CHAPTER 32

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ALKA

Targeting Glutamate Racemase of Mycobacterium Tuberculosis and Neisseria Gonorrhoeae: Experimenting New Tricks to Tackle Antibiotic Resistance Menace .

Supervisor : Prof. Uma Chaudhry

Th 24773

Abstract
(Not Verified)

Bacterial infections are increasing severe concern worldwide. The efficiency of available treatment is falling with the ever-evolving advent of multi-drug-resistant (MDR) bacterial pathogens. Most of the current studies highlight the self-adaptive mutations in the bacteria that account for alterations in several pathways in the progression of drug-resistant strains. Therefore, finding new classes of lead compounds and also significantly important to understand the mechanism of action of existing drugs so as to be better equipped for identifying new and novel targets. To tackle these problems, our study provides the first conclusive evidence of an additional target of ethambutol, namely glutamate racemase (MurI) a phase I PG biosynthesis enzyme. This protein target is in addition to the inhibition of arabinosyl transferases, an arabinogalactan polymerization enzyme, a well-established target of ethambutol. Collective studies using enzyme kinetics and structural analysis revealed that ethambutol inhibits the enzyme activity by binding competitively at its active site and thereby alters it conformation. The study could prove helpful in understanding the molecular mechanism of antimicrobial resistance of this established drug. Furthermore, since glutamate racemase is a conserved protein of the bacterial kingdom, ethambutol could also be explored as a broad spectrum antibiotic for many other bacterial diseases.Using combination of various in silico approaches and validating the observations by subsequent in vitro experiments, we have also identified natural lead compound against glutamate racemase of Mycobacterium tuberculosis and Neisseria gonorrhoeae. The membrane permeabilizing activity of chosen lead compounds described in this study may be used further in future endeavour to upsurge the effectiveness of antiquated antibiotics through augmenting their cellular uptake These molecules can serve as a robust platform for carrying out more rational modification and structure activity relationship studies. The strategy could be immensely promising in the current scenario of continuous emergence of resistant microorganisms and antibiotic crisis.

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natural compounds on glutamate racemase of Neisseria gonorrhoeae (NG-MurI) 7. Summary 8. Conclusion 9. References 10. Appendix 11. Publication 12. Conference and workshops 13. Awards and fellowships.

02. CHOWHAN (Rimpy Kaur)
Investigating the effect of pH (due to sub-cellular localization), redox state and genetic polymorphism on structure and multifunctionality of peroxiredoxin 6.
Supervisor: Dr. Laishram R. Singh
Th 24910

Abstract
(Not Verified)

Prdx6 is a multifunctional enzyme with the ability to function as glutathione peroxidase at cytosolic pH, and as calcium-independent phospholipase A2 (aiPLA2) and lysophosphatidylcholine acyltransferase (LPCAT) at lysosomal pH. By the virtue of its moonlighting activities, Prdx6 is involved in various crucial physiological processes including antioxidant defense, signal transduction pathways, inflammation, lipid metabolism, lung surfactant synthesis, cell wall repair, etc., and associated with several diseases, such as, chronic obstructive pulmonary disease, emphysema, Parkinson's disease, Alzheimer's disease, diabetes, prion diseases, cancer, etc. Interestingly, not all of these diseases implicate Prdx6 involvement due to its antioxidative behavior, there are various reports where Prdx6 is shown to behave as a pro-oxidant (and exacerbate diseases) due to excessive phospholipase A2 activity. A clear understanding of the protein structure and its modification under the pressure of trans-regulatory variables like environmental pH, redox state, and genetic polymorphism is believed to provide better insight into the functioning of this protein during physiological and disease conditions. Therefore, in the present research work, we have worked towards – (i) comprehending the impact of subcellular pH associated functional compartmentalization on Prdx6's structural allostery and oligomerization propensity, (ii) understanding the structural basis for redox regulation of Peroxiredoxin 6, and (iii) perform pathogenic prediction of single nucleotide polymorphism and their impact on Prdx6's structure and function. Our major observations revealed pH and enzyme redox state to influence the functionality of Prdx6 by altering its quaternary structure and stability. Also, we are reporting here for the first time, Prdx6 as an aggregation-prone protein with the self-association rate faster than that of β-amyloid (amyloidogenic peptide associated with Alzheimer's disease) at cytosolic pH and temperature conditions. Additionally, we have identified a naturally occurring polymorphism T177I of Prdx6 as a prospective beneficial variant for individuals with neurodegenerative diseases.

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03. GULATI (Parul)
Genomics and Transcriptomics of Maconellicoccus Hirsutus, a Model For Genomic Imprinting and Epigenetic Regulation.
Supervisor:Prof. Vani Brahmachari
Th 24772

Abstract
(Verified)

The mealybug system serves as a model for epigenetic regulation, with genomic imprinting being closely correlated with sex determination. In absence of sex chromosomes, the selective inactivation of the entire paternal genome in certain fertilized oocytes during cleavage stages results in male determination. We have utilized the mealybug, Maconellicoccus hirsutus to decipher epigenetics regulatory mechanisms. They are sexually dimorphic whereas the immature males and females are morphologically similar. The diploid genome consists of five pairs of chromosomes (2n=10). The complete genome sequencing, annotation and mining of selected classes of genes is the focus of the present thesis. We designed a novel approach to mine genomes deriving high-priority domains for each class of genes of our interest and performed meta-analysis on selected insect genomes which generated a comprehensive data set for reliable identification of epigenetic modifiers of histones in newly sequenced insect genomes. The sequencing data was generated for whole genome of M.hirsutus, assembled, annotated and analysed for the histone and histone variants, the epigenetic modifiers, in terms of the writers, readers and the erasers.The insecticide resistance properties conferring its survival in almost any kind of environmental conditions were also analysed and found to be members of the expanded class of genes in mealybug genome. The transcriptome sequencing and analysis of the differential expression of the genes between male and female M.hirsutus was also carried out. The primary data of genomics for genomic imprinting is generated. Therefore the work contributes towards creating a basal large scale genomics dataset for a model system and for epigenetic regulation to be utilized further in mechanistic studies.

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04. NITIN KUMAR
Synthesis and Physico-Chemical Interactions of Carbazole and Biscarbazole Derivatives to Evaluate Their Anticancer Activity in Human Glioma Cell Line .
Supervisor: Dr. Pratibha Mehta Luthra
Th 24911

Abstract
(Not Verified)

ABSTRACTGlioblastoma multiforme(GBM) is the most common and dangerous form of malignantprimary brain tumors in adults. The most commonly used chemotherapy agents areNitrosoureas (carmustine i.e., BCNU, lomustine i.e., CCNU), Procarbazine, Vincaalkaloids(vincristine, vinblastine) ,Platinum based drugs(cisplatin, carboplatin)Temozolamide(TMZ) etc. Despite ceaseless efforts by researchers and physicians, mostlypatients with GBM die within one and half years of diagnosis. We have investigatedcarbazoles as novel anticancer agents in the glioma therapy due to their specific andselective interaction with DNA. N-nitrogen containing tricyclic carbazoles derivativesexhibited antitumor, anti-oxidant, anti-inflammatory, antihistaminic and antibiotic activities.CONTENTSThe work presented in the thesis has been divided into three chapters: Chapter 1Introduction and review of literature on the subject, Chapter 2a Design, synthesis, DNAbinding and anticancer evaluation of novel substituted bis-carbazole derivatives againsthuman glioma U87 cell line Chapter 2b Design,synthesis, DNA binding studies andanticancer evaluation of 1,4-dimethyl-9-H-carbazol -3-yl)methanamine derivatives

againstU87 MG cell line Chapter 2c Design ,Synthesis, DNA binding studies and anticancerevaluation of substituted 1,4-dimethyl-9-H-carbazole tethered to carbamates derivativesagainst U87 MG cell line Chapter 2d Design , Synthesis, DNA binding studies andanticancer evaluation of substituted 1,4-dimethyl-9-H-carbazole tethered thiosemicarbazidesor semicarbazides derivatives against U87 MG cell line. Chapter 2e: Synthesis ofnovel phenanthridine based compounds and to study their in vitro physicochemicalproperties and anticancer activity. Chapter 3 contained the experimental methodology.

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05 VANDANA

Biochemical Characterization of Metacaspase-2 (MCA-2): A Novel Cysteine Protease from P. Falciparum.

Supervisor: Dr. Anju Katayal and Dr. Kailash C. Pandey

Th 24774

Abstract
(Not Verified)

The apoptosis mechanisms/s in protozoans belonging to genus Plasmodium revealed the presence of proteases with caspases like- activities, which are known as “metacaspases”. Although this family of cysteine proteases is structurally similar to caspases with Cys-His dyad but their evolutionary significance and functional relevance remains largely unknown. These proteases are proposed to be important therapeutic targets against malaria as metacaspases are not reported in humans. Therefore in the present study, we cloned, expressed and purified the PfMCA2 (Ser1508-Val1690) and studied its enzymatic characteristics and substrate specificity and its role in P. falciparum cell death in vitro. The results of enzymatic assays demonstrate that PfMCA-2 efficiently cleaved arginine/lysine specific peptide, but not classical caspase-specific substrate/s. Consistently, PfMCA-2 activity was sensitive to effector caspases inhibitor, Z-FA-FMK, and mildly inhibited by aprotinin and E-64. However, general caspase inhibitors such as Z-VAD-FMK and Z-DEVD-FMK had no effect on PfMCA-2 activity. Z-FA-FMK inhibits parasite growth with an IC50 value of 2.7µM along with the notable morphological changes. PfMCA-2 specifically expressed in schizonts and gametocyte stages and there was a notable depletion of PfMCA-2 expression in Z-FA-FMK treated schizonts and gametocytes stages of the parasite. Notably, PfMCA-2 cleaves a phylogenetically conserved protein, TSN (Tudor staphylococcal nuclease) and the proteolysis of PfTSN did not occur after treatment with the Z-FA-FMK. The production of a large number of reactive oxygen species in the presence of Z-FA-FMK caused oxidative stress which in turn leads to loss of cell viability. The oxidative stress further generates positive feedback for the occurrence of cell death in term of phosphatidylserine

externalization.Conclusively, PfMCA-2 expression level in the various stages contributes to the survival of parasite and its suboptimal expression can affect parasite viability and death. The parasite specific expression of PfMCA-2 makes it a lucrative therapeutic target for future drug development.

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