CHAPTER 32

MEDICAL SCIENCES
BIOCHEMISTRY

Doctoral Theses

391. DESHMUKH (Pravin S.)
Evaluation of Biological Response in Experimental Animals Following Microwave Exposure.
Supervisors : Prof. B. D. Banerjee, Prof. A. K. Tripathi and
Dr. Rafat S. Ahmed
Th 22330

Contents

1. Introduction 2. Aims and objectives 3. Review of literature 4. Materials and methods 5. Results 6. Discussion 7. Summary and conclusion. References. Appendices and publications.

392. JAISWAL (Neha)
Role of Forkhead Box M1 (FoxM1) Transcription Factor in Human Papillomavirus Mediated
Supervisor : Prof. Alo Nag
Th 22470

Contents

1. Introduction, review of literature and aims and objective 2. Elucidation of FoxM1 regulatory mechanism by SUMOylation and its modulation by HPVI6E7 3. To investigate the effect of HPVI6E7on APC/Cdh1 mediated degradation of FoxM1 and its singificance in HPV oncogenesis 4. Structure based screening of small molecule inhibitors against FoxM1, validation of interaction by in silico tools and effect on FoxM1 through cell based approaches 7. Summary and conclusion. Bibliography and appendix.

393. KAR (Ritika)
Development and Evaluation of Vaccines for Protection Against Tuberculosis.
Supervisor : Prof. Anil K. Tyagi
Th 22474

Abstract

Tuberculosis is a perilous infectious disease that claims millions of lives globally every year. Comprehensive control of tuberculosis urgently requires efficient vaccines given the unsatisfactory performance of the vaccine BCG, especially in India. This study was focused on the generation and evaluation of novel vaccines for the protection against tuberculosis. Our laboratory had reported enhanced protective

efficacy of a recombinant BCG strain overexpressing antigen 85C. The rBCG85C vaccine had been, in principle, recommended by TVCTEG, Department of Biotechnology, Ministry of Science and Technology, Govt of India, for eventual human clinical trials. However, the presence of antibiotic resistance marker in products intended for human application is discouraged. Thus, in this study, rBCG85C strain was modified to facilitate overexpression of antigen 85C in the absence of antibiotic resistance based selection. The strategy of auxotrophic complementation was employed for stable maintenance of plasmids devoid of antibiotic resistance gene. An auxotrophic mutant of BCG (BCGΔbioA) was generated by disrupting the bioA gene involved in biotin biosynthesis, essential for survival of mycobacteria. Subsequently, antibiotic marker free BCGΔbioA strain was generated via multiple steps involving plasmid curing and unmarking of antibiotic resistance gene, followed by complementation with an antibiotic marker free plasmid harboring the expression cassette for antigen 85C and BioA, resulting in the generation of the new modified rBCGA85C strain. Upon evaluation of protective efficacy, the modified strain exhibited significant protection against M. tuberculosis infection in guinea pigs. In this study we also generated an attenuated M. tuberculosis based vaccine strain by disrupting the bioA gene in M. tuberculosis. MtbΔbioA strain was found to be attenuated and avirulent in the susceptible guinea pig model of experimental tuberculosis. When employed as a vaccine strain, MtbΔbioA imparted significant protection against tuberculosis, which was comparable to the protection imparted by BCG, in guinea pigs.

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394. MEENAKSHI

Molecular Characterization of Natural and Engineered Photoactivated Cyclases from Microbes and Their Optogenetic Applications.

Supervisor : Dr. Suneel Kateriya

Th 22475

Abstract

Light provides a signals/stimulus for many biological processes. Living organisms possess the variety of photoreceptors to sense and respond to these light signals, which involved sensory transduction and for photoadaptive responses in many prokaryotes and in eukaryotes. The use of these genetically encoded natural photoreceptors as optogenetic tools has revolutionized the modern biology by allowing optically control of biological processes in a spatiotemporal manner with several advantages over traditional methods.
BLUF (Blue light sensors using FAD) domain containing proteins are flavin-based blue light photoreceptors. BLUF domains are also present in multidomain

architecture, where they fused with other effectors domain like GGDEF, EAL, CHD domain. BLUF domain linked to CHD (cyclase homology domain) domain is known as Photoactivated adenylyl cyclases (PAC). PACs have been reported in Euglena gracilis which mediates photobehavioral responses of the organism. These PACs were used as optogenetic tools for manipulating cAMP level simply by illumination in a controlled manner. Optical manipulation provides an opportunity to reversibly manipulate cAMP-mediated signaling in living cells, which are difficult to achievable in control manner using traditional pharmacological or genetic approaches. We have characterized PACs from amoeboflagellate protozoa Naegleria gruberi (named NgPACs) which consist of cyclase homology domain (CHD) and BLUF domain. In vitro, these PACs exhibits light regulated cyclase activity. We have used PACs as an optogenetic tool to modulate the biological functions in HEK293T cells and Dictyostelium. discoideum. These PACs could able to manipulate the intracellular cAMP level in HEK293T cells and D. discoideum. Increase in cAMP level in a light-dependent manner, activates the CREB transcription factor and regulate the expression of downstream targets genes (Cox-2 and cIAP2). In D. discoideum, overexpression of these PACs resulted in the alteration of the developmental life cycle of D. discoideum. These PACs have also been engineered to develop photoactivated guanylyl cyclases (PGC).

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395. MYTHILY S.

Biochemical and Immunochemical Studies to Assess the Effect of Murraya Koenigii (Curry Leaves) Extracts in Experimentally Induced Rheumatoid Arthritis.

Supervisors : Dr. Rafat S. Ahmed, Prof. Basu D. Banerjee and Prof. Vinod K. Arora

Th 22331

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396. SHARMA (Shingar)

Identification of Mycobacterium tuberculosis Proteins Presented in the Early Stages of Infection, and their Evaluation as Plasmid DNA Vaccine Candidates.

Supervisors : Prof. Anil K. Tyagi and Dr. Kanury V.S. Rao

Th 22473

Abstract

There is an urgent need for an effective vaccine against tuberculosis (TB), as the currently available BCG vaccine has not performed satisfactorily, especially against the adult form of the disease. Waning of BCG efficacy over time and poor induction of MHC class I restricted responses have prompted research for an alternative that can tide over these shortcomings. In this study we employed differential proteomics to obtain a list of potential vaccine candidate antigens. Bacterial epitopes being presented at early stages on MHC class I and class II molecules of macrophages infected with Mycobacterium tuberculosis (M.tb) were identified using iTRAQ labeling and reverse phase LC-MS/MS. The putative vaccine candidates thus identified were tested as plasmid DNA vaccines in mice to ascertain their protective efficacy against a challenge of aerosolized M.tb, based on the ability of the vaccine candidates to reduce the bacterial load in the lungs of infected mice. We report here that four of the seventeen selected antigens imparted significant protection against the challenge of M.tb. The four shortlisted antigens were further assessed in the more stringent guinea pig model, where too, they demonstrated significant protection when compared to the negative control (saline or empty plasmid vector). In this proof of concept study we show that combining a proteomics approach with in vivo assessment of vaccine candidates against experimental TB infection in animal models can be very valuable in identifying new potential candidates thus expanding the antigen repertoire for novel vaccine candidates against TB.

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397. SINGHAL (Pallavi)
Host and Viral Gene Interaction and Their Regulation During Development of Cervical Cancer.
Supervisors : Prof. Alo Nag and Dr. Mausumi Bharadwaj
Th 22472

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398. TIWARI (Pooja)
Evaluation of Various Polymer Based Nano-Carriers Loaded with Potential Anti-Malarials for Therapeutics.
Supervisor : Prof. Prahlad C. Ghosh
Th 22471

Abstract

The emulsion–solvent evaporation method used for preparation of spherical drug-loaded nano particles systems. For the first time comparative study of antimalarial compounds, Stearylamine/ monensin loaded in various polymeric nanoparticles was performed in an antimalarial setting. SA is working on the cell membrane of Plasmodium infected RBCs while monensin is disrupts the ionic gradient across the food vacuole in Plasmodium. Both are hydrophobic and toxic in high dose, that is why the need of making nanoparticles. Substantial inhibition of growth of P. falciparum by nanoparticles formulations was observed. In case of PLGA, it depends on the mol. wt. of PLGA. Malaria exists in many stages, so the efficacy of various formulations was also calculated in vitro. Maximum inhibition for monensin IC_{50} 3.4±1.2ng/ml was observed against trophozoit and ring stage. PEGylated PLGA monensin shown to be very effective in inhibiting the growth of P. falciparum in culture as well as P. berghei infection in mice. The entry of Coumarin – 6 loaded nanoparticles is clearly visible in parasitized RBC in case of drug loaded PLGA nanoparticles as compared to uninfected RBCs. Some of the polymers like PLA and PMMA nanoparticles the uptake was not selective. Overall study suggests that, for targeting various stages, in place of using combination of drugs, we can use combination of polymer loaded with same drug. So here we are proposing novel Idea of combination therapy. Significant distribution of theses nanoparticles may find a path for future therapies.

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399. ZUBERI (Mariyam)
Study of Genetic Alterations in Ovarian Cancer Patients.
Supervisor : Dr. Alpana Saxena, Dr. P. C. Ray and
Dr. Gauri Gandhi
Th 22466

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