nitrite reductase *in vivo*. During such reduction reactions, the hexacoordinated heme iron atom of *Syn*Hb is oxidized from the ferrous (Fe^{+2}) to ferric (Fe^{+3}) state and prevent damage by limiting the concentration of toxic metabolites such as nitrite. In order to perform these functions *in vivo*, there must be a mechanism that converts inactive Fe^{+3}-*Syn*Hb back to the active Fe^{+2}-*Syn*Hb to accomplish the nitrite reductase function. Here, we report a cognate reductase protein for *Synechocystis* hemoglobin which can reduce the Fe^{+3}-*Syn*Hb to Fe^{+2}-*Syn*Hb, thus lending a support to the proposed nitrite reductase function. This reductase is also able to reduce pentacoordinate Hbs such as myoglobin but with lower affinity compared to hexacoordinate *Syn*Hb. *In silico* model of reductase protein-cyanobacterial hemoglobin complex revealed that the heme active site of Hb faces the catalytic center of the reductase protein and several amino acids in the interface interacts non-covalently thus favoring their interaction. Overall, our *in vitro* study provides the basic foundation for the understanding of the specific molecular mechanism of action and interaction of the *Syn*Hb reductase protein, which need to be investigated in further detail.

1. Introduction

The extensive analysis of hemoglobins in the genomes from the three kingdoms of life revealed that the prominent function of globins is enzymatic in nature [1]. The reversible O_2 binding of mammalian myoglobin and hemoglobin was achieved by extensive diversification of the enzymatic functions of the hemoglobins to permit oxygen storage and transport, respectively [2]. Most importantly, their functions remain an enigma though various pieces of evidences have ruled out the oxygen storage and transport function which are commonly associated with hemoglobins. It has been assumed based on numerous reports that hemoglobins can perform various function by inducing conformation changes upon ligand binding which includes detection, scavenging, and detoxification of O_2 and O_2 – derived species (e.g., NO and CO) [3].

One function that has gained popularity over the years is the nitric oxide (NO) scavenging and nitrite related chemistries. It has been well documented that all hemeproteins can react with NO, one of the earliest and important function of hemoglobins. Physiological evidences of NO reaction with hemoglobins have come from blood hemoglobin (Hb) and myoglobin (Mb) and from bacterial and fungal flavohemoglobins (FHbs), which significantly expanded our understanding of the reaction of NO with hemoglobins [4–7]. Kinetic measurements demonstrated that cell-free hemoglobin is able to react with NO ~1000 times faster compared to RBC-encapsulated Hb, highlighting the NO scavenging function of hemoglobin [8]. In RBCs, endothelium-derived NO reacts with oxyhemoglobin (oxyHb, $Fe^{2+}O_2$Hb) to form methemoglobin (metHb, Fe^{3+}Hb) and nitrate (NO_3^-), thereby limiting the bioavailability of NO in the vascular compartment. Globins related in such function would be rapidly oxidized requiring the presence of cognate reductases to reduce them back to the ferrous form.

Synechocystis Hb (*Syn*Hb) is a hexacoordinate member of truncated hemoglobin family and displays hexacoordination in both the deoxygenated ferrous and ferric states [9–11]. Hargrove et al. [12] reported that *Syn*Hb exhibits complex biphasic ligand binding kinetics compared to monophasic ligand binding kinetics of Mb or leghemoglobin. Subsequently, the novel globin was crystallized and its three-dimensional structure in presence and absence of ligand was solved [13]. NMR structure of the globin was also solved [14]. Such structures demonstrated ligand-induced conformational change in *Syn*Hb is unprecedented in globin superfamily. His117 (H16) that covalently associated to heme vinyl group was discovered and investigated. The three His linkages to heme were unique for *Syn*Hb [15–17]. Various functions have been assigned to cyanobacterial Hb, but solid assignment of its physiological role remains elusive [18–20]. The functions vary from reversible binding

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Stability and Folding of the Unusually Stable Hemoglobin from *Synechocystis* is Subtly Optimized and Dependent on the Key Heme Pocket Residues

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Affiliations

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Abstract

Aims: The aim of our study is to understand the biophysical traits that govern the stability and folding of Synechocystis hemoglobin, a unique cyanobacterial globin that displays unusual traits not observed in any of the other globins discovered so far.

Background: For the past few decades, classical hemoglobins such as vertebrate hemoglobin and myoglobin have been extensively studied to unravel the stability and folding mechanisms of hemoglobins. However, the expanding wealth of hemoglobins identified in all life forms with novel properties, like heme coordination chemistry and globin fold, have added complexity and challenges to the understanding of hemoglobin stability, which has not been adequately addressed. Here, we explored the unique truncated and hexacoordinate hemoglobin from the freshwater cyanobacterium Synechocystis sp. PCC 6803 known as "Synechocystis hemoglobin (SynHb)". The "three histidines" linkages to heme are novel to this cyanobacterial hemoglobin.

Objective: Mutational studies were employed to decipher the residues within the heme pocket that dictate the stability and folding of SynHb.

Methods: Site-directed mutants of SynHb were generated and analyzed using a repertoire of spectroscopic and calorimetric tools.

Results: The results revealed that the heme was stably associated to the protein under all denaturing conditions with His117 playing the anchoring role. The studies also highlighted the possibility of existence of a "molten globule" like intermediate at acidic pH in this exceptionally thermostable globin. His117 and other key residues in the heme pocket play an indispensable role in imparting significant polypeptide stability.

Conclusion: Synechocystis hemoglobin presents an important model system for investigations of protein folding and stability in general. The heme pocket residues influenced the folding and stability of SynHb in a very subtle and specific manner and may have been optimized to make this Hb the most stable known as of date. Other: The knowledge gained hereby about the influence of heme pocket amino acid side chains on stability and expression is currently being utilized to improve the stability of recombinant human Hbs for efficient use as oxygen delivery vehicles.

Keywords: folding; hexacoordinate truncated hemoglobin; molten globule; site-directed mutagenesis; stability; synechocystis hemoglobin.

Related information

MedGen
PubChem Compound (MeSH Keyword)

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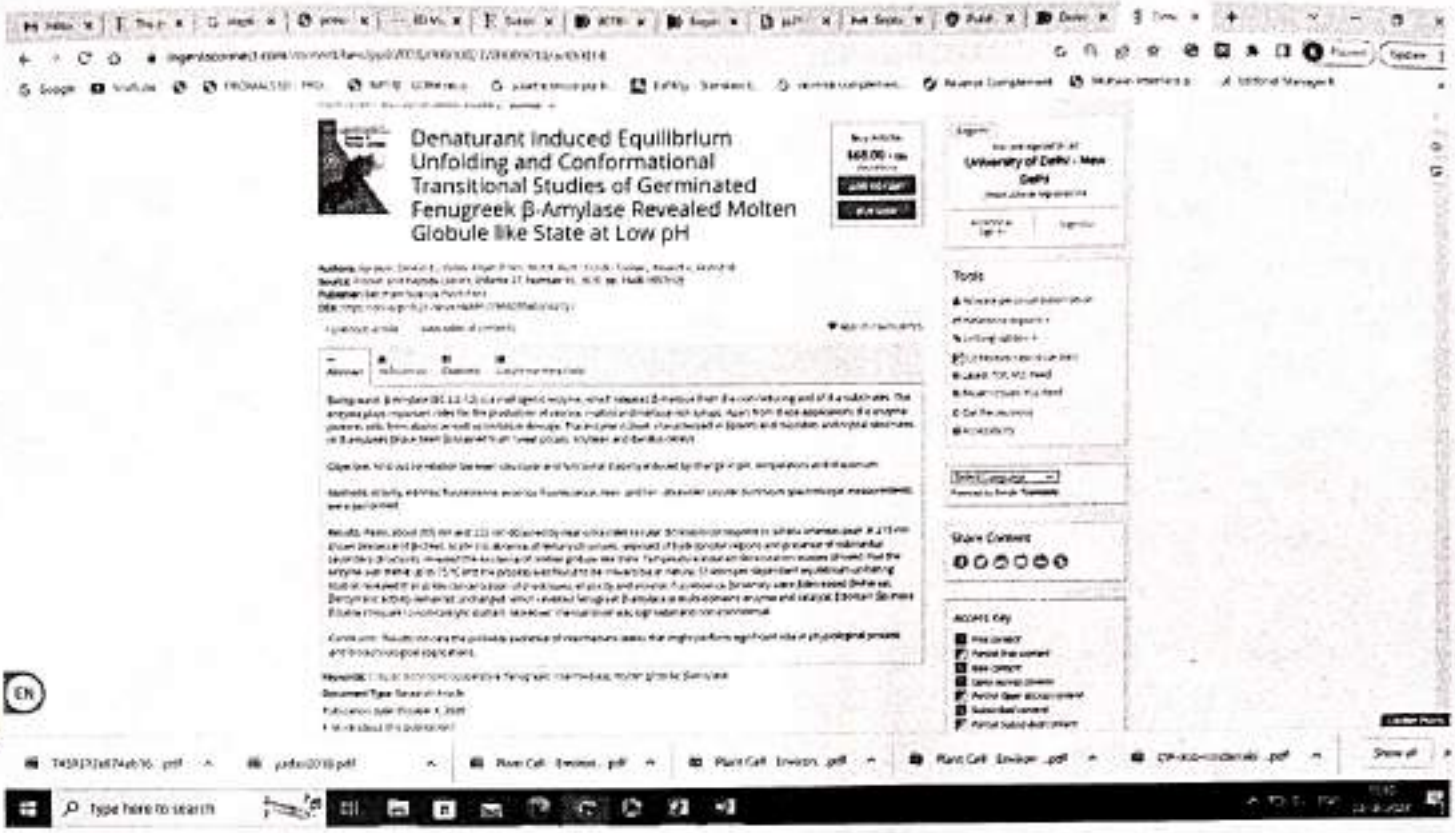

Denaturant Induced Equilibrium Unfolding and Conformational Transitional Studies of Germinated Fenugreek β-Amylase Revealed Molten Globule like State at Low pH

Unraveling the role of the transcriptional regulator VirS in low pH–induced responses of *Mycobacterium tuberculosis* and identification of VirS inhibitors

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Edited by Chris Whitfield

The ability of *Mycobacterium tuberculosis* to respond and adapt to various stresses such as oxygen/nitrogen radicals and low pH inside macrophages is critical for the persistence of this human pathogen inside its host. We have previously shown that an AraC/XylS-type transcriptional regulator, VirS, which is induced in low pH, is involved in remodeling the architecture of the bacterial cell envelope. However, how VirS influences gene expression to coordinate these pH responses remains unclear. Here, using a genetic biosensor of cytoplasmic pH, we demonstrate that VirS is required for the intracellular pH maintenance in response to acidic stress and inside acidified macrophages. Furthermore, we observed that VirS plays an important role in blocking phagosomal-lysosomal fusions. Transcriptomics experiments revealed that VirS affects the expression of genes encoding metabolic enzymes, cell-wall envelope proteins, efflux pumps, ion transporters, detoxification enzymes, and transcriptional regulators expressed under low-pH stress. Employing electrophoretic mobility-shift assays, DNA footprinting, and *in silico* analysis, we identified a DNA sequence to which VirS binds and key residues in VirS required for its interaction with DNA. A significant role of VirS in *M. tuberculosis* survival in adverse conditions suggested it as a potential anti-mycobacterial drug target. To that end, we identified VirS inhibitors in a virtual screen; the top hit compounds inhibited its DNA-binding activity and also *M. tuberculosis* growth *in vitro* and inside macrophages. Our findings establish that VirS mediates *M. tuberculosis* responses to acidic stress and identify VirS-inhibiting compounds that may form the basis for developing more effective anti-mycobacterial agents.

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The microarray data have been deposited to the GEO database and are available under accession ID GSE118508.

This article contains Tables S1–S6, Figs. S1–S3, and Excel Sheet X1.

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Mycobacterium tuberculosis is known to resist the acidic stress encountered in macrophages and multiply in these hostile conditions; however, the mechanisms for its survival in acidic conditions are poorly understood. There are a few genes that have been implicated in acid resistance in *M. tuberculosis*, which include serine protease Rv3671c, the OmpATb operon, and a putative magnesium transporter, MgtC (1–3). A transposon mutant of Rv3671c was shown to be impaired in the maintenance of intrabacterial pH under acidic conditions, suggesting its involvement in the acid resistance (1). OmpATb, an acid-responsive porin, was shown to be involved in the adaptation to acidic environment by mediating secretion of ammonia; however, its role was considered to be redundant, as knockout of OmpATb did not influence the virulence of the pathogen in mice (2, 4). *M. tuberculosis* lacking MgtC, a putative magnesium transporter, was found to be attenuated for growth under mild acidic conditions at low Mg^{2+} (3). Apart from *M. tuberculosis*, acid resistance has also been shown to be important for other bacteria, such as *Helicobacter pylori*, which colonizes in the human stomach, having an acidic environment; *Streptococcus pneumoniae*; and pathogenic strains of *Escherichia coli* and *Salmonella enterica*. Studies have shown the involvement of a few proteins in the acid resistance of these bacteria that include urease and ExbD in *H. pylori*, Gad proteins, and F_0F_1-ATPase in *E. coli* and Mg^{2+} transporter in *Salmonella* (5–9).

VirS (Rv3082c) of *M. tuberculosis* belongs to the AraC family of transcriptional regulators (10, 11). VirS is present divergently upstream of an acid-inducible operon termed the *mymA* operon, which comprises of seven genes (Rv3083–Rv3089) (12). The transcription of the *mymA* operon under acidic stress has been earlier shown to be regulated by VirS, which itself is regulated by acidic pH (12). Studies demonstrated that the *virS* mutant of *M. tuberculosis* exhibited altered cell-wall structure, altered mycolic acid content, defective intramacrophage survival, and reduced hematogenous dissemination *in vivo* (13). Importantly, *virS* expression was induced during chronic and reactivation phases of murine tuberculosis, implicating VirS in persistence and reactivation of tuberculosis (14). Despite these findings, mechanisms of how VirS exerts its influence on gene expression to elicit the response of *M. tuberculosis* under acid stress remain uncharacterized.

Here, our study has delineated the contribution of VirS in acid stress and how it mediates its influence on gene expression

FEBS Letters

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Site-specific phosphorylation of villin remodels the actin cytoskeleton to regulate Sendai viral glycoprotein-mediated membrane fusion

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Connivance of cellular factors during virus-host cell membrane fusion is poorly understood. We have recently shown that cellular villin plays an important role during membrane fusion of reconstituted Sendai virosomes with hepatocytes. Here, we employed villin-null Chinese Hamster Ovary (CHO) cells, where villin expression led to an increased fusion with virosomes, which was further enhanced due to tyrosine phosphorylation in the presence of c-src. However, the villin RRI mutant, lacking actin-severing function, failed to augment membrane fusion. Furthermore, quantitative mass spectrometry and detailed analysis revealed Tyr^{179} to be the key phosphorylation site of villin responsible for the enhancement of virosome-CHO cell fusion. Overall, our results demonstrate a critical role for villin and its cell-type dependent phosphorylation in regulating membrane fusion.

Keywords: actin; host pathogen interaction; membrane fusion; Sendai virosome; villin

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Sendai virus (SeV), a well characterized member of the *paramyxoviridae* family, binds to cellular sialoglycoprotein surface receptors through its haemagglutinin-neuraminidase (HN) protein which in turn promotes viral fusion (F) protein mediated entry into host cells in order to deliver the nucleocapsid directly into the cytoplasm [1]. A successful SeV infection is dependent on these two initial steps of viral entry mediated by HN and F proteins respectively. Reconstituted Sendai viral envelopes (virosomes) containing F and HN can successfully deliver bioactive molecules into host cell cytoplasm [2,3]. Furthermore, biochemical exclusion of HN (thus

Abbreviations

ABP, actin binding protein; CHO, Chinese hamster ovary; CTCF, corrected total cell fluorescence; DN, detergent resistant; HAU, haemagglutinating units; HNPs, haemagglutinin-neuraminidase fusion protein virosomes; HNPs-F^{-}, haemagglutinin-neuraminidase fusion protein virosomes lacking fusion activity; HP, head piece; FDQ, fluorescence dequenching; RITC, Rhodamine isothiocyanate; SeV, Sendai Virus.

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ORIGINAL ARTICLE

Identification of novel interaction between Promyelocytic Leukemia protein and human Alteration/Deficiency in Activation 3 coactivator and its role in DNA damage response

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Abstract
Human Alteration/Deficiency in Activation 3 (hADA3) is an interesting transcriptional coactivator adaptor protein with predominant nuclear localization. Even though it has been shown to be an important mediator of diverse cellular processes including embryonic development, cell cycle progression and maintenance of genomic stability, the molecular mechanisms underlying its role in apoptosis and DNA damage response remain elusive. Our study for the first time revealed that hADA3 exhibits punctate nuclear pattern which interestingly colocalizes with Promyelocytic Leukemia Nuclear Bodies (PML-NBs). We also provide novel evidence for the physical interaction between them, which is further enhanced following DNA damage. Moreover, we demonstrate that cells expressing a hADA3 mutant which is unable to interact with PML, displayed impaired apoptosis indicating a clear role of hADA3 in PML-mediated apoptosis. These findings, therefore, highlight a previously unappreciated function of hADA3 and establish its novel functional link with PML in provoking DNA damage response.

Keywords PML · ADA3 · Coactivator protein · DNA damage · Tumor suppressor

Introduction

A key feature of cellular systems is maintenance of genomic stability, which is brought about by several coordinated events including DNA replication, recombination and repair, all of which require proper access to the chromatin. Chromatin remodeling is an incessant event carried on by a complex interplay among various proteins, which involve several activators and co-activators to mediate their function. One such coactivator is the evolutionarily conserved hADA3 (Alteration/Deficiency in Activation 3) protein which, along with ADA2 and GCN5 (general control non-repressed 5), acts as an essential bridge between components of basal transcription machinery and Histone Acetyl Transferases (HATs) (Horiuchi et al. 1995; Mirza et al. 2012; Wang et al. 2008). Several HAT complexes, including SAGA (SPT-ADA-GCN5 acetylase), STAGA (SPT3-TAF$_{II}$31-GCN5L acetylase), TFTC (TATA-binding protein-free TAF-containing complex), ATAC (Ada2A containing) complex have been found to contain hADA3 as a conserved component (Gamper et al. 2009; Hardy et al. 2002; Martinez et al. 1998). Importantly, hADA3 acts as a transcriptional coactivator of the tumor suppressor protein p53 and participates in p300/CBP-associated factor (PCAF) and p300/CBP-mediated p53 acetylation and stabilization (Nag et al. 2007; Wang et al. 2001). Additionally, hADA3 is responsible for transactivation of estrogen receptor-α (ERα) and Retinoic X Receptor-α (RXRα), which are known to be targeted by high-risk human papillomavirus (HPV) 16E6 in E6-driven pathogenesis (Li et al. 2010; Zeng et al. 2002). Recent work from our lab highlighted the role of SUMOylation in regulation of hADA3. HPV16E6 was shown to accelerate SUMOylation-mediated ubiquitination and hence destabilization of hADA3 in cervical cancer cells (Chand et al. 2014).

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ORIGINAL RESEARCH

Peroxisome Proliferator Activated Receptor Gamma Sensitizes Non-small Cell Lung Carcinoma to Gamma Irradiation Induced Apoptosis

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The nuclear receptors known as peroxisome proliferator activated receptor gamma (PPARG) are lipid-activated transcription factors that have emerged as key regulators of inflammation. PPARG ligands have been shown to have an anti-proliferative effect on a variety of cancers. These ligands can induce apoptosis via TP53 (Tumor protein p53) or ERK1/2 (Extracellular signal-regulated kinases 1/2) (EPHB2) pathways. However, the exact mechanism is not known. PPAR, a type II nuclear hormone receptor deserves attention as a selective target for radiotherapy. Our study examines the potential of selective agonism of PPARG for radiation therapy in non-small cell lung carcinoma (NSCLC). We found that the overexpression of PPARG protein as well as its induction using the agonist, rosiglitazone was able to stimulate radiation-induced cell death in otherwise radio resistant NSCLC A549 cell line. This cell death was apoptotic and was found to be BAX (BCL2 associated X) mediated. The treatment also inhibited radiation-induced AKT (Protein Kinase B) phosphorylation. Interestingly, the ionising radiation (IR) induced apoptosis was found to be inversely related to TP53 levels. A relatively significant increase in the levels of radiation induced apoptosis was observed in H1299 cells (TP53 null) under PPARG overexpression condition further supporting the inverse relationship between apoptosis and TP53 levels. The combination of PPARG agonist and radiation was able to induce apoptosis at a radiation dose at which A549 and H1299 are radioresistant, thus confirming the potential of the combinatorial strategy. Taken together, PPARG agonism was found to invigorate the radiosensitising effect and hence its use in combination with radiotherapy is expected to enhance sensitivity in otherwise resistant cancer types.

Keywords: PPARG, radiosensitization, NSCLC, BAX, TP53, Hedgehog signaling

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Abbreviations: AKT, protein kinase B; BAX, BCL2 associated X, apoptosis regulator; BCL2, B-cell lymphoma 2, apoptosis regulator; **C4-2,** human papillomavirus-related cervical squamous cell carcinoma; CASP3, caspase 3; CDKN1A, cyclin-dependent kinase inhibitor 1A, p21; CDKN1B, cyclin-dependent kinase inhibitor 1B, p27; EPHB2, ephrin type B receptor 2; PARP, poly ADP ribose polymerase; PC3, prostate cancer cell line; PI3K, phosphoinositide 3-kinase; PI, propidium iodide; SW-48, SW human colon adenocarcinoma; TP53, tumor suppressor p53; TZD, thiazolidinediones.

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A conformational switch from a closed apo- to an open holo-form equips the acyl carrier protein for acyl chain accommodation

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ABSTRACT

Acyl carrier proteins (ACPs) play crucial roles in the biosynthesis of fatty acids, non-ribosomal polypeptides and polyketides. The three-dimensional NMR structure of *Leishmania major* holo-LmACP, belonging to the type II pathway, has been reported previously, but the structure of its apo-form and its conformational differences with the holo-form remain to be explored. Here we report the crystal structures of apo-LmACP (wild-type and S37A mutant) at 2.0 Å resolution and compare their key features with the structures of holo-LmACP (wild-type) and other type II ACPs from *Escherichia coli* and *Plasmodium falciparum*. The crystal structure of apo-LmACP, which is homologous to other type II ACPs, displays some key structural rearrangements as compared to its holo-structure. Contrary to holo-form, which exists predominantly as a monomer, the apo-form exists as a mixture of monomeric and dimeric population in solution. In contrast to the closed structure of apo-LmACP, holo-LmACP structure was observed in an open conformation as a result of reorganization of specific helices and loops. We propose that the structural changes exhibited by LmACP occur due to the attachment of the phosphopantetheine arm and may be a prerequisite for the initiation of fatty acid synthesis. The movement of helix 3 may also play a role in the dissociation of holo-LmACP from its cognate enzymes of the FAS II pathway.

1. Introduction

The long carbon chains of fatty acids in all organisms are assembled in a repeating four step sequence, catalyzed by the fatty acid synthase (FAS) enzymes [1]. Of the two types of FAS known, both type I and type II rely on a small carrier protein (70–100 amino acid long), called the acyl carrier protein (ACP), to carry out the process of fatty acid synthesis [2]. ACP is the central player of the pathway as it shuttles the fatty acid intermediate cargo from one enzyme to another in a typical FAS pathway [3]. These are small acidic proteins (Mr ~ 10 kDa) with the ability to covalently attach to acyl intermediates of fatty acid biosynthesis, channelling them into the synthetic pathway for their elongation. These fatty acids and their more complex forms play crucial roles in the virulence of protozoan parasites and their survival inside the mammalian host [4]. Hence, this pathway has recently gained attention in *Leishmania*, the causative agent of the deadly disease, leishmaniasis. The role of this pathway in the pathogenicity is not known [5–8]. Several genomic and proteomic approaches are required to decipher the role of fatty acid biosynthetic pathway in the infectivity of *Leishmania*. As a first step towards this, various biochemical and structural studies are being undertaken to uncover the importance of this pathway in *Leishmania* [9,10]. Such studies would also have the potential to lay the foundation for drug discovery initiative against FAS to combat leishmaniasis.

Leishmania major (Lm) genome encodes for the type II FAS pathway as revealed through genome sequencing of the parasite [11]. It codes for a single carrier protein, namely type II acyl carrier protein, LmACP. LmACP is a 150 amino acid protein comprising of an N-terminal mitochondrial signal sequence. A recent report highlighted the key structural features of the holo-form of the protein and emphasized on its unique interaction with its cognate phosphopantetheinyl transferase (PPT) [9]. NMR studies on holo-LmACP had shown that it shares a similar overall helical fold typical of ACP family, comprising three well defined loops consecutively connecting four helices arranged in a

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A conformational switch from a closed apo- to an open holo-form equips the acyl carrier protein for acyl chain accommodation

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ABSTRACT

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Ionophores as Potent Anti-malarials: A Miracle in the Making

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Affiliations

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Abstract

Plasmodium has a complex life cycle that spans between mosquito and human. For survival and pathogenesis it banks upon dynamic alterations in ionic transport across organelle and plasma membrane. Being a fundamental contributor of crucial biological processes in parasite, ionic balance facilitates parasite invasion, augmentation and transmission. Past few decades have witnessed tremendous advancement in understanding the relevance of ionic transit in parasites. Perhaps, not surprisingly, disruption of ionic homeostasis was thought to be detrimental for parasite. Compounds like ionophores are known to facilitate ionic transport across membrane down their electrochemical gradient. Despite continuous effort, malaria treatment is still a challenge particularly due to the development of resistance among parasites against existing therapeutic options. However, repurposing the existing drugs can be advantageous over de novo drug development programs in terms of cost and associated risk factors. Ionophores, being used in coccidiosis have proven to be of significance in the treatment of experimental models of malaria. Several recent reports have highlighted the attractive potential of ionophores such as Monensin, Maduramicin, Valinomycin, etc., that can act against multiple stages of malarial parasite's life cycle. Improved variety of these molecules may help in mitigating the drug resistance problems as well. This review is an attempt to examine the relevant literature and provide insight into the mechanism and prospects of different classes of ionophores as promising anti-malarial potpourri.

Keywords: Ionophore; Ions; Malaria; Parasite; Plasmodium; Therapeutics..

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Review

FoxM1: Repurposing an oncogene as a biomarker

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ABSTRACT

The past few decades have witnessed a tremendous progress in understanding the biology of cancer, which has led to more comprehensive approaches for global gene expression profiling and genome-wide analysis. This has helped to determine more sophisticated prognostic and predictive signature markers for the prompt diagnosis and precise screening of cancer patients. In the search for novel biomarkers, there has been increased interest in FoxM1, an extensively studied transcription factor that encompasses most of the hallmarks of malignancy. Considering the attractive potential of this multifarious oncogene, FoxM1 has emerged as an important molecule implicated in initiation, development and progression of cancer. Bolstered with the skill to maneuver the proliferation signals, FoxM1 bestows resistance to contemporary anti-cancer therapy as well. This review sheds light on the large body of literature that has accumulated in recent years that implies that FoxM1 neoplastic functions can be used as a novel predictive, prognostic and therapeutic marker for different cancers. This assessment also highlights the key features of FoxM1 that can be effectively harnessed to establish FoxM1 as a strong biomarker in diagnosis and treatment of cancer.

1. Introduction

The definition of a biomarker, as provided by the National Cancer Institute, designates it as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease", including cancer. A variety of biomolecules like proteins, metabolites, RNA transcripts, DNA, or epigenetic modifications of DNA hold tremendous potential as a biomarker [1]. The routine methods of detection of a biomarker are through biopsy or surgical resection of patient tissue samples, or some non-invasive approach, such as from body fluids. Biomarkers hold a significantly important role in the detection and management of patients suffering from cancer. The past decade has witnessed a paramount increase in research concerning discovery of signature biomarkers for the identification of individuals at increased risk of developing malignancy, better prognosis during initial diagnosis, recommending customized systemic therapy, predicting the outcome of therapy in the long run and monitoring the progression of disease. Few exemplary cancer biomarkers constitute of the human epidermal growth factor receptor 2 (HER2) oncogene for breast cancer [2], mutation in v-raf murine sarcoma viral oncogene homolog B1 (BRAF) for melanoma [3], the presence of a fusion between the echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene (EML4-ALK) in lung cancer [4], and serum PSA for monitoring disease progression of prostate cancer [5]. Cancer biomarker can be functionally classified into three major subtypes: predictive, pharmacodynamics and prognostic [6]. Predictive biomarker provides information on the plausibility of a response to a given chemotherapeutics or treatment regime. Pharmacodynamics biomarker reflects the effect of a therapy on the cancer proliferation and extent of apoptosis in tumor cell. Prognostic marker facilitates cancer diagnosis, enables assessment of tumor stage and monitoring of cancer progression and anticipates likely outcome of the illness. Due to concomitant advancement in the field of molecular biology, biomarker research has received a great stimulus. An ideal biomarker for clinical practice must possess high sensitivity, specificity, necessarily be accompanied with safe and easy means of measurement, preferably non-invasively, and improve management possibilities in conjunction with clinicopathological parameters. However, in reality, multiple biomarkers are needed for complete screening, diagnosis, prognosis, and prediction of the cancer type.

Mutations in the genes that encode for oncogene or tumor suppressor proteins have been well established as DNA-biomarkers. For example, oncogenes including K-RAS, EGFR, FGFR2 and HER2/neu and tumor suppressors such as p53, PTEN, p21 and p16 have been reported as biomarkers of endometrial, lung, breast cancer, etc [7,8]. A few well-established biomarkers of different cancer used in clinical practice in modern times have been summarized in Table 1. Alterations in both oncogene and tumor suppressor genes can define the molecular correlation between prognosis and clinical behavior of a particular cancer phenotype as shown by the accumulation of p53 mutant protein in

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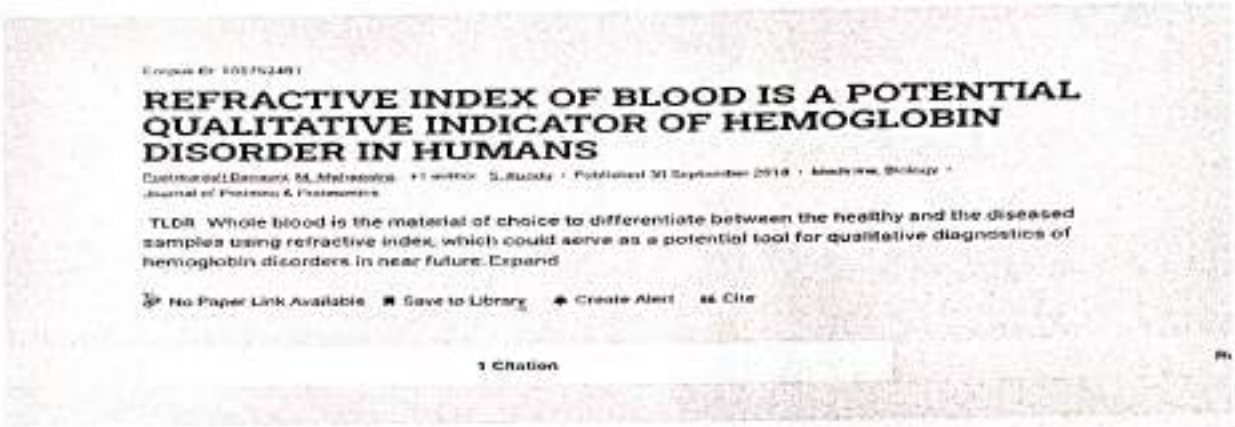

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REFRACTIVE INDEX OF BLOOD IS A POTENTIAL QUALITATIVE INDICATOR OF HEMOGLOBIN DISORDER IN HUMANS

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TLDR Whole blood is the material of choice to differentiate between the healthy and the diseased samples using refractive index, which could serve as a potential tool for qualitative diagnostics of hemoglobin disorders in near future. Expand

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ORIGINAL RESEARCH
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Necrosis Driven Triglyceride Synthesis Primes Macrophages for Inflammation During *Mycobacterium tuberculosis* Infection

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Pulmonary tuberculosis (TB) exhibits granulomatous inflammation, a site of controlling bacterial dissemination at the cost of host tissue damage. Intrigued by the granuloma type-dependent expression of inflammatory markers in TB, we sought to investigate underlying metabolic changes that drive amplification of inflammation in TB. Here, we show an association of higher inflammation in necrotic granulomas with the presence of triglyceride (TG)-rich foamy macrophages. The conspicuous absence of these macrophages in solid granulomas identified a link between the ensuing pathology and the metabolic programming of foamy macrophages. Consistent with *in vivo* findings, *in vitro* infection of macrophages with *Mycobacterium tuberculosis* (Mtb) led to increase in TG synthesis only under conditions of ~60% necrosis. Genetic and pharmacologic intervention that reduced necrosis prevented this bystander response. We further demonstrate that necrosis independent of Mtb also elicits the same bystander response in human macrophages. We identified a role for the human enzyme involved in TG synthesis, diacylglycerol *O*-acyltransferase (DGAT1), in this phenomenon. The increased TG levels in necrosis-associated foamy macrophages promoted the pro-inflammatory state of macrophages to infection while silencing expression of diacylglycerol O-acyltransferase (DGAT1) suppressed expression of pro-inflammatory genes. Our data thus invoke a role for storage lipids in the heightened host inflammatory response during infection-associated necrosis. Our data provide a functional role to macrophage lipid droplets in host defense and open new avenues for developing host-directed therapies against TB.

Keywords: necrosis, tuberculosis, macrophage, triglyceride, inflammation

INTRODUCTION

Tuberculosis (TB) is the major cause of worldwide mortality due to any single infectious agent (1). Inflammation in TB plays a dual role—onset of the innate inflammatory response is crucial for the recruitment and activation of macrophages while dysregulation of this response leads to disease exacerbation (2). The inflammatory response in TB also contributes to tissue necrosis and

Abbreviations: Mtb, *Mycobacterium tuberculosis*; TB, tuberculosis; MOI, multiplicity of infection; NCS, necrotic cell supplement; NAFMs, necrosis-associated foamy macrophages.

Nanoscale

PAPER

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Long circulatory liposomal maduramicin inhibits the growth of *Plasmodium falciparum* blood stages in culture and cures murine models of experimental malaria†‡

Mohsin Raza, Hina Bharti, Aakriti Singal, Alo Nag * and Prahlad C. Ghosh *

Malaria continues to be one of the deadliest infectious diseases and a global health menace. The emergence and spread of drug-resistant strains of malaria parasites have further made the process of disease management grimmer. Thus, there is an urgent need to identify promising antimalarial strategies that can target the blood stages as well as block parasite transmission. Maduramicin is one such ionophore selected out of a recent screen of gametocytocidal compounds that exhibit potent antiplasmodial activity. However, maduramicin's strong hydrophobic nature and associated toxicity restrict its application in chemotherapy. To alleviate this problem, we have developed a liposomal formulation loaded with the ionophore maduramicin for the treatment of chloroquine sensitive and resistant *Plasmodium* infections. Here, we show that maduramicin in PEGylated liposomal formulations displayed enhanced antiplasmodial activity *in vitro* compared to free maduramicin. Significantly, four consecutive doses of 1.5 mg kg^{-1} body weight of PEGylated maduramicin loaded lipid vesicles completely cured cerebral and chloroquine resistant murine models of malaria without any obvious toxic effects and suppressed the key inflammatory markers associated with the progression of the disease. PEGylated liposomal maduramicin also exhibited a prolonged plasma clearance rate, implying a greater chance of interaction and uptake by infected RBCs. Furthermore, we also provide evidence that the detrimental effect of liposomal maduramicin on parasite survival is mediated by increased ROS generation and subsequent perturbation of parasite mitochondrial membrane potential. This study presents the first report to demonstrate the potent antimalarial efficacy of maduramicin liposomes, a strategy that holds promise for the development of successful therapeutic intervention against malaria in humans.

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Introduction

Malaria remains a worldwide health concern, being a leading cause of global morbidity and mortality. The number of cases of this infectious disease is on the rise, primarily due to high prevalence of resistant strains of the causative parasite, thereby imposing a significant health and socio-economic burden.[1,2] Strategies for efficient management of this disease include vector control using insecticide treated bed nets, early diagnosis, and adequate treatment with artemisinin-based combination therapy (ACT).[3] Mefloquine, quinine and lumefantrine are other commonly used antimalarials for human chemotherapy.[4] However, this parasite has developed resistance against most of the commonly used antimalarials and the emergence of artemisinin resistance in malaria-endemic regions has posed a significant threat to disease control.[5–9] In 2015, a malaria vaccine (RTS,S/ASO1) came into limelight after receiving a positive response from the European Medicines Agency; however, it was moderately effective and was not recommended for future use.[10] In the therapy front, several drug candidates majorly effective at the blood schizont stage are in the pipeline for clinical trials.[11] Additionally, continuous efforts are being made to identify effective antimalarials employing a structure-based drug development approach using essential proteins of the parasite as the drug target.[12,13] For instance, target-based screening of compounds against *P. falciparum* cyclic GMP-dependent protein kinase led to the identification of an inhibitor that could effectively clear the

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† The patent was filed with the Indian Patent Office on February 15, 2018. Title: "Novel antimalarial liposomal formulation". Inventors: Prahlad Chandra Ghosh, Alo Nag, Mohsin Raza, Aakriti Singal and Hina Bharti. Application Number: 201711016131.

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Biochemistry

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Article

The 'recognition helix' of the type II Acyl Carrier Protein (ACP) utilizes a 'ubiquitin interacting motif (UIM)' like surface to bind its partners

Usha Yadav, Richa Arya, Suman Kundu, and Monica Sundd

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PPARγ-targeting Potential for Radioprotection

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Abstract

Background: Peroxisomal proliferator receptor gamma (PPARγ) is a class of nuclear hormone receptor family involved in insulin sensitization. In addition, PPARγ has a key role in the protection against oxidative stress and inflammation through regulation of NFkappaB levels and crosstalk with the Nrf2 pathway. Also, its role in the modulation of immune response is substantial.

Objective: Radiation-induced oxidative stress is the sole determinant of damage to hematopoietic, gastrointestinal system and immune system suppression. Uncontrolled exposure to normal cells during radiotherapy raises the demand for novel and efficient radioprotectors. In this review, we will present an overview of the involvement of PPARγ in radiation-induced damage and inflammation with major emphasis on whether PPARγ can serve as a suitable radiomodification target.

Conclusion: Through this review, we have justified that PPARγ having both radioprotective as wells as radiotherapeutic potential, may serve as an attractive target for the development of novel and more effective therapies.

Keywords: NFkappaB; PPARγ; cancer; inflammationnitrosylation; oxidative stress; radiation..

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RESEARCH ARTICLE

Biotin-tagged proteins: Reagents for efficient ELISA-based serodiagnosis and phage display-based affinity selection

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Abstract

The high-affinity interaction between biotin and streptavidin has opened avenues for using recombinant proteins with site-specific biotinylation to achieve efficient and directional immobilization. The site-specific biotinylation of proteins carrying a 15 amino acid long Biotin Acceptor Peptide tag (BAP; also known as AviTag) is effected on a specific lysine either by co-expressing the *E. coli* BirA enzyme *in vivo* or by using purified recombinant *E. coli* BirA enzyme in the presence of ATP and biotin *in vitro*. In this paper, we have designed a T7 promoter-lac operator-based expression vector for rapid and efficient cloning, and high-level cytosolic expression of proteins carrying a C-terminal BAP tag in *E. coli* with TEV protease cleavable N-terminal deca-histidine tag, useful for initial purification. Furthermore, a robust three-step purification pipeline integrated with well-optimized protocols for TEV protease-based H10 tag removal, and recombinant BirA enzyme-based site-specific *in vitro* biotinylation is described to obtain highly pure biotinylated proteins. Most importantly, the paper demonstrates superior sensitivities in indirect ELISA with directional and efficient immobilization of biotin-tagged proteins on streptavidin-coated surfaces in comparison to passive immobilization. The use of biotin-tagged proteins through specific immobilization also allows more efficient selection of binders from a phage-displayed naïve antibody library. In addition, for both these applications, specific immobilization requires much less amount of protein as compared to passive immobilization and can be easily multiplexed. The simplified strategy described here for the production of highly pure biotin-tagged proteins will find use in numerous applications, including those, which may require immobilization of multiple proteins simultaneously on a solid surface.

Introduction

Over the last two decades, specific and efficient capture of biotin-tagged proteins on streptavidin surface by virtue of extremely high-affinity interaction has found use in a diverse range of

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ORIGINAL RESEARCH

Identification of *Mycobacterium tuberculosis* BioA inhibitors by using structure-based virtual screening

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Background: 7,8-Diaminopelargonic acid synthase (BioA), an enzyme of biotin biosynthesis pathway, is a well-known promising target for anti-tubercular drug development.

Methods: In this study, structure-based virtual screening was employed against the active site of BioA to identify new chemical entities for BioA inhibition and top ranking compounds were evaluated for their ability to inhibit BioA enzymatic activity.

Results: Seven compounds inhibited BioA enzymatic activity by greater than 60% at 100 µg/mL with most potent compounds being A36, A35 and A65, displaying IC_{50} values of 10.48 µg/mL (28.94 µM), 33.36 µg/mL (88.16 µM) and 39.17 µg/mL (114.42 µM), respectively. Compounds A65 and A35 inhibited *Mycobacterium tuberculosis* (*M. tuberculosis*) growth with MIC_{90} of 20 µg/mL and 80 µg/mL, respectively, whereas compound A36 exhibited relatively weak inhibition of *M. tuberculosis* growth (83% inhibition at 200 µg/mL). Compound A65 emerged as the most potent compound identified in our study that inhibited BioA enzymatic activity and growth of the pathogen and possessed drug-like properties.

Conclusion: Our study has identified a few hit molecules against *M. tuberculosis* BioA that can act as potential candidates for further development of potent anti-tubercular therapeutic agents.

Keywords: *Mycobacterium tuberculosis*, BioA, virtual screening, drug discovery

Video abstract

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Introduction

Mycobacterium tuberculosis (*M. tuberculosis*), the etiological agent of tuberculosis (TB), has been threatening mankind for millennia and is amongst the deadliest diseases in the world.[1] The remarkable capacity of this pathogen to evade the host immune responses makes it a successful and difficult pathogen. The current chemotherapy is a multidrug regimen consisting of rifampicin, isoniazid, pyrazinamide and ethambutol that requires 6–9 months to achieve high cure rates. Currently available standard treatment of TB has a poor compliance due to prolonged treatment duration resulting in the emergence of multidrug-resistant and extremely drug-resistant strains.[2,3] Hence, there is an urgent need to improve the treatment by identifying new chemical entities with potent activities against *M. tuberculosis*.

Biotin is an essential cofactor required for fatty acid metabolism, amino acid biosynthesis and gluconeogenesis.[4] *M. tuberculosis* harbors four necessary genes, namely, *bioF*, *bioA*, *bioD* and *bioB*, which encode enzymes required for the biosynthesis of biotin from pimeloyl-CoA.[5,6] The second step of biotin biosynthesis pathway is catalyzed by 7,8-diaminopelargonic acid synthase (BioA), a pyridoxal-5′-phosphate (PLP)-dependent aminotransferase, which is a crucial enzyme involved in the

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A combination of docking and cheminformatics approaches for the identification of inhibitors against 4′ phosphopantetheinyl transferase of *Mycobacterium tuberculosis*†

Akshay Rohilla,[a] Garima Khare[*a] and Anil K. Tyagi[*ab]

4′ Phosphopantetheinyl transferase (PptT) is involved in post translational modification by carrying out phosphopantetheinylation of proteins in non-ribosomal peptide synthesis and polyketide synthesis pathways of various organisms. PptT was recently shown to be crucial for the survival as well as persistence of *Mycobacterium tuberculosis* (*M. tb*) in mice models thus demonstrating it to be an attractive drug target. By employing Autodock 4.2 and Glide, we virtually screened the filtered NCI library against the active site of PptT and out of the 205 molecules tested *in vitro*, 13 molecules exhibited potent enzyme inhibition with $IC_{50} \leq 10$ μg ml^{-1}. Further evaluation of the molecules against the *in vitro* growth of *M. tb* resulted in the identification of six compounds that exhibited inhibition of both enzyme activity as well as *M. tb* growth. Subsequently, a cheminformatics based structure similarity approach led to the identification of 5 analogues of P-52 (IC_{50} – 2.25 μg ml^{-1} and MIC_{90} – 77.5 μg ml^{-1}) with $IC_{50} \leq$ 1 μg ml^{-1} thereby establishing *N,N*-diethyl-*N*′-(2-methylquinolin-8-yl)propane-1,3-diamine as one of the potent inhibitory scaffolds of PptT. The inhibitors were further evaluated for their MIC_{90} values as well as cytotoxicity against various mammalian cell lines. PS-40 (NSC-328398), an analogue of P-52, emerged as a potent inhibitory molecule which exhibited an IC_{50} of 0.25 μg ml^{-1}, MIC_{90} of 10 μg ml^{-1} and negligible cytotoxicity with a selectivity index >10 against three mammalian cell lines tested. Thus, our study identified potent inhibitory scaffolds against 4′ phosphopantetheinyl transferase of *M. tb*, an important drug target of *M. tb*.

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1. Introduction

Mycobacterium tuberculosis (*M. tb*), the causative agent of tuberculosis, killed 1.4 million people in the year 2015 worldwide with an additional 0.4 million people that were co infected with HIV.[1] These massive numbers highlight the high rate of morbidity and mortality caused by tuberculosis worldwide.[2] Although, an effective chemotherapeutic regimen for drug susceptible TB and a recently discovered drug bedaquiline for multi drug resistance (MDR-TB) are available, the number of patients suffering from drug susceptible as well as drug resistant forms of *M. tb* is ever increasing.[2,3] Thus, the need to fill the drug pipeline with as many new scaffolds with distinct mechanisms of action cannot be overemphasized in order to curtail this deadly disease.

4′ Phosphopantetheinylation is an important post translational modification prevalent in organisms ranging from bacteria to humans. It is responsible for the activation of proteins belonging to fatty acid synthases, polyketide synthases and non-ribosomal peptide synthases.[4–6] In the case of mycobacteria, 4′ phosphopantetheinylation is a post translation modification that immensely contributes to the survival and virulence of the pathogen as this modification converts inactive apo forms of proteins to active holo (phosphopantetheinylated) forms of proteins involved in important pathways of *M. tb*. These pathways are involved in the formation of mycolic acid (a key cell wall component of mycobacteria), fatty acids, mycobactins and carboxymycobactins (siderophores). Mycobacteria encodes two types of transferases, AcpS type transferase that activates the proteins belonging to fatty acid synthase (Fas) systems[4,5] and Sfp type transferase (PptT) that activates type-I polyketide synthases[6] and non-ribosomal peptide synthases.[7,8] By constructing knockouts in various mycobacterial and corynebacterial species, Chalut *et al.* demonstrated that these two proteins are non-redundant and they activate distinct subset of proteins.[9] Their study also exhibited that both AcpS and PptT individually are essential for the viability of *M. smegmatis*.[9]

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REVIEW ARTICLE

Ionophores as Potent Anti-malarials: A Miracle in the Making

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Abstract: Plasmodium has a complex life cycle that spans between mosquito and human. For survival and pathogenesis it banks upon dynamic alterations in ionic transport across organelle and plasma membrane. Being a fundamental contributor of crucial biological processes in parasite, ionic balance facilitates parasite invasion, augmentation and transmission. Past few decades have witnessed tremendous advancement in understanding the relevance of ionic transit in parasites. Perhaps, not surprisingly, disruption of ionic homeostasis was thought to be detrimental for parasite. Compounds like ionophores are known to facilitate ionic transport across membrane down their electrochemical gradient. Despite continuous effort, malaria treatment is still a challenge particularly due to the development of resistance among parasites against existing therapeutic options. However, repurposing the existing drugs can be advantageous over de novo drug development programs in terms of cost and associated risk factors. Ionophores, being used in coccidiosis have proven to be of significance in the treatment of experimental models of malaria. Several recent reports have highlighted the attractive potential of ionophores such as Monensin, Maduramicin, Valinomycin, *etc.*, that can act against multiple stages of malarial parasite's life cycle. Improved variety of these molecules may help in mitigating the drug resistance problems as well. This review is an attempt to examine the relevant literature and provide insight into the mechanism and prospects of different classes of ionophores as promising anti-malarial potpourri.

Keywords: Ionophore, Malaria, Parasite, Ions, Plasmodium, Therapeutics.

1. INTRODUCTION

Malaria is a life-threatening infectious disease caused by the parasites of the genus Plasmodium. Presently, there are five strains of Plasmodium that are known to cause malaria in humans, namely *P. falciparum, P. malariae, P. vivax, P. ovale* and *P. knowlesi*. This disease has been afflicting numerous human lives since ages and has a negative effect on human health and longevity making it one of the most serious and life-threatening maladies. According to the available statistics, malaria is responsible for the death of at least three quarters of a million people worldwide every year and is the most common cause of serious imported infection in non-endemic areas. In 2016, 216 million cases of malaria were reported and 90% of cases were reported in Africa alone, making the continent most vulnerable to the disease burden followed by south-east Asia that recorded 7% cases in the same year [1]. Infants below 6 months were the most susceptible owing to their poorly formed immune system and loss of maternal antibodies. The protozoan parasites are transmitted mainly by the bite of an infected female mosquito belonging to the Anopheles genus.

Subsequent to the blood meal, the motile sporozoites glide through sinusoids to invade hepatocytes [2]. These infective Plasmodia reside in hepatocytes wherein they multiply to give rise to thousands of merozoites (as a result of asexual fission/schizogony) which ultimately disseminate into the bloodstream. Inside the erythrocytes, these merozoites undergo erythrocytic schizogony to replicate. Some merozoites differentiate into mature male or female gametocytes, causing parasite transmission by vector mosquito when it feeds on human blood [3]. The inception of clinical symptoms usually occur 7-10 days post initial mosquito bite. Depending on the manifestation of the symptoms, the disease can be classified as asymptomatic, uncomplicated or severe [4]. Asymptomatic malaria is caused by almost all species of Plasmodium and the infected person normally does not manifest symptoms. In uncomplicated cases, the infected person exhibits moderate and non-specific symptoms such as fever, chills, diarrhoea, sweating, and nausea *etc.* Severe cases are usually due to infection with *P. falciparum* (less frequently by *P. vivax* and *P. knowlesi*) and accompanied by many life-threatening complications including severe anemia, organ failure, cerebral malaria, coma, hypoglycemia, kidney injury and edema [5]. The outcome of this form of malaria is often fatal.

Inspite of the severe outcomes of the disease, currently, there exists no effective licensed vaccine against malaria [6] due to poor understanding about anti-malarial immunity, genetic diversity of malarial parasite and immune correlation of protection [7]. Past years have seen an upsurge in research towards development of anti-malarial vaccine. The most extensively tested vaccine candidate till date,

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RESEARCH ARTICLE

Contrasting Function of Structured N-Terminal and Unstructured C-Terminal Segments of *Mycobacterium tuberculosis* PPE37 Protein

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ABSTRACT Pathogens frequently employ eukaryotic linear motif (ELM)-rich intrinsically disordered proteins (IDPs) to perturb and hijack host cell networks for a productive infection. *Mycobacterium tuberculosis* has a relatively high percentage of IDPs in its proteome, the significance of which is not known. The *Mycobacterium*-specific PE-PPE protein family has several members with unusually high levels of structural disorder and disorder-promoting Ala/Gly residues. PPE37 protein, a member of this family, carries an N-terminal PPE domain capable of iron binding, two transmembrane domains, and a disordered C-terminal segment harboring ELMs and a eukaryotic nuclear localization signal (NLS). PPE37, expressed as a function of low iron stress, was cleaved by *M. tuberculosis* protease into N- and C-terminal segments. A recombinant N-terminal segment (P37N) caused proliferation and differentiation of monocytic THP-1 cells, into CD11c, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin)-positive semimature dendritic cells exhibiting high interleukin-10 (IL-10) but negligible IL-12 and also low tumor necrosis factor alpha (TNF-α) secretion—an environment suitable for maintaining tolerogenic immune cells. The C-terminal segment entered the macrophage nucleus and induced caspase-3-dependent apoptosis of host cells. Mice immunized with recombinant PPE37FL and PPE37N evoked strong anti-inflammatory response, validating the *in vitro* immunostimulatory effect. Analysis of the IgG response of PPE37FL and PPE37N revealed significant immunoreactivities in different categories of TB patients, viz. pulmonary TB (PTB) and extrapulmonary TB (EPTB), vis-a-vis healthy controls. These results support the role of IDPs in performing contrasting activities to modulate the host processes, possibly through molecular mimicry and cross talk in two spatially distinct host environments which may likely aid *M. tuberculosis* survival and pathogenesis.

IMPORTANCE To hijack the human host cell machinery to enable survival inside macrophages, the pathogen *Mycobacterium tuberculosis* requires a repertoire of proteins that can mimic host protein function and modulate host cell machinery. Here, we have shown how a single protein can play multiple functions and hijack the host cell for the benefit of the pathogen. Full-length membrane-anchored PPE37 protein is cleaved into N- and C-terminal domains under iron-depleted conditions. The N-terminal domain facilitates the propathogen semimature tolerogenic state of den-

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RESEARCH ARTICLE

An attenuated quadruple gene mutant of *Mycobacterium tuberculosis* imparts protection against tuberculosis in guinea pigs

Ritika Kar Bahal[1,‡], Shubhita Mathur[1,‡], Priyanka Chauhan[1,*] and Anil K. Tyagi[1,2,§]

ABSTRACT

Previously we had developed a triple gene mutant of *Mycobacterium tuberculosis* (*Mtb*Δ*mms*) harboring disruption in three genes, namely *mptpA*, *mptpB* and *sapM*. Though vaccination with *Mtb*Δ*mms* strain induced protection in the lungs of guinea pigs, the mutant strain failed to control the hematogenous spread of the challenge strain to the spleen. Additionally, inoculation with *Mtb*Δ*mms* resulted in some pathological damage to the spleens in the early phase of infection. In order to generate a strain that overcomes the pathology caused by *Mtb*Δ*mms* in spleen of guinea pigs and controls dissemination of the challenge strain, *Mtb*Δ*mms* was genetically modified by disrupting *bioA* gene to generate *Mtb*Δ*mmsb* strain. Further, *in vivo* attenuation of *Mtb*Δ*mmsb* was evaluated and its protective efficacy was assessed against virulent *M. tuberculosis* challenge in guinea pigs. *Mtb*Δ*mmsb* mutant strain was highly attenuated for growth and virulence in guinea pigs. Vaccination with *Mtb*Δ*mmsb* mutant generated significant protection in comparison to sham-immunized animals at 4 and 12 weeks post-infection in lungs and spleen of infected animals. However, the protection imparted by *Mtb*Δ*mmsb* was significantly less in comparison to BCG immunized animals. This study indicates the importance of attenuated multiple gene deletion mutants of *M. tuberculosis* for generating protection against tuberculosis.

KEY WORDS: Multi-gene mutant, BCG, Tuberculosis, Attenuation, Auxotrophic vaccines, Biotin

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of human tuberculosis (TB), continues to be a major cause of mortality. The BCG vaccine provides effective protection against severe forms of TB in children but shows variable efficacy against adult pulmonary tuberculosis (Zodpey and Shrikhande, 2007). The difference in antigenic repertoire of *M. tuberculosis* and BCG leads to the generation of different host immune responses which might be responsible for the limited impact of BCG on control of TB. Live attenuated vaccine strains mimic the natural course of infection and maintain a balance between attenuation and immunogenicity. Several attenuated mutants of *M. tuberculosis* have been tested as TB vaccine strains, only few are able to generate protection equivalent to BCG such as the *panCD*, *cysH*, *proC* and *trpD* mutants of *M. tuberculosis* (Sambandamurthy et al., 2002; Senaratne et al., 2007; Smith et al., 2001). Strains such as Δ*lysA*Δ*panCD* and Δ*leuD*Δ*panCD* demonstrated negligible multiplication in mouse organs, yet generated protection equivalent to BCG (Sambandamurthy et al., 2005; Sampson et al., 2004). On the contrary, their prototype Δ*lysA* and Δ*leuD* strains failed to reduce the bacillary load as much as BCG (Hondalus et al., 2000; Pavelka et al., 2003). Though the attenuated *M. tuberculosis* strains such as MTBVAC (Arbues et al., 2013; Solans et al., 2014; Spertini et al., 2015) and *Mtb*Δ*sigH* (Kaushal et al., 2015) have shown promising results, the success rate of TB vaccine in clinical trials is low (Tameris et al., 2013). Thus, novel strains with new combinations of gene deletions need to be evaluated for their potential as vaccine against TB.

We had previously constructed a triple gene mutant of *M. tuberculosis* (*Mtb*Δ*mms*), having deletions in genes encoding for phosphatases *mptpA*, *mptpB* and *sapM* that are involved in host-pathogen interaction (Bach et al., 2008; Chauhan et al., 2013; Vergne et al., 2004; Zhou et al., 2010). The mutant *Mtb*Δ*mms* demonstrated bacillary growth in the spleens of guinea pigs at 4 weeks post-intradermal administration along with concomitant pathological damage to spleen (Chauhan et al., 2013). Further, animals vaccinated with *Mtb*Δ*mms* generated a sustainable and superior protection as compared to BCG in lungs. However, *Mtb*Δ*mms* was unable to control hematogenous dissemination of challenge strain to spleen with no significant difference from sham-immunized animals (Chauhan et al., 2013).

In order to overcome the pathology caused by *Mtb*Δ*mms* during the early phase of infection, and to generate a strain that controls dissemination of challenge strain, *Mtb*Δ*mms* strain was modified to generate an auxotrophic mutant by disrupting *bioA* gene involved in biotin biosynthesis (Attwood and Wallace, 2002; Beckett, 2007; Knowles, 1989; Tang et al., 2014; Mann et al., 2009, 2013). Several studies have demonstrated essentiality of *bioA* for survival of mycobacteria (Keer et al., 2000; Sassetti et al., 2003; Woong Park et al., 2011). We have earlier reported that disruption of *bioA* renders *M. tuberculosis* severely attenuated for growth and virulence in guinea pig along with negligible granulomatous pathology (Kar et al., 2017). Immunization with *Mtb*Δ*bioA* imparted significant protection in lungs and spleen when compared to sham-immunized animals demonstrating an efficient control over the dissemination of infecting strain to the spleen (Kar et al., 2017). In this study, we generated a quadruple gene mutant (*Mtb*Δ*mmsb*) by disrupting *bioA* gene in *Mtb*Δ*mms* strain. Further, we evaluated *in vivo* attenuation and assessed the protective efficacy of *Mtb*Δ*mmsb* against virulent *M. tuberculosis* challenge in guinea pigs.

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A combinatorial approach for robust transgene delivery and targeted expression in mammary gland for generating biotherapeutics in milk, bypassing germline gene integration

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Affiliations
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Abstract

Protein expression in the milk of transgenic farmed animals offers a cost-effective system for producing therapeutics. However, transgenesis in farmed animals is not only cumbersome but also involves risk of potential hazard by germline gene integration, due to interruptions caused by the transgene in the native genome. Avoiding germline gene integration, we have delivered buffalo β-casein promoter-driven transgene construct entrapped in virosomes directly in the milk gland through intraductal perfusion delivery. Virosomes were generated from purified Sendai viral membrane, containing hemagglutinin-neuraminidase (HN) and fusion factor (F) proteins on surface (HNF-Virosomes) which initiate membrane fusion, devoid of any viral nucleic acids. Intraductal delivery of HNF-Virosomes predominantly transfected luminal epithelial cells lining the milk duct and buffalo β-casein promoter of the construct ensured mammary luminal epithelial cell specific expression of the transgene. Mammary epithelial cells expressed EGFP at lactation when egfp was used as a transgene. Similarly, human interferon-γ (hIFN-γ) was expressed in the mammary gland as well as in the milk when hIFN-γ was used as a transgene. This combinational approach of using Sendai viral membrane-derived virosomes for entrapment and delivery of the transgene and using buffalo β-casein promoter for mammary gland specific gene expression provided a better option for generating therapeutic proteins in milk, bypassing germline gene integration avoiding risks associated with animal bioreactor generated through germline gene integration.

Keywords: Animal bioreactor, In vivo gene delivery, Mammary gland specific expression, Therapeutic protein, β-Casein promoter

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