CHAPTER 29

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ARORA (Geetika)
Development and Clinical Validation of in Vitro Naat-Based for Diagnosis of Infectious Diseases.
Supervisor: Prof. Daman Saluja
Th 25809

Abstract

Substantial progress has occurred in recent years to bring down the morbidity and mortality associated with communicable diseases yet the South-East Asian region continues to carry the burden the major proportion of infectious that occur globally. Every year, almost 6% of Indian population encounters one or the other Sexually Transmitted Infections (STIs). Furthermore, 58000 lives of newborns are lost every year in India alone int the battle against drug-resistant infectious diseases. Perhaps, we were barely managing the burden of existing communicable diseases that the COVID-19 pandemic disrupted the control over them in a myriad of ways including hindering the routine vaccination programs.

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02. DINESH KUMAR
To Investigate the Role of miRNAs in the Pathogenesis of Coronary Artery Disease.
Supervisor: Prof. Daman Saluja
Th 25808

Abstract

Coronary artery disease (CAD) is a multifactorial disease which has remained a major threat and a global health burden while being a primary cause of mortality worldwide. CAD is characterised by the formation of atherosclerotic plaque, which get accumulated in the coronary arteries and thus termed as 'atherosclerosis'. It is a progressive disorder which generally initiate in childhood and starts manifesting its clinical characteristics in middle to old age where it tends to gain it's severe to worse stages. This demands for its timely biomarker-based early prediction and diagnosis which still remains a major unmet clinical challenge. Recently, there have been numerous evidences manifesting the clinical implications and association of small non-coding RNAs such as 'miRNAs' with CAD. MicroRNAs have been reported to be involved in various complications associated with pathophysiological processes of atherosclerosis. MicroRNAs evidently regulate several pathophysiological events of atherosclerosis by targeting various protein coding genes involved in maintaining vascular integrity as well as in causing endothelial dysfunction which results in vascular damage and plaque build-up. Studies have highlighted microRNAs to be able to secrete into the circulation and reflect

pathophysiological state of certain tissues. These are known to be cell or tissue specific as their profile correlate with disease phenotype and thus hold a potential to represent a novel class of biomarkers. With the increasing CAD incidences and lack of reliable and specific biomarker, we aimed to assess the potential association of circulatory miRNAs with CAD and its pathogenesis, in order to establish their potential as diagnostic biomarkers. Two of the candidate miRNAs, miR-133b and miR-21, were selected through an online algorithm based resource along with the literature mining approach. These two miRNAs were selected due to their implication in atherosclerosis, endothelial dysfunction as well as their robust expression in various tissues associated with coronary artery complications. Further in this attempt, we sought to predict and identify the potential target genes of candidate miRNAs through bioinformatic analysis in order to connect their expression profiles to the molecular mechanisms of CAD. Quantitative real time assays were performed in order to access the expression profile of candidate miRNAs. A total of 147 subjects were recruited which includes 78 subjects with angiographically proven CAD, 15 pre-atherosclerotic normal coronary artery (NCA) subjects and 54 healthy individuals. To better understand the functional role of both the candidate miRNAs, we sought to validate their predicted target genes through assessing their expression pattern at protein level in CAD patients as compared to controls. Expression pattern of predicted genes at protein level were also assessed in THP1 macrophage cell lines treated with respective miRNA inhibitors as compared to un-treated macrophages.

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03. GUPTA (Noopur)
Radiation Induces Alteration in Gut Microflora and its Modification by Hdac Inhibitor.
Supervisors: Prof. Manisha Tiwari and Dr. Paban. K. Agrawala
Th 25813

Abstract

Acute radiation syndrome (ARS) is a collection of pathological conditions as a output of exposure to highly sensitive ionizing radiation (IR). Gastrointestinal (GI) system is very prone to IR exposure and the symptoms include anorexia, nausea, vomiting and severe diarrhea and can result in multiple organ failure. If remain untreated, it may result into death within 2 weeks with prominent cause being infection, dehydration and electrolyte imbalance. GI tract is inhabited by number of commensal bacteria and damage to the GI system facilitates bacterial translocation to other organs due to loss in its epithelial integrity. Bacterial translocation results in conversion of commensals into opportunistic pathogens which release various types of lethal toxins culminating in multiple organ failure. Our study focused on elucidating consequences of radiation exposure to GI system, the microbiota inhibiting GI and critical analysis of data from different studies done so far to counter those consequences. The group radiation+drug (TAS and DAS) showed reduced susceptibility to radiation injury as well as microbiota related anomalies compared to the irradiated alone group. This was described by increased microflora in different parts of the GI tract in the radiation+drug group compared to the irradiated group and reduced histopathological and physiological damages in the jejunum. Drug administration (TSA and DAS) in radiation+drug group activated many repair pathways by activating different TLRs. Also, a reduced percentage of translocated bacteria were found in different organs of

radiation+drug group animals. HDACI TSA and DAS treatment post-irradiation could effectively control bacterial translocation as well as GI injury in mice by activating many repair pathways.

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1. Introduction 2. Review of literature 3. Hypothesis and objectives 4. Materials and methods 5. Results6. Discussion 7. Conclusion. References.

04. GUPTA (Surbhi)
CRISPR-Cas System's Functional Role in Regulation of Antibiotic Resistance and Virulence in Uropathogenic E. coli.
Supervisor: Prof. Manisha Yadav
Th 25806

Abstract

In many Bacteria and most Archaea, CRISPR act in a sequence-specific manner by providing acquired immunity against viruses and plasmids. Given CRISPR's involvement in inhibiting horizontal gene transfer, it's intriguing to consider how these loci can influence the development and emergence of pathogenic bacterial strains. In the present work, we have analysed the CRISPR content between Uropathogenic E. coli and commensal E. coli isolates collected from urine of suspected UTI patients and faecal matter of healthy individuals respectively. Comparative analysis revealed UPEC has more CRISPR array content than commensal E. coli. A total of 751 unique spacers were extracted from both Commensal and UPEC isolates. Though UPEC spacers has more viral, bacteriophage and plasmid hits, the uniqueness was more in Commensal spacers. In addition, mapping of these spacers to viral genomes, phage genomes and plasmids shed light on the biological relevance to the current understanding of CRISPR content. Interestingly, some important host- pathogen interaction proteins were identified which may aid in the development of novel antiviral and antibacterial therapies. Moreover, we identified plasmid hits from almost all the major classes of antibiotics ranging from carbapenems, fluroquinolones, tigecycline, colistin and others. Interestingly, self-targeting spacer hits were more in UPEC spacers implying the pathogenic potential of these isolates. In addition, to explore the therapeutic potential of CRISPR against UPEC, we developed a highly efficient novel CRISPR based gene editing strategy. CRISPR-cas was conjugated with carbon quantum dots to develop CRISPR-dots for targeting an important virulence factor of UPEC, Fimbriae Adhesion (papG gene). CRISPR-dots could effectively reduce the adhesive properties as well as pathogenic properties of UPEC. This study has not only gained insights about the CRISPR-cas system in UPEC pathogenesis, but also proposed a new category of antimicrobials that can specifically and effectively target pathogenic strain without affecting commensal strain

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1. Review of literature 2. Understanding the CRISPR repeat array of uropathogenic E.coli from UTI patients in comparison to commensal E.coli from healthy individuals 3. Understanding the association of CRISPR repeat array and antibiotic resistance of uropathogenic E. coli form UTI patients in comparison to commensal E.coli from healthy individuals. 4. To exploit CRIPR-cas gene editing strategy against virulence gene papG of uropathogenic E.coli 5. Summary and Conclusion. References.

05. JYOTI RANI
Computational approach for drug repurposing in tuberculosis and pathway modeling for type 2 diabetes associated tuberculosis.
Supervisor: Prof. Urmi Bajpai and Dr. S. Ramachandran
Th 25807

Abstract

With an alarming rate of emergence of bacterial resistance to available antibiotics and given the paucity of new antibiotics being introduced, we have entered into the postantibiotic era. The rapid rise in the emergence of drug resistant strains of Mycobacterium tuberculosis (Mtb) mandates the discovery of novel tuberculosis (TB) drugs. Further the slow and expensive drug discovery process aggravated the situation. Drug repurposing is a promising approach where known drugs are examined for a new indication. Investigating the non-antibiotics for antibacterial/antimicrobial activity and repurposing them for microbial. Mur enzymes, identified as essential proteins in Mtb, are considered potential drug targets. However, none of the clinical drugs have yet been developed against these enzymes. Initially, we have screened FDA-approved drugs from DrugBank and eLEA3D against Mtb MurB and MurE enzymes. Our study found Sulfadoxine (–7.3 kcal/mol) and Pyrimethamine (–7.8 kcal/mol) to show stable interaction with MurB while Lifitegrast (–10.5 kcal/mol) and Sildenafil (–9.1 kcal/mol) showed most reliable interaction with MurE. Furthermore, binding free energy (DGbind), RMSD and RMSF data and the number of hydrogen bonds corroborated the stability of interactions and hence these drugs for repurposing should be explored further. Our study not only provides sufficient evidence in support of these drugs, but we also believe that the combination of various approaches adopted could also serve as a helpful strategy to screen drug banks for any desired target protein. We have also screened an anti-tubercular library against these enzymes. For experimental validation, the top hits obtained on in silico screening were screened in vitro, using Mtb Mur enzyme-specific assays. In all, seven compounds were found to show greater than 50% inhibition, with the highest inhibition observed at 77%, and the IC50 for these compounds was found to be in the range of 28–50 μM. Compound 5175112 showed the lowest IC50 (28.69 ± 1.17 μM), and on the basis of (1) the binding affinity, (2) the stability of interaction noted on molecular dynamics simulation, and (3) an in vitro assay, MurE appeared to be its target enzyme. We believe that the overall 2 strategy followed in this study and the results obtained are a good starting point for developing Mur enzyme-specific Mtb inhibitors. This study describes an efficient strategy that employs a combination of structure-based screening followed by in vitro assay to test anti-tubercular compounds. We believe that this methodology could be used for HTS of larger compound libraries, while ensuring that computational results are corroborated by experimental analysis. In type 2 diabetes mellitus (T2DM) patients, chronic inflammation underlies susceptibility to tuberculosis and results in poor control in TB infection. Secondly, we aimed to use an integrative pathway based approach in order to investigate the perturbed pathways in T2DM patients rendering susceptibility to TB. First of all we assembled the genes implicated in the Type 2 diabetes associated co-morbidity tuberculosis (T2DMTB) from literature and obtained 36 T2DMTB genes and 7 microRNAs associated with these T2DMTB genes were obtained. 275 host TB susceptible genes were also obtained from the literature. Resulted genes and miRNAs were analyzed for their differential expression in T2DM patients and differentially expressed entities further searched for implicated pathways. Total 77 pathways were simulated using BioNSi. Perturbed genes were mapped to pathways and Necroptosis pathway exhibited maximum perturbations. The necroptic pathway reported for enabling dissemination of M. tuberculosis and including its role in chronic inflammation renders susceptibility to TB in T2DM patients

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06. MISHRA (Smita)
Isolation and characterization of the bioactive constituents of justicia adhatoda nee vasica for antimycobacterial activity on mycobacterium smegmatis and mycobacterium bovis (BCG).
Supervisor: Dr. Varsha Mehra
Th 25810

Abstract

TB has proved to be a great suffering. It has plagued human society since the Neolithic age. The disease is airborne and chronic, which usually infects the lungs and gets disseminated to the other organs if not treated properly. The causative agent is a small, aerobic, rod-shaped bacterium named Mycobacterium tuberculosis, which usually infects the human respiratory system and gets disseminated through air droplets.4 Overall, 2 billion people are affected by TB, and the disease causes 1.5 to 2 million people deaths every year.5 Hence, it will not be an overstatement that TB is the one of the most lethal infectious disease globally. The disease has surpassed HIV/AIDS patients, affecting less than 10% of the total TB patient of the worldwide population, and was declared as a global emergency by WHO in 1993.6 The disease is still beyond cure despite the number of drug regimens designed to cure the disease. There has been no preventive vaccine against the illness except BCG (Bacillus Calmette Guerin) since 1921, which has proved ineffective in most cases.7 With the surge of Drug-Resistant TB (Rifampicin resistant and Multi-Drug Resistant - MDR-TB) cases, the existent drug regimen is rendered useless as the bacilli rapidly gain resistance against them. Recently there has been a transformation into XDR-TB (Extremely Drug-Resistant) and TDR-TB (Totally Drug-Resistant). India accounts for an immense TB burden, having 2.69 million TB cases, approximately 27% of the total world TB cases. According to the World Health Organization (WHO), 1.8 billion, i.e., over a quarter of the world population, is infected with Mycobacterium tuberculosis. In 2019, 10 million people were affected, and 1.4 million died due to the disease. People with associated diseases like HIV, diabetes, malnutrition, and other co-infections have a higher risk of getting TB infection 8,9.

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07. SINGH (Indu)
Small Molecule- and Polymer- Based Bioactives: Design, Synthesis and Evaluation.
Supervisor: Prof. Gagan Dhawan
Th 26509

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08. VARMA (Diksha Awadhesh)
Targeting the c-MET receptor in cervical cancer using natural lead compound: an in sili approach.
Supervisor: Prof. Manisha Tiwari
Th 25804

Abstract

As cervical cancer is among the leading cause of death in female population after breast, lung and colorectal cancer. Apart from exhaustive screening and vaccination, finding its treatment is the only way to control the disease progression and reoccurrence. The natural compounds repositories provide the indispensable source of unique molecular scaffolds that serves as an effective chemotherapeutic solution against cervical cancer. In the present study, the cutting-edge computational tools of Discovery Studio 4.0 have been utilized to model the structure-based pharmacophore of c-MET C-terminus catalytic domain. The pharmacophore model built was used to virtually screen the Super Natural II database to retrieve the potential hit compound Brazilein that was further evaluated for its in vitro anti-cancer activity in HeLa and SiHa cells by targeting the C-terminus catalytic domain of c-MET and inhibiting its phosphorylation. The study strongly reinforces the previously available evidence on the anti-cancer potential of Brazilein. In addition to this Brazilein has been studied to induce apoptosis in cervical cancer cells in a concentration dependent manner at 72 hours of treatment without having an inhibitory effect on the cell cycle of the cervical cancer cells. All these effects are related to the strong inhibition of c-MET which is responsible for cell growth, proliferation and metastasis. The study also evaluates the anti-migratory and anticlonogenic potential of Brazilein in HeLa and SiHa cells. Thus, the work performed suggests Brazilein as a potential lead that can be developed as an effective chemotherapeutic drug molecule against cervical cancer. Further studies may be performed to determine the detailed cytotoxic mechanism, molecular events associated with Brazilein induced downregulation of c-Met phosphorylation and pharmacokinetic/pharmacodynamics properties of this molecule in vitro and in vivo. In addition to this, the anti-cancer effects of Crizotinib in cervical cancer cells has been studied. Crizotinib was found to cause programmed cell death in HeLa and SiHa cells through increased production of ROS and decrease in mitochondrial potential. Though Crizotinib was found to induce apoptotic death in cervical cancer cell lines in vitro, the clinical significance of these effects needs to be studied further. In addition, evaluation of the expression profiles and activity of potential targets of Crizotinib, namely c-MET, ALK and ROS-1 in cervical cancer in future investigations would describe the chemotherapeutic effects of the drug at molecular level.

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09. VANDANA
Investigations into the Role of SUMOulation during Mycobaterial Infection.
Supervisor: Prof. K. Natarajan
Th 26189

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10. VASHISHTHA (Vidhi)
GSK3 Isoform Selectively Regulates Deubiquitinating Enzyme Dependent Expression of RNA Binding Proteins.
Supervisor: Prof. Daman Saluja
Th 26508

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1. To study the function of GSK3β and its inhibitors in Glioma cell progression 2. To investigate the role of GSK3 isoform in Glioma cells 3. To explore the role of Deubiquitin enzymes and its interaction with RNA binding protein in Glioma 4. To access the role of hnRNPA1 variants in Glioma cell apoptosis. Summary and Appendices.