CHAPTER 60

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. BANDYOPADHYAY (Upasana)
Characterization of Immune Response Induced by Mycobacterium Tuberculosis Antigens Displaying Similarity to Mammalian Proteins.
Supervisor : Prof. K. Natarajan
Th 24191

Abstract
(Verified)

Slow but steady pathogenesis of *Mycobacterium tuberculosis* (*M. tb*), makes it one of the most successful pathogens in human history. This is attributed to its wide array of immune evasive mechanisms. These mechanisms suppress the pro-inflammatory responses enhancing the survival of intracellular *M. tb.* Toll like Receptors (TLRs) are central to the innate immune responses. TLR2 and TLR4 are known to be upregulated in tuberculosis infection. Downstream signaling of the TLR2 pathway that proceeds via MyD88, IRAK, involving, pERK, MAP Kinase pathway finally leading to NF-κB, upregulates the pro inflammatory responses of the cell. Microorganisms are known to utilize molecular mimicry as a measure thwarts protective responses by the host where, they incorporate sequences and domains in their genes/proteins that have similarity to various domains of the host proteins. In this study, we report that *M. tb*protein Rv3529c exhibits significant similarity to the death domain of MyD88. Incubation of macrophages with Rv3529c specifically inhibited TLR2 mediated pro inflammatory responses. This included attenuated oxidative burst, reduced phosphorylation of MAPK-ERK, reduced activation of transcription factor NF-κB and reduced secretion of pro inflammatory cytokines IFN-γ, IL-6, and IL-17A with a concomitant increased secretion of suppressor cytokines IL-10 and TGF-β. Importantly, Rv3529c significantly inhibited TLR2-induced association of MyD88 with IRAK1 by competitively binding with IRAK1. Further, Rv3529c mediated inhibition of apoptosis and phagosome– lysosome fusion. As a cumulative effect of above mentioned immune suppression, incubation of macrophages with Rv3529c increased bacterial burden inside macrophages. Rv3529c also interacts with crucial cell signaling molecules L-type VGCC, pP38, pPKC and SOCS. This show a strategic molecular mimicry evolved by *M. tb*toward immune evasion; along with a strategy of protein repurposing, where one of its proteins involved in the metabolic pathways within the bacterial cell is being effectively used in immune evasion inside the host cell.

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02. BHATTACHARYA (Kausik)
Genome sequence Variation in a Multidrug Resistant Clinical Isolate of Mycobacterium Tuberculosis: Analysis and Evaluation of Functional Implications.
Supervisors : Prof. Vani Brahmachari and Prof. Mandira Varma Basil
Th 24297

Abstract
(Verified)

We have completed the sequencing and the comparative analysis of the genome of a multi-drug resistance (MDR) clinical isolate of M.tuberculosis from VPCI, India. The single nucleotide variations, (SNVs) are mapped to the genic and potentially important intergenic regions including essential genes. We compared the SNVs in VPCI clinical isolate with SNVs in more than 500 clinical isolates in the database leading to the identification of invariant sequence stretches and also SNVs unique to VPCI clinical isolate. We have evaluated the effect of SNVs in VPCI isolate in two cases of genic variation and one of intergenic variation for their functional implication. The structural alteration was seen in MprA and its variant and RpoB. The variation in RpoB led to altered affinity for binding of Rifampicin. With MprA we demonstrate the unique localization of the pathogen protein into the host cell nucleus, leading to altered transcription of host genes, IL17A and TGFβ. This leads to the decrease in phago-lysome fusion and rescue from apoptosis. Thus all the parameters that favour the survival of the pathogen showed enhanced expression by the nuclear localization of MprA and these responses were further increased by the SNV containing protein. We have examined the effect of intergenic variation mapping between Rv3793 and Rv3794, known to enhance the binding of EmbR thus contributing to ethambutol resistance. In the last section the presence of two promoters, P1 and P2 in the mce1 operon, coding for genes that are essential for the entry of the bacillus was identified. A promoter upstream of fadD5 (Rv0166) was also identified. The occupancy of RNA polymerase on both the promoters, P1 and P2, was shown, aiding differential expression of the operon. This work adds new knowledge towards the understanding of the biology of Mycobacterium tuberculosis.

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03. GAUTAM (Amardeep)
Evaluating Protective Role of Hypericum Perforatum Hypobaric Induced Cognitive Dysfunction and Developing a Drosophila Model to Study High Illness.
Supervisor : Dr. Anju Katyal
Th 24192

Abstract
(Not Verified)

With decrease in atmospheric pressure, the amount of oxygen available to tissues is reduced, creating hypoxic conditions and manifested as Acute Mountain Sickness, High Altitude Cerebral Edema and High Altitude Pulmonary Edema. The exact mechanism of the pathology of high attitude illness is not clear, but the role of reactive oxygen species is well established. Pharmacological interventions such as acetazolamide and dexamethasone provide symptomatic relief. Thus a more tolerable and wide spectrum natural treatment is preferred. *Hypericumperforatum*is an effective anti-depressant with nootropic properties. We evaluated the efficacy of ethanolic extracts of *H. perforatum*in preventing simulated hypobaric hypoxia induced cognitive dysfunction in mice. Treatment with *H. perforatum*extracts reduced neuronal cell death in hippocampal region of hypoxic mice. It ameliorated hypobaric hypoxia induced behavioral abnormalities in mouse (associative memory in passive avoidance test and spatial learning in elevated plus maze). It reduced oxidative stress in the brain indicated by lipid peroxidation and nitric oxide production in brain. *Drosophila*is used as a model organism to study human disease as it provides a very extensively studied biology. We established a simple setup to simulate hypobaric hypoxia in *Drosophila*. The hypoxia sensitive flies responded to hypoxic insult as assessed by behavioral abnormalities and increased mortality. They seem to employ reduction in metabolism as the strategy to compensate for reduced oxygen. The proposed setup is easy and can be utilized by other laboratories to study hypobaric hypoxia pathology.

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04. NASREEN BANO
Role of NF-κB, miRNA and HPV and Their Cross Talk in Cancer and Cancer Stem Cells During Oral Carcinogenesis.
Supervisors : Dr. Manisha Yadav and Dr. Laishram R. Singh
Th 24254

Abstract
(Not Verified)

A small subpopulation of cancer stem-like cells (CSCs) present in almost all tumors is responsible for drug resistance and tumor recurrence. The role of NF-kB and miRNA in close association with essential risk factors, tobacco, alcohol and high risk HPV infection during oral carcinogenesis and its prognosis is not well understood. We have isolated cancer stem like SP cells from both HPV+/-ve oral squamous cell carcinoma (OSCC) cell lines and primary tumors, which formed orospheres, expressed stemness markers Oct4, Sox-2, CD44, CD133 and CD117. These cells showed differentially upregulated expression of NF-kB proteins and selective overexpression of viral oncogenes E6/E7 specifically in HPV16+ve cells which formed higher number of orospheres, overexpressed c-Rel and selectively activated p65 that heterodimerized with p50 to show higher DNA binding activity. Further, selective over expression of miR-21 and miR-155 and downregulation of miR-34a were demonstrated by HPV+ve CSCs which overexpress HPV16 oncogene E6 that is responsible for the maintenance of stemness. While, HPV-ve CSCs show exclusively p50 homodimeriztion, poor differentiation and worst prognosis, HPV infection induced participation of p65 along with deregulated expression of specific miRNAs along with sensitization of HPV-CSCs by curcumin led to well differentiation of tumors and better prognosis.

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05. VERMA (Vivek)
Characterization of Inflammasomes and its Role in Pathogenesis of Uropathogenic Escherichia Coli Causing Urinary Tracts Infections.
Supervisors : Dr. Manisha Yadav and Dr. Rakesh Singh Dhanda
Th 24255

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1. Introduction 2A. Mechanism of NLRP3 inflamma some activation in UPEC stimulated THP-1 derived macrophages 2B. Cloning of UPEC alpha hemolysin 2C. Role of UPEC alpha hemolysin in the activation of inflammasome 3. Investigation of the NLRs role in pathogenesis of UPEC infected UTI patients 4. Summary, conclusion, references, appendix, list of publication, list of conference and workshops.