CHAPTER 60

ZOOLOGY

Doctoral Theses

01. ARAFAT HUSSAIN
Developing an Infection model for Mycobacterium Smegmatis in fish and Understanding its Pathogenicity.
Supervisor:Prof. Shibnath Mazumdar
Th 24800

Abstract
(Not Verified)

Infections. Hitherto, majority of research on M. smegmatis has been done on mammalian systems. In this study, we developed an alternate in vitro infection model to study M. smegmatis pathogenesis using headkidney macrophages (HKM) from Clarias gariepinus (Catfish). The interaction between M. smegmatis and HKM was studied at the cellular level that can be extended to understand mechanisms underlying the pathogenesis of other mycobacterial species. Live M. smegmatis induce HKM apoptosis in a time, MOI and growth-phase dependent manner. M. smegmatis induced TLR-2 mediated (Ca2+)c surge along with the cross-talk between ER-stress and O2- potentiates HKM pathology by amplifying pro-inflammatory TNF-α and IL-1β production. The pro-oxidant environment triggers NO release which prolonged ER-stress and TNF-α production, culminating in HKM apoptosis and bacterial clearance. Using Danio rerio (zebrafish) we noted that immunization with live M. smegmatis and subsequent challenge with M. fortuitum activates innate immune response genes and generates robust T-cell response conferring protection against piscine mycobacteriosis. Thus, we surmise that our HKM model to study the pathogenesis of M. smegmatis not only provides a comprehensive view of mycobacteria-macrophage interaction but also mimics several aspects of mammalian macrophage responses to mycobacteria, implying it to be a convenient model to study host-mycobacterial interactions. Also, our results with zebrafish immunization-challenge study suggest M. smegmatis to be a potential vaccine candidate against piscine mycobacteriosis.

Contents

02. GAUR (Mohita)
Assessment of different Risk Factors of Tuberculosis and its Diagnostic Chanlllenges.
Supervisor:Prof. Yogendra Singh
Th 24799

Abstract
(Not Verified)

Tuberculosis (TB) is a persistent problem and the leading cause of death in the developing world. As of now, the best way available to treat this deadly disease is prompt identification and early treatment of active TB cases. The pulmonary TB (PTB) is mainly diagnosed by microbiological, radiological, or molecular assays on the patient's sputum sample. Moreover, the diagnosis of extra-pulmonary TB (EPTB), the rapidly-emerging manifestation of TB, is substantially more challenging because of unapparent and nonspecific symptoms. This study applied a commercial nucleic acid amplification test, the GeneXpert® system (Xpert), and also evaluated the diagnostic utility of in-house IS6110 PCR based detection to a non-invasive sample viz. stool obtained from TB patients. The diagnostic yield of the stool Xpert and stool PCR was assessed against the confirmed PTB patients, few EPTB, and also among healthy individuals. The overall sensitivity of 88.71% and 73.3% for stool-PCR and Xpert respectively shows that stool as a potential sample in TB diagnosis providing early identification in microbiologically unconfirmed cases reducing the possibility of inadvertent misdiagnosis. Also, we have performed a retrospective study that compares patients with EPTB and PTB at three tertiary centres in Delhi region by analyzing their demographic data and clinical factors underlying the disease. Clinical data of 22,898 patients were analysed to understand the prevalence of different forms of TB and various risk factors present in the population under study. Comparative analysis of such clinical data of TB patients from India and the world would help expand our demographic knowledge regarding the disease. This will go a long way in studying better management strategies in tackling TB in endemic regions.

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03. IMRAN MOIN
Metabolic Pathways Altered by Etoposide Loaded Gelatin Nanoparticles in breast Cancer (Assessing ROS induced apoptosis or necroptosis and in-vivo tumour regression).
Supervisor: Dr. Anita Kamra Verma
Th 24795

Abstract
(Not Verified)

Despite awareness, early diagnosis, advanced therapeutics, breast cancer cure is yet elusive, being the leading cause of death worldwide. Etoposide(Etop) is used against diverse tumors but its clinical use is limited by lipophilicity and adverse effects. Hence, novel strategies and therapeutic molecules against breast cancer urgently need to be investigated. Gelatin nanoparticles(GNPs) were synthesized by two-step desolvation. The size distribution, PDI and zeta potential of GNPs were 150±12nm, 0.257±0.14, and -31.4±4.6 mV, Entrapment-Efficiency~70%. Biotoxicity of Etop, Etop loaded Gelatin Nanoparticles(EGNP) on breast cancer(MCF-7) and human embryonic kidney cells(HEK) was elucidated by MTT Assay. The IC50 of EGNPs and Etop was 74.14µg/mL and 142.67µg/mL post 24hrs in MCF-7 cells. Maximum DNA-fragmentation was induced by EGNP in MCF-7, as compared to HEK. Reduced GSH, GPx, GR, SOD levels and increased GST, LDH and NO levels were observed in MCF-7 treated by EGNP, whereas HEK were unaffected. EGNP enhanced intracellular ROS generation as seen by DCFDA in

MCF-7 leading to increased ER-stress with significant loss of Mitochondrial Membrane Potential. FACS analysis indicated 6% & 25% cells in Apoptosis, 23% & 5% Necrosis, 66% & 2% necroptosis, post-treatment with EGNP and Etop. Cell death mechanism induced by Etop per se and EGNP was assessed with inhibitors Z-VAD and Nec on MCF-7 cells through RT-PCR. EGNPs augments the activity of Etop by inducing high ER-stress, Mitochondrial impairment causing bioenergetic catastrophe, ROS generation and calcium homeostasis dysregulation preferentially in cancer cells culminating in necroptosis, thereby modulating the cell-death mechanisms from apoptosis to necroptosis. Biodistribution showed no retention in tissues. Tumour was efficiently regressed in Balb/c mice model by EGNP. The susceptibility of cancer cells to metabolic insults offers a selective therapeutic window to be exploited to abrogate the cell-survival mechanisms by EGNPs, Physiological levels of ROS in normal cells rendered them ineffective to oxidative insults.

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04. POOJA VIJAY

Protein Profiling, Gene Expression and In-Silico Analysis of Multiple Forms of Vitellognin and Choriogenin in the Murrel, Channa Punctatus (Bloch).

Supervisor: Prof. Neeta Sehgal

Th24909

Abstract

(Not Verified)

Estradiol-17 β is released into circulation under the influence of environmental factors that regulates the transcription of vg and chg genes in hepatocytes of male murrel. Partial transcripts of vga, vgb, chgH, chgL genes and full-length transcript of vgc gene have been sequenced from liver and their derived protein sequences have been characterized. Three-dimensional structure of VgC shows presence of helix, sheets and loops. Both chg as well as vgc genes are expressed first, with low levels of E2. Whereas mRNA levels of vgb and vga genes are increased when titre of E2 is significantly high in the plasma. All the genes fail to express in the absence of E2. Time-dependent study shows that chgH, chgL and vgc express earlier than vgb followed by vga. Both vg and chg genes are translated into proteins in hepatic tissue,respective proteins are released into the circulation which finally get deposited into the oocytes. The yolk protein, lipovitellin, has been isolated from the egg-yolk extract of murrel shows sequence similarity with precursor proteins, VgA and VgB. Threonine and alanine are present in maximum concentration among fifteen free amino acids detected in the yolk-extract of murrel. Structural analysis of egg-envelopes elucidates the presence of two layers. Outer surface has number of pores and single micropyle whereas inner layer has proteinaceous deposition. The egg-envelope proteins resemble with Choriogenin and Transmembrane proteins of other fishes. In addition to three forms of Vg proteins, Chg is also present in plasma of murrel. Phosphorylated proteins (VgA and VgB) and non-phosphorylated proteins (VgC and Chg) are eluted as two discrete peaks (Peak 'I' and Peak 'II' respectively) on Ultrogel AcA 34 column. Corresponding to these proteins, respective genes have

been sequenced. Expression pattern of these genes is chgH, chgL, vgc, vgb, vga sequentially during annual ovarian cycle.

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05. PRABHAT (Abhilash)
Effects of Light and Food Environment on Behaviour and Reproduciton in Zebra Finches Taeniopygia Guttata (Vieillot 1817).
Supervisor:Prof. Vinod Kumar
Th 24797

Abstract
(Verified)

The adaptive changes are faithfully reflected in the behaviour and physiology. In last some years, a great deal of attention has been directed to understand how disruption in the daily light environment affects the behaviour and physiology. Less is understood of changes in behaviour and physiology in diurnal species in response to disruptions in the light and food environment. A key question is how brain and other organs (e.g. liver, gonad) respond to altered light and food conditions and, in turn, affect the downstream processes that control the reproduction and associated behaviour. The present research aimed to investigate this using zebra finches (Taeniopygia guttata), which are as an ideal experimental system for the following reasons. They are non-seasonally breeding and non-photoperiodic species in the sense that gonadal maturation and birth of young ones are not rigidly tied to photoperiod changes. This is significant since the measured effect of changes in the food condition will not be confounded with those of the photoperiod, and possibly the temperature. We demonstrated that loss of night affected the overall daily behaviour, decreased the overall activity output in both males and females, negatively affected the males' song (an important sexual female choice trait) and gene expression patterns. Interestingly, hypothalamic gene expression rhythms were abolished under LL in both sexes, suggesting dissociation between the behavioural and mRNA expression pattern. There appeared to be a homeostatic adaptation to restricted feeding, perhaps suggesting re-wiring of the overall machinery. There was also an overall negative effect of the nutritional quality on the reproduction. A high protein diet maintained general health, but reduced the overall reproductive performance, shown by sex steroid levels, egg quality and failure to hatch. Present results significantly add to the understanding how food availability and nutrition alters the metabolism and affect reproductive success in diurnal vertebrates.

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06. SAKSENA (Komal)
Role of Ascorbic Acid in Lymphoma for Epiqenetic Regulation and Immunomodulation.
Supervisors : Prof. Anju Shrivastava
Th 24793

Cancer is better managed with treatments having multiple targets and ascorbic acid (AA) is supposedly one such treatment. It is an antioxidant, free radical scavenger, and an essential cofactor in numerous enzymatic reactions. Over the years, epidemiological studies have emphasized the correlation between high dietary intakes and high plasma levels of AA with decreased incidence of cancer and improved patient's survival. But studies on its antitumor property reflect inconsistency which is attributed to its pharmacokinetic, route of administration, dose and cancer type. Based on above background and the fact that serum levels of ascorbic acid drops in cancer patients, we aimed to evaluate the anticancer effect of ascorbic acid on lymphoma and in parallel tried to develop an electrochemical probe for its detection in the biological fluids. Dalton's lymphoma-bearing mice were used for studying the anti-tumor activity of AA. Tumor growth, blood parameters, anatomical & histological changes and survival was evaluated. For mechanism of action HDACs expression & activity and tumor-immunoregulation were assessed with AA treatment. For detection of AA in biological samples, we developed a chiral electrochemical sensor via an electro-generated molecularly imprinted polymer-based ultrathin film using L-ascorbic acid as a template. The results from the present study for the first time revealed that ascorbic acid when infused directly into tumor may be very efficacious against lymphoma. It provides novel insight regarding its mode of action that is by altering HDAC expression and not by occupying the catalytic sites of HDACs like SAHA. We convincingly showed that ascorbic acid can also influence tumor-immunoregulation by modulating the functions of tumor-associated macrophages. And most importantly we developed a chiral electrochemical sensor for detection of AA with detection limit of 1μM, which offers excellent prospects in biomedical fields for simple, fast and cost effective quantification of ascorbic acid in serum samples.

Contents

07. SARKAR (Rajesh Kumar)
Role of Meisi and CTCF in Regulation of sertoli Cellmediated Spermatogenesis using functional Genomics.
Supervisors:Prof. Umesh Rai
Th 24796

Abstract
(Verified)

Spermatogenesis is a complex process that requires coordination between the developing Germ cells (Gc) and somatic cells of the testis. Germ cell division and differentiation is

dependent on adequate hormonal (FSH and testosterone) supply and the entire process involves a complex neuro-endocrine regulation. Sertoli cells (Sc) are the somatic cells of the seminiferous epithelium which provide architectural support, functions as an immunological barrier, nourishes the developing Gc and provides a niche that is conducive for spermatogenesis. During infancy, Sc are highly proliferative, immature and they do not respond to FSH and T and hence cannot support spermatogenesis. Sc maturation occurs at the onset of puberty, when these cells start responding to FSH and T and are stimulated to secrete factors which are essential for spermatogenic progression. Infant and pubertal Sc differ in their gene expression patterns. Analysis of microarray data (generated previously in our lab) of infant (5 day) and pubertal (12 day) rat Sc revealed the differential expression of a large cohort of genes in the two age groups. 663 genes were up-regulated and 735 genes were down-regulated in pubertal Sc as compared to infant Sc. In order to understand the regulation of the differentially expressed genes, the promoters of the up- and down-genes were analyzed using TRANSFAC to determine the potential transcription factor binding sites on the genes. Analysis of TRANSFAC data revealed enrichment of binding site for twenty transcription factors on the promoters of genes up regulated in pubertal Sc. We selected two transcription factors- MEIS1 and CTCF from this list to decipher their roles in Sc maturation and spermatogenesis. We found that Meis1 knockdown specifically in Sc affect Gc homeostatic development and leads to sub-fertility. In order to study Meis1 functions in the spermatogenesis we have done fertility assessment by sperm count.

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08. SHARMA (Aakansha)
Neural and Metabolic Plasticity Underlying Seasonal Life History States in Latitudinal Songbird Migrants.
Supervisor:Prof. Vinod Kumar
Th 24798

Abstract
(Verified)

survival chances during the year. Migrants also show an amazing feat of endurance for long flights, navigation and night-sleep deprivation (diurnal songbirds migrate at night). These naturalistic observations have attracted scientists over many decades. The present research investigated mechanistic bases of the transition between seasonal states using two long-distance songbirds migrants, the blackheaded bunting (Emberiza melanocephala) and redheaded bunting (E. bruniceps), overwintering in India. We focused on changes at the transcriptional levels with photoperiod-induced alteration in the seasonal migratory and reproductive states in captive buntings. In particular, the hypothalamus and liver transcriptomes revealed transcriptional changes in the regulatory and functional pathways between non-migratory to migratory states. Differences in mRNA expression of genes further provided evidence for the metabolic plasticity that migrants undertake to accomplish two similar annual journeys in the spring and autumn. Higher expression of genes associated with fat mobilization and energy generation in parallel with increased body fattening in spring, compared to the autumn migration, suggested differential activation of the metabolic pathways or alteration in the efficiency of existing functional machinery during different seasonal life history states. The effect of testes removal on development of spring and autumn migratory states further suggested a functional linkage between the seasonal migration and reproductive state. Overall, present results provide molecular insights into mechanism(s) underlying the seasonal homeostasis during the year, which is exhibited by almost all long-lived species. In a broader sense, these laboratory findings can be useful to make predictions for the seasonal ecology of animals.

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1. General introduction 2. Section I: Transcriptome wide changes in central and Peripheral tissues 3. Study1: Early response to photostimulation in blood and hypothalamus 4. Study2: Difference in hypothalamus and liver between seasonal life history states 5. Study3: Difference in testes between seasonal life history states 6. Section II: Molecular correlates of the photoperiod induced migratory states 7. Study1: Photoperiodic induction of the spring migratory state 8. Study2: Differences between photoperiod induced spring and autumm migratory states 9. Study3: Role of testes in photo-induction of the spring and autumn migratory state, Summary, References, Publications and Presentations.

09. SINGH (Priya)
Assessment of Biophysiological Impairments in Psychiatric disorders using Nuclear Magnetic Resonance and Advanced Magnetic Resonance Imaging Techniques.
Supervisor:Prof.Rina Chakrabarti
Th 24794

Abstract
(Not Verified)

Major Depressive Disorder (MDD) and Post Traumatic Stress Disorder (PTSD) have become the prime debilitating psychiatric disorders, affecting a large percentage of the population across the globe, irrespective of an individual's age, gender, socio-economic background and accounting for huge economic and social losses. The present study focuses on investigation of metabolic and structural alterations associated with Major Depressive Disorder (MDD) and Post Traumatic Stress Disorder (PTSD) in animal model as well as humans. A multiparametric MagnteicResonance (MR) approach was undertaken for the examination of biophysiological underpinnings in these two psychiatric disorders. In the present study, the development of animal model (rats) for PTSD was conducted using the underwater trauma regime. For this purpose, 1H NMR spectroscopy approach was undertaken for the profiling of urine samples obtained from and classification ofmetabolic changes brought in body due to the PTSD trauma in rats. In the final experiment, the cellularity changes in young adult onset (GYOA) and elder adult onset (GEOA) MDD patients were assessed using the Diffusion Kurtosis Imaging (DKI) and Diffusion Tensor Imaging (DTI). Findings from this study illustrate the differential pattern of manifestation of MDD in both the younger onset and elder onset adults.

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1. Introduction 2. Review of literature 3. Priciples of nuclear magnetic resonance 4. Development of animal model 5. Nuclear magnetic resonance spectroscopy based urinary metabolic profiling in post traumatic stress disorder rats. 6.Microstructural manifestation of younger and elder onset adult major depressive disorder patients: diffusion kurtosis imaging and diffusion tensor imaging analysis, Summary and Conclusion, References.

10. SINGH (Sujata)
Modecular Analysis of Defense Response during Spodoptera Litura-Maize Interaction.
Supervisor:Dr. Indrakant K. Singh
Th 24792

Contents

1. Introduction. 2. Review of literature 3. Feeding, growth, development and gene expression profiling of midgut digestive enzymes of spodoptera litura in castor (control Plant) and zea mays (test plant) 4. Global transcript profiling of defense response in zea mays upon S. Litura feeding 5. In silico analysis – role of S. litura midgut-derived chemosensory proteins in perceiving toxic metabolic from food resources, Conclusion and future perspectives, List of publications.

11. SINGHVI (Nirjara)
Comparative Proteomic Analysis of Amycolatopsis Mediterranei (Insights into the mechanism of production of rifamycin B and its analog, 24-desmethylrifamycin B.
Supervisor: Prof. Yogendra Singh and Prof. Rup Lal
Th 24907

Abstract
(Not Verified)

Combining the information availed from the genome sequences and by generating proteomics data. In addition, a mutant strain of S699 (SCO2-2) where rifamycin B gene cluster was inactivated, used as a control to reveal the genes that are up and down-regulated during rifamycin production. The proteomics analysis and in-silico protein-protein interaction approach revealed the relative abundance profile, relationship between structural and regulatory proteins, and major regulatory hubs that are involved in regulating rifamycin B production in wild type strain. Among other proteins, RifA, an important structural protein was found to be the major hub regulating the entire rifamycin biosynthetic cluster network. Relative expression values revealed that genes of the rifamycin biosynthetic cluster encoding RifC-RifI and transporter protein, RifP were expressed well in S699 and down-regulated in DCO#34. The transcription factors involved in this process were also identified and their role was investigated. This study also elucidated the mechanisms leading to reduced production of analog, 24-desmethylrifamycin B, by DCO#34 due to activation of negative feedback mechanism (it was noted that DCO#34 produced only 20 mgL-1 of analog when compared to the S699 producing 50 mgL-1 of rifamycin B). The investigations carried out will form the basis for knocking-out or silencing rifQ and overexpressing rifP to upscale the production of 24-desmethylrifamycin B, semisynthetic derivatives of which were found to be more effective against rifampicin-resistant strains of M. tuberculosis.

Contents

1. Introduction. 2. Review of literature 3. Material and methods 4. Results 5. Discussion, Summary, References, Appendices, List of publications.

12. TYAGI (Aakriti)
Bio-Evaluation of Metabolic Activities by Altering the Core in Quantum Dots (QDs) Against Hepatocellular Carcinoma (In vitro mechanistic studies and in vivo tumor inhibition.
Supervisor: Dr. Anita Kamra Verma
Th 24908

Abstract
(Not Verified)

GST, GSH, GPT, NO levels, induced ER-stress, disturbed intracellular calcium homeostasis, reduced Mitochondrial Membrane Potential indicated apoptotic signals. Further enhanced levels of pro-apoptotic protein Bax, reduced levels of Bcl-2, increased Cytochrome-C, caspase-9 and caspase-3 expressions were also assessed. Based on comet assay, DPA and DNA fragmentation assay, it was confirmed that these QDs triggered apoptosis in Hep3Bcells. Serum stability tests and long blood-circulation time revealed the stable nature of ZnSe/CdSe-QDs. Biodistribution studies of radiolabelled-QDs exhibited maximum accumulation in Liver after 1hour, that was reduced by half within 4hours and complete clearance within 24hours post i.v. injections. Molecular imaging supported the biodistribution data. Oxidative stress was evaluated post 21 days of injection in tumor bearing mice by various biochemical assays. Enhanced GST levels indicated reduced oxidative stress in tissues. Decreased GSH GPx and GR levels in tissue homogenates indicated detoxification capacity of cells. Decreased NO levels clearly depicted no oxidative stress in body tissues. Enhanced SOD levels supported negligible stress Ultimately, in vivo studies demonstrated therapeutic efficiency of QDs; therefore, our QDs indicated interesting results which warrant detailed evaluation on the signalling pathways to ascertain the extent of interdependence.

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