CHAPTER 34

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ENIYAN K

Development of a One-pot Assay for Screening Multi-target Inhibitors of Mur Enzymes of *Mycobacterium Tuberculosis*

Supervisor: Dr. Urmi Bajpai

Th 23711

Abstract
(Not Verified)

The cell wall of *Mycobacterium tuberculosis* (Mtb) consists of peptidoglycan, arabinogalactan and mycolic acids. The cytoplasmic steps in the peptidoglycan biosynthetic pathway, catalyzed by the Mur (A-F) enzymes, involve the synthesis of UDP-N-acetylmuramyl pentapeptide, a key precursor molecule required for the formation of the peptidoglycan monomeric building blocks. Mur enzymes are indispensable for bacterial cell integrity and the lack of their counterparts in eukaryotes suggests that they are promising Mtb drug targets. However, the caveat is that most of the current assays utilize a single Mur enzyme, thereby identifying inhibitors against only one of the enzymes. Here, we report development of a one-pot assay that reconstructs the entire Mtb Mur pathway in vitro and has the advantage of eliminating the requirement for nucleotide intermediates in the pathway as substrates. The MurA-MurF enzymes were purified and a onepot assay was developed through optimization of successive coupled enzyme assays using UDP-N-acetylglucosamine as the initial sugar substrate. The assay has been biochemically characterized and optimized for high-throughput screening of molecules that could disrupt multiple targets within the pathway. Furthermore, we have validated the assay by performing it to identify D-Cycloserine and furan-based benzene-derived compounds with known Mur ligase inhibition, as inhibitors of MurE and MurF. We also present the crystal structure of *Mycobacterium tuberculosis* MurB (MtbMurB) with FAD as the prosthetic group. The structure has been solved and refined at 2.0Å resolution. Comparison of MtbMurB structure with the structures of the *Escherichia coli* MurB (in complex with UDP-GlcNAc-enolpyruvate) and *Pseudomonas aeruginosa* MurB (in complex with NADPH) showed all three structures to share similar domain architecture and residues in the active site. The structure of the MtbMurB provides a useful template for the design of Mtb specific inhibitors that might be developed into promising drug candidates.

Contents

02. JOON (Deepali)

Development and Evaluation of Loop Mediated Isothermal Amplification Combined with Lateral Flow Dipstick for Rapid Diagnosis of Pulmonary and Extra Pulmonary Tuberculosis.

Supervisor: Prof. Daman Saluja
Th 23709

Abstract
(Verified)

Rapid detection of tuberculosis is essential to improve management of infected patients and avoid transmission. To address the need for diagnostic test for tuberculosis, the present study was carried out to develop rapid and sensitive loop mediated isothermal amplification (LAMP) based diagnostic method. The assay was evaluated for diagnosis of pulmonary and extrapulmonary tuberculosis (EPTB).In the present study, in-house *sdaA* LAMP assay was applied for analysis of clinical specimens for diagnosis of EPTB. The method was evaluated against culture and composite reference standard (CRS) comprising of culture and PCR results. The *sdaA* LAMP assay showed highest sensitivity and specificity in comparison with culture and CRS. Novel method for detection of amplification using lateral flow dipstick (LFD) was applied to the assay which led to sequence specific detection of LAMP amplified products in user friendly and rapid format. The LAMP-LFD assay was tested in culture confirmed specimens for pulmonary tuberculosis with positive results. The diagnostic accuracy of the method was also evaluated in comparison with GeneXpert MTB/RIF assay using 107 clinical specimens from suspected patients of pulmonary tuberculosis. Out of 107, 15 specimens were positive with both the methods showing high concordance. The increasing resistance to drugs used in antitubercular treatment is cause of concern to global tuberculosis control efforts. Therefore, LAMP assay was developed for amplification of 81 bp region of *rpoB* gene mutation in which imparts resistance to rifampicin. The assay was combined with LFD using specific probe. This assay was multiplexed with *sdaA* LAMP assay as proof-of-concept for simultaneous amplification of both targets leading to diagnosis of tuberculosis as well as screening for drug resistance. In conclusion, *sdaA* LAMP-LFD assay is a potential diagnostic test for diagnosis of PTB and EPTB, owing to its speed, simplicity, sensitivity and specificity, especially in resource limited settings.

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03. KAK (Gunjan)
Deciphering the Role of Interferon-gamma (IFN-y) during *Mycobacterium Tuberculosis* Infection.
Supervisor: Dr. K. Natarajan
Th 23707

Abstract
(Verified)

In this study, we decipher the role of Interferon-gamma (IFN-γ) during *Mycobacterium tuberculosis* (*M. tb*) infection. We show that depending upon its site of action in the infected macrophage, IFN-γ plays a protective or suppressive role. On the surface of the infected macrophages, IFN-γ induces protective responses, while inside the macrophage it induces suppressive responses. Strategically, *M. tb* down-modulates Interferon-gamma receptor (IFN- γR) within 24h of infection and concomitantly induces the expression of IFN-γ inside macrophages. Interestingly, the down modulation of IFN-γR and the expression of IFN-γ are regulated similarly by TLRs, second messengers, calcium, Protein Kinase C and the MAPK pathways. Importantly, the intracellular IFN-γ inhibits apoptosis in order to establish persistent infection, inhibits phagosome-lysosome fusion and host defense responses such

as ROS production and autophagy. In contrast, IFN-γ on the cell surface induces mirror responses by promoting apoptosis and autophagy, induces Reactive Oxygen Species (ROS) burst and phagosome-lysosome fusion. Knockdown of intracellular IFN-γ significantly resolves the infection inside macrophages. Thus, by modulating the site of action of IFN-γ, *M. tb* has evolved a powerful and strategic immune evasive mechanism.

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04. NAMRATA KUMARI
Study the Molecular Mechanism of Neuroprotection Mediated through Adenosine A_{2A} Receptors in Therapy of parkinson's Disease.
Supervisor: Dr. Pratibha Mehta Luthra
Th 23751

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05. PANDEY (Renu)
Elucidating the Role of SIN-3 Mediated ROS in Regulation of Autophagy, Longevity and Healthspan in Caenorhabditis Elegans.
Supervisor: Prof. Daman Saluja
Th 23752

Abstract
(Verified)

Aging is the manifestation of dysfunction in the synchronised and coordinated regulation of genes critical for fitness and longevity of an organism. Any change in the environmental steady state, or irregularity in homeostasis at the cellular and genetic levels have direct implications on fecundity and determine the lifespan of an organism. Reactive oxygen species (ROS) long reflected to be mere toxic spin-offs of oxidative metabolism have now been established to be essential mediators in tightly controlled signalling cascades. ROS not only are signalling molecules but have been shown to play an important role in regulation of various molecular processes. Sin3 is a transcriptional regulator which serves as a scaffold for various chromatin modifying enzymes comprising a multiprotein complex. Sin3 has been implicated in metabolic dysregulation and mitochrondrial dysfunction. Using *Canerhabditis elegans* as a model system, our work provides evidence that elevated ROS and enhanced rate of aging in *C. elegans* is attributable to SIN-3. Elevated ROS though augmented autophagic flux but it fails to restore the normal lifespan. Supplementation with antioxidant, vitamin C not only restored the lifespan in *sin-3* mutant worms but also brought various metabolic enzymes and metabolites near basal levels. We provide evidence for same at biochemical, molecular and physiological levels. Complementing the high intracellular stress in the background of *sin-3* deletion, the worms also demonstrate an age associated decline in stress tolerance, protein homeostasis, respiration, mitochondrial function and muscle integrity. *sin-3* deletion further leads to accumulation of lipids and enhanced age associated death pigments like lipofuschin. *sin-3* is also critical for maintenance of DNA integrity as loss of *sin-3* coupled with chronic ROS and dysfunctional homeostatic cellular machinery of

the organism predisposes it to further DNA damage. Our study provides critical insight into the *sin*-3 mediated regulation of aging in *C. elegans.*

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06. PANDEY (Sanjay)
Effects of Dietary 2- Deoxy-D-Glucose (2-DG) on Inflammatory and Immune Responses in Chronic Inflammation and Induced Tumors In Mice.
Supervisors: Prof. K. Natarajan and Dr. B.S. Dwarakanath
Th 23705

Abstract
(Not Verified)

Inflammation is body's immediate response to damage to its tissues and cells by pathogens, noxious stimuli such as chemicals, or physical injury. Many cancers arise from sites of infection, chronic irritation and inflammation. Present study is an effort to study the effect of dietary 2-DG (an energy restriction mimetic agent) on inflammatory status and the immune events in various induced tumor and chronic inflammation mice models. Dietary administration of 2-DG (in drinking water) reduced the inflammation induced tumorigenesis, which correlated with the reduced inflammation in the 2-DG fed mice. 2-DG showed a marked decrease in the papilloma frequency and incidences which was found correlating with reduced systemic levels of pro-inflammatory cytokines. Dietary administration of 2-DG also reduced the leukemia incidences from 23.8% to 4.16%. 2-DG increased the 300 days survival in radiation treated mice from 47.6% to 84%. 2-DG also reduced the proliferation of leukemia virus transformed myeloid macrophage (RAW264.7). Dietary 2-DG (0.4%) reduced induced LPS induced (endotoxemia associated) death significantly. Administration of dietary 2-DG decreased the endoteximia induced deaths in mice up to 40% and prolonged the survival. 2-DG also reduced the oxidative stress by strengthening the anti-oxidant defense in lung. Administration of 2-DG decreased the systemic TNF and IL-6 levels in serum and broncheo-alveolar lavage fluid (BALF). 2-DG also downregulated the in-vivo stimulation of splenic macrophages, and reduced infiltration of neutrophils (polymorph-nuclear cells). Furthermore, 2-DG fed mice showed a significantly reduced incidences and severity in arthritis when immunized with the rat collagen. Mice which were fed on the HFD with administration of 2-DG showed reduced levels of proinflammatory adipokine Leptin. 2-DG also reduced the HFD induced mean body weight and the adiposity markers in serum. 2-DG also prevented the growth of 3-MCA induced sarcoma and B16 allograft tumors.

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07. SHARMA (Deepika)
Role of Calcium Homeostasis during Mycobacterial Infection with Emphasis on L-type Voltage Gated Calcium Channel.
Supervisor: Prof. K. Natarajan
Th 23710

Abstract
(Verified)

Mycobacterium tuberculosis (*M.tb*) is known to modulate the host machinery for its own survival. With the emergence of Multi Drug Resistance and Extreme Drug Resistance (XDR) TB, it is becoming imperative to understand the host pathogen interactions to come up with better therapeutic interventions. In the recent times, the regulation of calcium homeostasis by Voltage Gated Calcium Channel (VGCC) upon *M. tb* infection has recently assumed importance. Taking a cue from these results, we investigated the impact of VGCC on the protective responses of macrophages during mycobacterial infection. To that end, we monitored the effect of VGCC activation (via BAYK8644, VGCC agonist) on various processes like ROS generation, apoptosis, autophagy, phagosome-lysosome fusion and cytokine production by the host macrophages. Costimulation of macrophages with both *M. bovis* BCG and BAYK8644 synergistically attenuated ROS production which was dependent on TLR pathways; calcium influx from external medium, PKC and internal calcium sensing machinery of the host macrophages. A similar analogy was observed for calcium flux inside the macrophages upon channel activation and infection. Cell survival was further monitored by MTT assay and JC1 staining. These results further reiterated a synergistic role of Ltype VGCC activation and *M. bovis* BCG infection in decreasing apoptosis, thereby creating conditions conducive for prolonged survival inside macrophages. Costimulation of macrophages with *M bovis* BCG or costimulation of BMDMs with *M. tb* H37Rv infection along with BAYK8644 further reduced phagosome-lysosome fusion. Collectively these results point to a unique strategy adopted by *M. tb* to subvert the host defence mechanisms. This begins with the expression of proteins/antigens that enhance the cell surface expression of VGCC. The enhanced expression and subsequent activation of VGCC shifts the otherwise protective responses into immune suppressor responses leading to the establishment of persistent infection.

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08. SHARMA (Gurumayum Surajkumar)
Structural Consequences and Mechanism of functional Loss of Proteins Due to Modification by Homocysteine.
Supervisor: Dr. Laishram Rajendra Kumar Singh
Th 23712

Abstract
(Verified)

Elevated levels of homocysteine (Hcy) are known to be associated with several neurodegerative and cardiovascular complications. One likely mechanism of Hcy toxicity is the modification of proteins lysine residues by its metabolite, homocysteine thiolactione (HTL). Furthermore Hcy is also known to posses the ability to modify cysteine residues in proteins. However, the structural consequences and the mechanism of functional loss of such modifiactions are not yet clearly understood. Our studies with HTL lead to the conclusion that acidic proteins are easily modifiable suggesting that cell or tissues rich in acidic proteins could be major target for HTL, whereas basic proteins tend to be resistant towards structural alterations upon HTL-binding. Furthermore, studies using Cyt c revealed that HTL induced certain alterations in Cyt c with disrupted tertiary interactions and reduced heme center, which led to the induction of peroxidise function, thus inducing an apoptotically competent species. The induction of peroxidise function was due to the disruption of heme-Met80 ligation. The findings provide an insight into the mechanism for cyt c release and cell death under homocystinuric conditions. Finally, we report our finding with Hcy mainly targeting heme-proteins, whereas non-heme proteins did not show any alterations in structure or function upon incubation with Hcy. Our findings provide new

avenue towards Hcy-induced toxicity targeting heme proteins and possible linkage ofheme biosynthesis and homocystinuria.

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1. Review of literature 2. HTL-induces protein structural and functional alterations 3. Conformational status of cytochrome c upon N-homocysteinylation 4. Hey-induced protein structural and functional alterations. Summary, references and publications

09. TARUN KUMAR
Protein N-homocysteinylation: Stuctural Consequences and Preventive Strategies
Supervisor: Dr. Laishram Rajendra Kumar Singh
Th 23708

Abstract
(Verified)

Homocystinuria is a disorder of methionine metabolism caused by cystathionine β-synthase (CBS) deficiency, leading to elevated levels of homocysteine (Hcy) in serum and urine. Hyperhomocysteinemia is simply the elevation of homocysteine levels in blood. This condition may also exist without homocystinuria. Manjor symptoms of homocystinuria include arteriosclerosis, osteoporosis, mental retardation, thrombosis, dislocated eye lenses and neurodegenerative pathologies such as dementia, Parkinson's and Alzheimer's diseases. Major cause of Hcy toxicity is due to modification of proteins by its metabolite homocysteine thiolactone (HTL). HTL forms amide bonds with ε-amino group of lysine residues of protein in a nonenzymatic mechanism; a process referred to as ''protein N-homocysteinylation'' leading to loss of protein function and aggregation. Therefore, detoxification of HTL is important for the purpose of maintaining biological integrity. In the present thesis, we studied the effect of HTL induced protein covalent modification on the native state structure of proteins and we found that covalent modification of cyt-c and α-LA by HTL induced the formation of molten globule state while no such changes were observed in case of lysozyme. MG state formation was also found to be responsible for the formation of protein aggregates and hence for the toxicity of HTL modified proteins. The overall findings have been discussed in chapter 2 of the present thesis. In the third chapter of the thesis, we studied role of proline in prevention of HTL induced toxicity on proteins and cellular systems. Proline was found to inhibit protein N-homocysteinylation by the hydrolysis of HTL into less reactive Hcy thereby conferring protective effect of proline on HTL induced toxic effect on proteins and cytotoxicity in cells. The study raises the possibility for the use of proline and proline based analogues for the therapeutic intervention of homocystinuria or hyperhomocysteinemia associated pathological conditions.

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1. Review of literature 2. Formation of molten globule state upon N-homocysteinylation 3. Prevention of protein N-homocysteinylation by proline. Summary, references and publications

10. YADAV (Nalini)
Synthesis, Characterization and biological Evaluation of New 1,3, 4-oxadiazole Thione Derivatives and Sitosteryl Esters are Potential Anticancer Agents.
Supervisor: Dr. Madhu Chopra
Th 23706

Abstract
(Verified)

Targeted anticancer therapies are being developed to overcome the side effects of traditional chemotherapy. Natural products such as vinca alkaloids, paclitaxel etc. have been used as anticancer agents. Synthetic anticancer drugs are also in clinical use such as cisplatin, methotrexate etc. However, there is need for new chemotherapeutic agents due to serious side effects of the present drug entities. Aims and objectives of the present study included: Docking, synthesis and biological evaluation of CDK 1/cycB1 receptor complex inhibitors (1, 3, 4-oxadiazoles thiones) and Docking, synthesis and biological evaluation of Selective Estrogen Receptor Modulators (Sitosteryl esters). The molecular docking studies tools were employed for preliminary screening of a set of 15 oxadiazole thione analogues with the help of Discovery Studio. The poses obtained were ranked according to scores and c-docker energies. The compounds were synthesized and characterized with spectroscopy. Synthesized derivatives were studied for their cytotoxicity and Structural Activity Relationship on 4 cancerous cell lines. Compounds bearing nitro and fluoro at the para position of benzene ring, displayed highest activity against HeLa and further experiments with these compounds showed apoptosis via G2-M arrest in Hela cells. Next, sitosterol and their esters were checked for their affinity for Estrogen Receptor (ER) by docking studies. Sitosteryl butanoate showed selectivity in binding to ER β active form, dimerisation of which leads to anti-proliferation of cells, thus suggesting that it could function as a weak ER β agonist. Out of all the synthesized analogues tested sitosteryl butanoate was showing an affinity for ER β with an EC value of 8.932 x 10 M which was five fold lower than estradiol (natural ligand). Results concluded that the esters were successfully synthesized. Butanoate, pentanoate and decanoate showed an IC value of 60-70 μM on U-87 while IC of these compounds was above 250 μM on HEK

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