CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

01. DASAUNI (Pushpanjali)
Identification and Characterization of Hemoglobin Disorders using Spectroscopic, Mass Spectrometric and 2D-DIGE Methods.
Supervisor : Prof. Suman Kundu
Th 24077

Abstract
(Verified)

Hemoglobin (Hb) disorders are genetic diseases. Approximately 7% of the world population is affected with Hb disorders. Any change in the hemoglobin protein sequence that results in loss of Hb function (oxygen transport) give rise to Hb disorders. Common symptoms are anemia, splenomegaly, pain syndromes, poor growth, heart, bones problem etc. To counter these symptoms no feasible cure is available; only symptomatic treatment could be provided. The tools commonly used for diagnosis are isoelectric focusing (IEF) and high performance liquid chromatography (HPLC). Recently, mass spectrometry and DNA analysis are also being used, but their clinical usage is very limited due to some method/technical challenges. Nevertheless, no novel tool was developed in the last several years. Thus, there is an urgent need of development of novel tools for regular screening of Hb disorders while improvising the existing tools as well. With such objectives, in the present study we employed various spectroscopic and biophysical tools to identify and characterize Hb disorders. Blood samples were collected after obtaining written consents from volunteers based on appropriate ethical clearances. Sample for Hb disorders identified by HPLC/ IEF/ DNA analysis/ RBCs staining (from hospital records) were obtained. Initial analysis was performed by recording the refractive index of blood samples. Subsequently, other qualitative tools like FTIR and 2D-DIGE were used to further differentiate between the healthy controls and the Hb disorder blood samples. Final confirmation was achieved by MS/MS analysis of Hb peptides where the identification of signature peptide lead to detection of diseased (variant). High sequence coverage of Hb chains was achieved by screening methods for sample preparation. A dedicated database was built with protein sequences for Hb variants which fastened the search. This study has the potential to establish “novel” tools/technique for the regular diagnosis/screening of Hb disorders, which were not explored systematically earlier.

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02. GUPTA (Nidhi)

Identification and Characterization of Promoters for Toxin-Antitoxin Loci of Mycobacterium Tuberculosis.

Supervisor : Dr. Amita Gupta

Th 24083

Abstract
(Verified)

Tuberculosis, caused by pathogen *Mycobacterium tuberculosis,* continues to be the most deadly disease in the world. Poor understanding of the causative agent, poor efficacy of existing drugs against latent TB infection and accelerated development of drug-resistant strains due to long treatment regimen and poor compliance have aggravated the problem. One set of genes implicated in the development of drug tolerance and persistence in bacteria is the Toxin Antitoxin loci (Singh, R. et. al., 2010; Tiwari, P. et. al., 2015). Stochastic induction of Toxin Antitoxin loci has been shown to induce development of persisters (Dahl. J. L., et. al., 2003).*M. tuberculosis* genome harbors 79 Toxin Antitoxin loci (Sala, A. et. al., 2014). Previous studies in the lab have led to elucidation of the killing effect of these toxins and their reversal by cognate antitoxins. Further transcription profiling has revealed differential expression of these loci in the bacterium under stress (Gupta, A. et. al., 2017). To understand the regulatory mechanisms that lead to differential activation of these Toxin Antitoxin loci in *M. tuberculosis*, an attempt has been made to identify and characterize the promoters for these Toxin Antitoxin loci. The work carried out in this direction in this thesis can be summarized as following : 1. An EGFP reporter vector system was developed and it was used to identify promoters of five Toxin Antitoxin loci encoded by *Mycobacterium tuberculosis.* 2. Promoter for *higBA1* Toxin-Antitoxin locus of *Mycobacterium tuberculosis* was characterized. 3. A Promoter reporter vector system based on selection was developed and optimized for identification of genome wide promoters of *Mycobacterium tuberculosis.* Whole genome libraries of *Mycobacterium tuberculosis* were constructed, promoters were selected and using next generation sequencing technology promoters were mapped for genome-wide Toxin-Antitoxin loci.

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03. MATHUR (Shubhita)

Development and Evaluation of Vaccines against Tuberculosis.

Supervisors : Prof. V. K. Chaudhary and Prof. Anil K. Tyagi

Th 24086

Abstract
(Not Verified)

Tuberculosis is a major cause of death worldwide. Failure of BCG vaccine to protect against adult pulmonary TB necessitates the development of a superior vaccine. Previously, we had developed a triple gene mutant of *M. tuberculosis* (*MtbΔmms*) harbouring disruption in three genes namely *mptpA, mptpB*and *sapM*. In order to overcome the pathology caused by *MtbΔmms*in the spleens of guinea pigs and also to control the dissemination of the challenge strain, *MtbΔmms*was genetically modified by disrupting *bioA*gene to generate *MtbΔmmsb*strain. We found that *MtbΔmmsb*was highly attenuated for growth and virulence in guinea pigs. Vaccination with *MtbΔmmsb*generated significant protection in comparison to sham-immunized animals at 4 and 12 weeks post-infection in lungs and spleens of guinea pigs. However, the protection imparted by *MtbΔmmsb*fell short in comparison to the protection observed in BCG immunized animals. Furthermore, we employed Mtb8.4 DNA and Rv3407 DNA vaccines in combination with chemotherapeutic drugs to study their influence on shortening the treatment duration. We show that mice infected with *M. tuberculosis,* when treated with DNA vaccines encoding either Mtb8.4 or Rv3407 antigen in conjunction with chemotherapeutic drugs, exhibit no relapse of the disease when compared to mice treated with four months of chemotherapy alone. Our data suggests that adjunctive immunotherapy with either Mtb8.4 DNA or Rv3407 DNA vaccine holds promise in shortening the duration of chemotherapeutic regimen from six months to four months. Further, these DNA vaccines were evaluated as booster vaccines to assess whether DNA vaccines encoding antigen Mtb8.4 or Rv3407 could improve the efficacy of BCG vaccine in guinea pigs. Our findings, however, show that as booster they do notconfer any significant enhancement in protective efficacy of BCG in lungs and only one vaccine (Mtb 8.4 DNA) shows significant enhancement of BCG efficacy in spleens.

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04. SHARMA (Shivani)
Elucidating Role of miR-191 in Pathogenesis and Liposomal Delivery of Anti miR-191 for Breast Cancer Treatment.
Supervisor : Prof. Prahlad C. Ghosh
Th 24082

Abstract
(Not Verified)

Breast cancer is the most common malignancy in woman. Despite tremendous advances, breast cancer treatment still remains a challenge due to development of resistance to various therapies. Therefore, there is a need to understand breast tumor biology in entirety and to identify novel mediators involved in the pathogenesis. miRNAs play a key role in various cellular processes such as development, differentiation, migration, and apoptosis. They are involved in the pathogenesis of various diseases including cancer. A vast amount of literature show specific miRNAs to be abnormally expressed in various cancers by acting as oncomiRsor tumor suppressor miRNAs. Thus, miRNAs show huge potential in cancer

diagnosis, prognosis and therapy. As miRNAs have enormous therapeutic potential in cancer therapy. However, the delivery mechanism of miRNAs or anti-miRNAs have encountered several challenges such as poor penetration, stability in blood, serum and other body fluids, off target effects and immune toxicity. Therefore, efficient delivery systems have been required form iRNA therapeutics in cancer. Lipid based carriers, liposomes are one of the most widely used delivery vehicle in vitro and in vivo. For efficient delivery of miRNA cationic, anionic and neutral lipids can be used to form lipoplexes/liposomes. Cationic lipids (structure of positively charged head group with hydrophobic tail region) have gained great attention in gene delivery mainly due to charge based interactions with negatively charged nucleic acid by facilitating high loading efficiency and efficient transfection reagents. Overall, the work identifies miR-191 as a p53 downregulated miRNA that inhibits apoptosis and act as anti-apoptotic miRNA in breast cancer. Thus, miR-191 was found to be key participant of p53 signaling pathway as part of a negative feedback loop. Moreover, we have developed stearylamine based cationic liposome formulation for the efficient delivery of miR-191 antagonist (anti-miR-191) to breast cancer cells.

Contents

05. SINGH (Swati)

Characterization of Novel Drug and Identification of Inhibitory Molecules against Mycobacterium Tuberculosis

Supervisors : Dr. Garima Khare and Prof. Anil K. Tyagi

Th 24080

Abstract
(Verified)

Drug resistance against currently available anti-mycobacterial drugs leads to the failure of control measures against tuberculosis. Hence, identification of novel chemical entities exhibiting potent antimycobacterial activity is urgently needed. In this study, structure-based virtual screening approach has been employed for the identification of new inhibitory molecules against *Mycobacterium tuberculosis* (*Mtb)*. The targets employed in the study included (i) BioA (Rv1568), a crucial enzyme of biotin biosynthesis pathway (ii) VirS (Rv3082c), a transcriptional regulator of *Mtb*which is believed to mediate acidic responses of the pathogen and (iii) DHPS (Rv3608c), an important enzyme of folate biosynthesis pathway. Virtual screening against BioA has identified three potent moieties (A35, A36 and A65), which inhibited BioA activity and *Mtb*growth with compound A65 as the most promising molecule in the study. We investigated the role of VirS in acid induced responses of *Mtb*and found that its absence reduced pathogen's ability to survive in acidic conditions, arrested phagosomal maturation in activated macrophages and led to disturbance in the intrabacterial pH maintenance under acidic stress thereby suggesting its involvement in evading stressful acidic conditions. Identification of downstream targets of VirS by microarray studies provided insights into the regulation of multiple genes by VirS in coordinating pH responses, which could help bacteria face the harsher conditions. Mutagenesis studies identified four crucial residues of VirS that can be explored for the rational design of inhibitors and structure-based virtual screening has identified two promising inhibitors of VirS*i.e.* compounds V7B and V15B (IC -1.8 µg/ml and 31.21 µg/ml; MIC -40 µg/ml and 5 µg/ml,respectively). Virtual screening against pterin binding site of DHPS has identified five potent inhibitory molecules against *Mtb*growth with compound G90

(MIC – 1.25 µg/ml) as the most promising molecule. The identified molecules can act as potential candidates for further development of potent anti-tubercular agents.

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