CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

01. BHALLA (Nikhil)
A Targeted Sequencing Approach for Detection and Comprehensive Profiling of Drug Resistance-Associated Mutations in M. Tuberculosis.
Supervisors: Prof. Amita Gupta
Th 27201

Abstract

Tuberculosis is a significant public health concern in India with the highest number of cases globally. The risk factors for TB include poverty living in substandard housing conditions smoking exposure to second hand smoke and solid fuel usage co-morbidities such as diabetes prior history of TB, HIV status alcohol and drug abuse and gender. Most of these factors can be addressed and that can help eliminate TB (Kumar et. Al. 2018 MOHFW 2020. Pathak et al. 2021 Singh et.al. 2018 Smith 2003 Thomas et.al. 2015,2021) However it is important to note that non-primate animals can also be infected with TB and can act as a reservoir for the disease posing the risk of zoonotic outbreaks even if it gets eliminated form humans. Additionally the emergence of drug resistant TB threatens the programs to eliminate the disease within the next 5-10 years. The ernergence of DR is often the result of a patient's refusal to comply with treatment. Poor antibiotic regiments can also lead to selective pressure enabling the DR strains to outgrow. The genes encoding drug targets and their regulatory regions may harbour DR associated mutations that can result in DR phenotypes. These mutations can cause a loss of binding affinity between the drug and its target loss of drug activation properties for converting prodrug to the active metabolite overexpression of the drug target to compensate for the target that has been bound to the drug molecule and enhancement of intrinsic drug resistance mechanisms. Screeing these genomic loci for certain mutations helps in predicting DR profiles.

Contents

1. Review of literature 2. Development of a targeted sequencing approach for the detection of drug resistant tuberculosis. 3. Optimization and validation of the targeted sequencing approach for detection of drug resistant M. Tuberculosis. 4. Whole genome sequencing and correlation with targeted sequencing for Drug resistant M. Tuberculosis isolates. 5. Summary and Conclusions.

02. KAUR (Simran)
Devising Intervention Strategies to Combat Tuberculosis.
Supervisors: Prof. Garima Khare
Th 26998

Abstract

With a larger aim to devise intervention strategies against TB, we carried out studies to understand the mechanisms of M.tuberculosis (M.tb) persistence and drug evasion in the unconventional niche of bone marrow mesenchymal stem cells (BM-MSCs). In this study, we have demonstrated that M.tb when present inside macrophages, which we have shown to be a result of upregulation and increased activity of host ABCG2 efflux pumps. We have also identified various pathways/ processes that are modulated by M.tb infection in BM-MSCs which favors bacterial persistence and survival inside this niche.We also attempted to identify novel inhibitors against M.tb by using a natural product based approach. Towards this, we have characterized the anti-mycobacterial properties of a component of a fungal specie aspergillus terreus and have identified a cell wall hydrolysing protein secreted by the fungus that inhibits M.tb growth.In chapter I. we made an attempt to understand the responsiveness of M.tb residing inside mouse bone marrow derived mesenchymal stem cells (BM-MSCs) to anti-TB drugs and have delineated one of the probable mechanisms responsidble for the reduced responsiveness of M.tb to anti-TB drugs when residing inside BM-MSCs.

Contents

1. Introduction 2. Review of literature 3. Aims and objectives 4. Results and discussion 5. Evaluation of responsiveness of mycobacterium tuberculosis (M.tb) residing inside bone marrow mesenchymal stem cells (BM-MSCs) to anti-TB drugs and determination of the role of ABCG2 efflux pumps in unresponsiveness to drugs. 6. Delineating host proteins/ pathways modulated by mycobacterium tuberculosis inside bone marrow mesenchymal stem cells by using label free-MS based protemomics approach to identify host-directed therapy targets for intervention of tuberculosis. 7. Characterization of an anti mycobacterical component of aspergillus terreus. Summary and conclusions. Appendix.

03. PANWAR (Neha)
Targeted Killing of Tumorous Hepatocytes by Anti-Cancer Drug Loaded in Polymeric Nanoparticles Through Sendai f-Yirosomes.
Supervisors: Prof. Alo Nag, Prof. Debi P. Sarkar and Dr. Amulya K.Panda
Th 26572

Abstract

The present study represents a formulation of polymeric nanoparticles based hybrid virosomes (IPLGS-PTX-Virosome) for delivering drugs at targeted sites. Paclitaxel is FDA approved, an anti-proliferative and a chemotherapeutic drug effective against wide-range of tumors but due to its poor solubility and systemic side-effect, its therapeutic application is limited. Therefore, various nonoformulations were prepared so as to enhance its aqueous solubility, retention time at the tumor site and to overcome all the limitations associated with paclitaxel, however, paclitaxel based nanoformulations have also resulted in the various limitations which majorly includes the target specificity of the particles and therefore to overcome issues related to nanoformulations, sendai virus based reconstitiuted virosomes were prepared. The aim of fabricating hybrid virosomes entrapping paclitaxel loacded PLGA nanoparticles was to combine the potential functions of both the nanoparticles was to combine the potential functions of both the nanoparticles for controlled, sustained and targeted drug release using sendai F-virosomes. The formulation was characterized using transmission electron microscope (TEM), scanning electron microscope (SEM) and its cytotoxicity was

assessed on three different cell lines HepG2, CHO and HeLa cells using MTT assay. The results depicted the spherical shaped virosomes with smooth surface and the size ranging between 170nm 280nm. It has shown 45% entrapment efficiency of PTX loaded PLGA nanoparticles. The viability of HepG2 cells was significantly inhibited by F-Virosome entrapped PTX loaded PLGA nano formulations in a dose dependent manner at a specific time. The results have demonstrated the targeted and side specific drug delivery with high efficacy and significant toxicit mediated by the F-Virosome entrapping PTX loaded PLGA nanoparticles in HepG2 cells. Thus, it provides the futuristic potential as a promising formulation for drug delivery in treating hepatocellular carcinoma (HCC).

Contents

1. Introduction and objectives 2. Review of literature 3. Formulation and characterization of polymeric nanoparticles entrapping chemotherapeutic drug 4. Fabrication, characterization of virosomes entrapping drug-loaded polymeric nanoparticles and evaluating an in vitro fusion potential of virosomes mediated drug delivery 5. Summary and Conclusions.

04. SINGAL (Aakriti)

Characterization of Plasmodium Falciparum SUMO Conjugating Enzyme, *Pf*UBC9 and Elucidation of the Mode of Action of Maduramicin with Implication in Malaria Therapy.

Supervisor: Prof. Alo Nag

Th 26573

Abstract

The malaria parasite P. falciparum poses a significant global health challenge, necessitating robust efforts to mitigate its impact and transmission. Accumulating evidence underscores the vital role of post-translational machinery in the parasite's survival. The focus of our investigation was to gain a deep understanding of the function and characteristics of malarial parasite specific SUMO conjugating enzyme, PfUbc9, found in Plasmodium falciparum. Our goal was to unravel crucial insights into the inherent traits of PfUbc9, encompassing its resilience and robustness with the intricate cellular milieu. Through this research, we anticipate shedding light on the remarkable abilities of PfUbc9 to adapt and persist within the malaria parasite, gain a deeper understanding of the functioning of this enigmatic enzyme and interaction with its specific substrates. These interactions play a vital role in the malaria parasite's life cycle and overall pathogenesis. Our findings established cytoplasmic expression of PfUbc9 across asexual stages of the parasite and revealed its oligomeric forms. We showed that PfUbc9 can perform SUMOylation utilizing human E1 and HsSUMO2 and identified critical amino acid residues for its activity. Together, our data furnishes significant insights into PfUbc9's structure-function relationship. Additionally, we investigated the mechanisms underlying the anti-malarial action of Maduramicin in P. falciparum. We have explored the alterations in the proteome of P. falciparum triggered by Maduramicin drug treatment, employing both 2D gel electrophoresis and iTRAQ-coupled mass spectrometry techniques. The identified dysregulated proteins were validated through quantitative real-time polymerase chain reaction (qRT-PCR). Our research provides the basis for understanding the lethal activity of Maduramicin on P. falciparum. Collectively, by delving into these intricate aspects of *Pf*Ubc9 and Maduramicin, our study contributes valuable insights into the intricate mechanisms underlying malaria pathogenesis and potential therapeutic interventions.

Contents

1. Background to the problem 2. Review of literature 3. Expression and characterization of the E2 conjugation enzyme of plasmodium falciparum, *Pf*Ubc 9 4. Mutational analysis of *Pf*Ubc9 to determine its crucial interfacial residues for substrate interaction 5. Elucidation of the mechanism of action of maduramicin using proteome analysis 6. Summary and Conclusion. Future Perspectives, Bibliography, Appendix, Publications and Patent.

05. YADAV (Sanjeev Kumar)
Investigation of Regulation of Ligamd Binding and stability Determinants in Novel hemoglobins with Applications in Blood Substitutes.
Supervisors: Prof. Alo Nag and Prof. Suman Kundu
Th 26574

Abstract

Hemoglobins are extremely well known and extensively investigated protein molecules that primarily store and transport oxygen in mammals and other organisms. Of late, they have been found to be ubiquitous across all life forms and their diverse structure, sequence and chemistry support diverse and previously unheard of functions for hemoglobins. Our laboratory uses various spectroscopic methods, bioinformatics tools, fast kinetics and X-ray crystallography to discover, characterize and understand structurefunction relationship and stability of novel hemoglobins (Hbs), to acquire new knowledge that could be engineered in recombinant Hbs for the production of stable hemoglobin based oxygen carriers (HBOCs). Here, we report the identification and characterization of two novel Hbs from extremophilic organisms, one from thermo-acidophilic algae, Galdieria sulphuraria (GsuHbt) and another from thermophilic cyanobacteria, Thermosynechococcus elongatus-BP1 (SynelHb). We hypothesized that proteins from extremophilic organisms could provide us with stable globin and information about specific factors that dictate its stability, which helps the organisms to survive in stressed condition. The X-ray crystal structure (2.15Å) of SynelHb suggested the presence of a globin domain with a pre-A helix similar to the sensor domain (S) family of Hbs, implying new function for a cyanobacterial Hb. Synel Hb displayed higher resistance to structural perturbations induced via external stresses like pH and GuHCl compared to myoglobin or human Hb. However, SynelHb exhibited lower thermal stability compared to mesophilic Hbs for unknown reasons. GsuHbt was thermally less stable in vitro relative to its mesophilic counterparts as well. The globin was, however, stable against pH and GuHCl like myoglobin. Additionally, attempt was made to evaluate the amyloid forming ability and its mechanism for another novel globin, cytoglobin. Apo-cytoglobin formed amyloids that were more toxic as compared to holo-cytoglobin and its physiological relevance remains to be explored.

Contents

1. Introduction statement of problem, review of literature, aims and objectives 2. Discovery, characterization of a novel single sensor haemoglobin domain from the thermodphilic cyanobacteria thermosynechococcus elongates BP-1 3. Investigation of a novel haemoglobin from a thermo acidophilic algae (Galdieria sulphuraria) previously identified in our laboratory, for insight into its stabiligy and ligand binding 4. Characterization of amyloid fibrils formed by cytoglobin.