CHAPTER 29

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. AARTI
Characterizing the Role of Toll Like Receptors (TLRs) in Mediating Immune Responses to Mycobacterial Infection.
Supervisor: Prof. Krishnamurthy Natarajan
Th 27622

Abstract

Tuberculosis is one of the oldest known calamities caused by the etiological agent Mycobacterium tuberculosis (M. tb). Despite being preventable and curable, it is still one of the largest causes of death around the globe. Mycobacterium tuberculosis (M. tb) infects mainly through the mucosal tissue of the respiratory tract. The mucosal surface provides the primary site for host-pathogen interactions. The epithelial layer present at the mucosal surface acts as a physical barrier that prevents invasion of the organism by various defense responses. The epithelial cells are known to express all known human TLRs on their surface. These TLRs activate downstream signaling pathways that trigger immediate innate immune responses and also prime and orchestrate antigen-specific adaptive immune responses. Thus, they act as a mediator between innate and adaptive immunity. Although TLRs are considered pivotal in eliciting innate responses, they are found to be involved in the pathogenesis of several infections like malaria, candidiasis, autoimmunity, and asthma. Thus, TLRs are known to play dual roles, as they act both in activation and suppression of innate immunity. Mycobacteria have several mechanisms for evasion of protective responses mounted by the host. In this study, we unravel yet another mechanism that is mediated by Toll-Like Receptors TLR2, TLR4, and TLR7 in epithelial cells. We report a negative role for TLR2, TLR4 and TLR7 on lung epithelial cells which might be exploited by BCG to survive within the host cells. We show that BCG infection of epithelial cells at higher MOI results in an increase in the expression of the above TLRs. This increase in TLR expression corresponds to increased rates of BCG internalization early on in the infection but stabilizes at later time points. This increased uptake may contribute towards increased inhibition of proximal defense response such as ROS generation, apoptosis and autophagy. These results clearly point towards the strategy of BCG to increase the expression of TLRs upon infection and to use this increased expression to downmodulate defense responses. We also showed a direct role of the key TLR signaling intermediate MyD88, whose inhibition led to the induction of ROS and a significant reduction in the intracellular mycobacterial survival. TLR signalling through MyD88 activates key MAP kinases, which further regulate downstream processes. We checked for pERK MAP kinase which has been reported to play a significant role in cell proliferation and mounting anti-apoptotic effects. Interestingly, BCG infection decreased pERK activation, but TLR4 and 7 stimulations increased its activation. Significantly, inhibiting ERK activation resulted in decreased BCG survival inside epithelial cells, thus, pointing towards a strategy of BCG to employ TLRs for immune evasion.

Collectively, our data points towards negative role of TLRs utilized by BCG in inhibiting protective defense responses by various mechanisms.

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02. BAREJA (Reshmee)
An Integrative Approach to Identify P73-Regulated Biomarkers in Colorectal Cancer and to Unravel Their Mechanism in Cancer Progression.
Supervisor: Prof. Daman Saluja
Th 27624

Abstract

This work aims to decipher p73-regulated biomarkers for a prompt diagnosis of colorectal cancer (CRC) by employing a combination of integrative bioinformatics and expression profiling technologies. Transcriptome profile of HCT116 cell line p53−/− p73+/+ and p53−/− p73 knockdown was performed to identify differentially expressed genes (DEGs) and long non-coding RNAs followed by cross-checking with CRC tissue expression datasets available in Gene Expression Omnibus and TCGA. KEGG and Gene ontology were performed on differentially expressed transcripts obtained via the transcriptome profile and intersected genes. The PPI network was constructed via Cytoscape to extract hub genes. Kaplan-Meier plots assisted in investigating the prognostic significance of the hub genes. qPCR was carried out for expression. Promoter analysis was employed for the identification of p73 binding sites in the selected upregulated or downregulated lncRNAs which was further confirmed by Luciferase reporter, and ChIP assay. ChIP showed promoter enrichment of the selected lncRNAs. Machine learning algorithms were employed to perform TNM-stage classification. Transcriptome profiling revealed 1,289 upregulated and 1,897 downregulated genes. KEGG showed enrichment of the DEGs in metabolic process, fatty acid biosynthesis, etc. The PPI network constructed assisted in identifying 20 hub genes. The deep learning model achieved a TNM-stage classification accuracy of 0.75 using 20 hub genes. Our study provides insights into the differential regulation of lncRNAs in a p73-dependent manner which may provide a mechanism of their action at the genome level. We utilized the StemChecker database to investigate any possible effect of p73 deletion on the stemness in cells displaying mesenchymal features. We observed embryonic and hematopoietic stem cell-related genes to be the most overlapped genes with our dataset. This is a novel study utilizing transcriptomics, publicly available tissue datasets, and machine learning to unveil key CRC-relevant genes that may be important for patients' prognosis and diagnosis.

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03. JENA (Harekrushna)
Design and Development of Synthetic Vectors for Delivery of Biomolecules.
Supervisor: Prof. Sunit K. Singh
Th 27626

Abstract

The cationic, non-toxic, and biocompatible nature of low molecular weight branched polyethyleneimine (LMW bPEI, 1.8 kDa) has received considerable attention in the design and development of carriers for nucleic acids. Due to the inadequate nucleic acid complexation ability, transportation across the cell membrane, and non-specificity, it exhibits poor transfection efficacy, limiting its widespread use in clinical applications. To overcome these limitations, we have synthesized three series of amphiphilic conjugates by varying the percent grafting of three different hydrophobic ligands onto bPEI polymer, i.e., retinoyl-bPEI (RP), PEI-Dabsyl (P-Dab), and N-[3-(2-pyridyldithio)] propionoyl polyethylenimine (PDPP) conjugates. These conjugates have been characterized physicochemically and in aqueous environment, these amphiphilic conjugates form core-shell nanocomposites after self-assembly with cationic surface charge. These nanocomposites bind plasmid DNA, carry it inside the cell, and protect it in the cellular environment. The transfection efficiency of these nanocomposites/pDNA complexes was evaluated on the mammalian cells. RP/pDNA complexes showed receptor-mediated gene delivery in HepG2 cells due to the presence of all-trans-retinoic acid in its structures which acts as a ligand for over-expressed retinoic acid receptor-α of HepG2 cells. P-Dab nanocomposites containing azo moieties showed stimuli-responsiveness under the influence of UV-Vis light and azoreductase enzyme, found in abundance in the colonic area. The PDPP nanocomposites containing disulfide linkages showed glutathione, found in much higher concentration in cancerous tissues than in healthy physiological tissues, responsiveness. All these complexes showed many-fold higher transgene expression in both cancerous and normal cells as compared to native LMW bPEI without compromising the cytocompatibility. Further, the hydrophobic cores of both P-Dab and PDPP nanostructures helped in the encapsulation of the model hydrophobic drug, ornidazole, and showed faster drug release in response to the external stimuli, azoreductase enzyme, and reductive conditions, respectively.

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04. KRITIKA
Investigating the Role of Brain Metabolites, Methylamines as Potential Ligands for Carbonic Anhydrase and Acetylcholinesterase: A Study Based on Dementia.
Supervisor: Prof. Madhu Chopra
Th 27628

Abstract

Dementia is a syndrome characterized by the progressive decline in cognitive ability of the individual thereby interfering with the capability of the brain to function normally. To date, there has been no clear molecular-level understanding of the pathophysiology of dementia and hence its treatment strategies. It has been previously reported that many metabolites are known to be upregulated or downregulated in dementia patients adding to the pathology of dementia. One such

class of molecules that has been given much attention in humans is methylamines (e.g., trimethylamine N-oxide, TMAO; sarcosine; dimethylglycine, DMG; and betaine). In the present work, we have systematically investigated the structural and functional consequences of different methylamines on Carbonic anhydrase (CA) and acetylcholinesterase (AChE) and also examined if they are appropriate ligands for the enzymes. Our results confirmed that TMAO could be an ideal ligand of CA that is specifically targeted to its folding intermediate, Uf by affecting prolyl cis-trans isomerization of CA-II and hence affecting its function in neuronal cells. We also observed that other methylamines, betaine, DMG, and sarcosine are ligands of AChE and competitively inhibit it. Our results on cell viability, ROS levels, and apoptosis of HT22 cells depict that betaine and DMG (but not sarcosine) protect HT22 cells against Aβ-induced cytotoxicity by inhibiting AChE. Since betaine and DMG are FDA-approved supplements, the results implicate that they could be employed independently or as an AChEi-methylamine mixture for the treatment of dementia.

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05. RAJNISH KUMAR
Rapid Detection, Characterization and Enumeration of Microbial Load on Fresh Produce.
Supervisor: Prof. Uma Chaudhry
Th 27630

Abstract

Consequent to the rise in lifestyle disorders, the consumption of fresh produce has increased considerably in India which can also lead to serious cases of foodborne illnesses as these are mainly consumed raw. A wide range of foodborne pathogens can be transmitted via fresh produce which also includes bacterial pathogens. Detecting bacterial pathogens using traditional methods like microbiological culture, can be expensive, time-consuming, and labor-intensive. Rapid detection of bacterial pathogens in fresh produce is crucial for preventing outbreaks of diseases. In the present study, we used a two-pronged approach to identify the pathogens prevalent in fresh produce using microbiological and molecular methods. Five hundred samples of fresh produce were procured from retail and local markets of Delhi and NCR region from 2018 to 2023. Most of the samples exhibited the presence of Escherichia coli, Staphylococcus aureus, and Salmonella sp as per the standard microbiological protocols that were used and also evaluated by the in-house developed multiplex-PCR which was more specific and less time-consuming. The relationship between the nutritional status of fresh produce and the microbial load was analyzed and it was found that produce rich in glucose accelerated the bacterial load. Isolated bacterial samples were subjected to antimicrobial susceptibility profiling. Most of the isolated bacteria were found to be resistant to available antibiotics. Since most of the tested samples exhibited a high abundance of Escherichia coli which were also found to be resistant to many antibiotics, we decided to identify a novel target of food pathogenic E. coli O157:H7 and found a moonlight protein, glutamate racemase, that could be used to design natural product inhibitors. The present study delved into the specificities of the Antimicrobial resistance challenge prevalent in fresh produce, identified the potential strategies for mitigating the crisis, and evaluated the broader implications for public health.

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06. RANA (Ankush Kumar)
Role of Short Chain Fatty Acids in Mediating Protective Responses to Mycobacteria.
Supervisor: Prof. Krishnamurthy Natarajan
Th 27631

Abstract

Tuberculosis is a well-known life-threatening disease that significantly affects millions of humans lives worldwide. Mycobacterium tuberculosis (M. tb.) is a causative pathogen of this disease. It enters the host pulmonary system through aerosols and resides primarily in macrophages and dendritic cells. After successful infection, mycobacterium does needful alterations in the defence system of immune cells for disease progression and prolonged survival. These include inhibiting phagosome lysosome fusion, retarding pro-inflammatory responses, delaying T- cell-mediated immunity, and modulating calcium channels for granuloma formation. On the other hand, gut microbiota modulates host defence responses against infections in order to maintain immune homeostasis. This host-microbe crosstalk is regulated by gut metabolites such as butyrate, propionate and acetate. Butyrate is one such small chain fatty acid produced by gut microbes upon fermentation that has the potential to influence immune responses. Here we investigated the role of small chain fatty acids (SCFAs) and carbon substrates in macrophages during mycobacterial infection. Results demonstrate that butyrate and propionate significantly suppress the growth kinetics of mycobacteria in culture medium as well as inhibit mycobacterial survival inside macrophages. On the other hand, however, sodium succinate promotes growth kinetics of mycobacteria in culture medium and enhances mycobacterial survival inside macrophages. Interestingly, butyrate and propionate alter the pentose phosphate pathway by inducing a higher expression of Glucose-6-Phosphate Dehydrogenase (G6PDH) resulting in a higher oxidative burst. Further, butyrate supplementation decreased Super Oxide Dismutase-2 (SOD-2) and increased NADPH oxidase-2 (Nox-2) expression. Butyrate induced G6PDH also mediated a decrease in mitochondrial membrane potential. This in turn led to an enhanced induction of apoptosis as measured by lower expression of the anti-apoptotic protein Bcl-2 and a higher expression of pro-apoptotic molecule cytochrome C. Overall, the results in the study indicate that butyrate alters the metabolic status of macrophages and induces protective responses against mycobacterial infection.

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07. SHARMA (Shakshi)
Understanding the Altered Gene Expression of Splicing Factors and their Effect on Splicing of SIN3B in Oral Cancer and to Establish sIL2R as a Marker of HLH in Indian Population.
Supervisor: Prof. Daman Shaluja
Th 27632

Abstract

Head and Neck Squamous Cell Carcinoma is the sixth most common cancer worldwide developing from mucosal epithelium in the oral cavity, pharynx, and larynx. By 2030, the incidence rate is anticipated to increase by 30%. More promising diagnostic techniques and novel biomarkers are required connoting the need for early diagnosis and better prognosis. Sin3B is a global transcriptional regulator responsible for gene repression through chromatin-modifying complexes. This results in the regulation of important biological processes such as genomic stability, cell cycle progression, homeostasis, and embryonic development as a result of its interaction with a vast number of repressors and co-repressors leading to modulation of transcription and chromatin structure. Our study suggests the presence of a novel alternatively spliced form of Sin3B in several transformed cell lines and oral cancer tissue samples. The presence of alternatively spliced forms of Sin3B was also observed in some malignant and pre-malignant cases of oral carcinoma and a few mammalian cell lines connoting the importance of their presence in the transformed phenotype. We also observed the downregulation of Sin3B and its spliced variants in clinical samples when compared between Pre-cancer stages and late cancer. Our data suggests the critical involvement of SRSF family members especially, SRSF1 and SRSF11 in alternative splicing of SIN3B, highlighting their potential as key players in the dysregulated splicing landscape associated with cancer progression. Raised soluble interleukin-2 receptors (sIL2R, CD25) are one of the biomarkers of Hemophagocytic Lympho-histiocytosis (HLH), a cellular immune dysregulation caused by underlying genetic defects or triggered by infection, malignancies or rheumatological conditions. However, sIL2R normal values are so far not known in Indian population. So, this study was undertaken to measure sIL2R in healthy children and adults to establish age-related reference values. Our results indicate that age appropriate sIL2R values should be taken into consideration rather than a static cut-off of 2400IU/ml to improve the accuracy for the diagnosis of HLH.

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08. SINGHAL (Shelly) Nee Shelly Aggarwal
Diagnostic Potential of Sort1 Gene and Designing Pcsk9 Inhibitors as Therapeutic Strategy for Coronary Artery Disease.
Supervisors: Prof. Daman Saluja and Dr. Kamna Srivastava
Th 27623

Abstract

Coronary artery disease (CAD) encompasses a range of conditions, from silent atherosclerosis to life-threatening events like heart attacks, presenting a significant global health challenge. CAD claims numerous lives annually, emphasizing its status as a major public health issue. The multifaceted nature of CAD etiology, influenced by factors such as age, gender, and lifestyle, complicates both risk assessment and diagnosis. Early and accurate diagnosis, along with risk stratification, are crucial for initiating effective treatment. Various risk factors contribute to CAD development, with lipid-lowering therapy playing a pivotal role in disease management. However, challenges persist in lipid management and in identifying reliable diagnostic and prognostic biomarkers for CAD. The SORT1 gene, situated on chromosome 1, encodes the Sortilin protein, involved in intracellular protein trafficking and linked to cardiovascular risk. Sortilin interacts with PCSK9, a key player in LDL cholesterol metabolism and a target for CAD therapy. PCSK9 inhibitors offer promise in lowering LDL cholesterol and preventing cardiovascular events. Research highlights the interconnectedness of PCSK9, SORT1, and LDLR pathways in CAD development. Our study aims to explore the differential expression of the SORT1 gene in CAD patients compared to healthy individuals, potentially serving as a diagnostic or prognostic biomarker. Additionally, we identify small molecules targeting PCSK9 at allosteric sites, which could reduce LDL cholesterol levels and CAD risk. Through molecular docking, compounds like Sofosbuvir, Benazepril, Tirofiban, and Quinapril have been identified as potential PCSK9-LDLR interaction inhibitors. Molecular dynamics simulations provide insights into their stability and interactions with PCSK9. In vitro experiments confirm the inhibitory activity of Benazepril and Quinapril against PCSK9-LDLR binding, suggesting therapeutic potential for CAD and laying the groundwork for future drug development. This comprehensive approach, integrating genetic studies with drug design strategies, holds promise for advancing CAD diagnostics and developing innovative therapies.

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09. YADAV(Nitin)
Understanding the Molecular Mechanisms of Epileptogenesis in Drug-Resistant Focal Epilepsy for the Identification of Pathology-Specificpotential Biomarkers.
Supervisor: Dr. Aparna Dixit
Th 27633

Abstract

Focal drug-resistant epilepsy (DRE) is often associated with Focal Cortical Dysplasia (FCD) and Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS), which accounts for one-third of surgical cases. The inability to precisely localize epileptogenic zones (EZs) is a major contributor to poor surgical outcomes in FCD. Liquid chromatography coupled with high-resolution tandem mass spectrometry was used to identify altered lipid profiles in resected FCD and MTLE-HS tissues, obtained during electrocorticographically (ECoG)-guided surgery, compared to autopsy. A total of 1224 lipids were detected, with 607 in positive mode and 617 in negative mode. Thirteen lipids were significantly altered in FCD tissues ($p<0.05$, fold-change ≥2). Upregulated lipids included neutral triacylglycerols (TAGs), while downregulated lipids comprised phosphatidylcholine (PC) and phosphatidylethanolamine (PE). TAGs

were notably upregulated across all FCD subtypes and in MTLE-HS brain tissue, and their levels were significantly higher in peripheral blood plasma of FCD and MTLE-HS patients. These findings suggest that distinct lipid signatures could be leveraged in REIMS-based techniques for defining EZs in FCD. In parallel, the extracellular matrix protein FN1 mediated α5β1integrin-Src kinase-NMDAR signalling was significantly upregulated in the hippocampus of MTLE-HS patients. Further FN1-α5β1-Src kinase-NMDAR signalling was also altered in both hippocampus and ATL regions in a pilocarpine-induced rodent model of chronic TLE. In-vivo knockdown of FN1 led to decreased levels of FN1 and NR2B, reduced EEG frequency, and attenuated dendritic growth in the pilocarpine model. Similarly, in-vivo knockdown of Src downregulated NR2B phosphorylation in the hippocampus and ATL regions and decreased EEG frequency. These findings suggest that TAGs, FN1, and Src could be potential targets for drug discovery in MTLE-HS and FCD. Further studies on larger cohorts will help us establish TAGs, FN1 & Src as potential target molecules for drug discovery.

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