CHAPTER 31

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ARORA (Rashi)
Development of Diagnostic Assay for Detection of AMLI-ETO Translocation and Elucidation of Mode of Action of Esculetin, a Potential Anticancer Drug.
Supervisors: Prof. Daman Saluja and Prof. Sameer Bakhshi
Th 22778

Abstract
(Not Verified)

The havoc of cancer is increasing significantly due to the monetary burden and cytotoxic side effects associated with chemotherapy. Herein, we have made an attempt to address these two issues in cancer care. A part of this work is dedicated to develop a cost effective diagnostic assay for detection of genetic lesion using AML1-ETO translocation, known in 20% of AML patients, as a model system. The dependency of modern WHO classification system of AML on the underlying genetic abnormality and the increasing development taking place in molecular targeted therapy for this form of AML highlights the importance of its detection. None of the available methods are routinely performed in developing countries mostly due to lack of infrastructure or expertise or sufficient funds, and therefore, it becomes imperative to develop a cost effective, easy and highly sensitive method for its detection in AML patients. Herein we have successfully developed such an assay for the same by combining the technique of RT-PCR with fluorescent labeled molecular beacon probe. The assay so developed is highly specific and sensitive; and has been evaluated using 140 clinical samples. The other part of this work projects a naturally occurring coumarine derivative, esculetin to be a potential anticancer drug. We could observe antiproliferative, apoptotic and antioxidant effect of this compound in PANC-1 cells. Depleted levels of NF-κB were observed that could account for apoptosis and inhibitory effect on cell cycle as it is established to regulate both. We could get sufficient proves through in silico as well as in vitro studies to establish binding potential between esculetin and KEAP1, an inhibitory protein of transcription factor Nrf2. The interaction between the two was found to be disrupted in PANC-1 cells upon esculetin treatment, which further led to increased nuclear accumulation of Nrf2 that mediates antioxidant response.

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02. CHOUDHARY (Richa)
Investigation of the Role of 12/15 Lipoxygenase (LOX) in Hypobaric Hypoxia Induced Cognitive Dysfunction and Neuronal Damage.
Supervisor: Dr. Anju Katyal
Th 23211

Abstract
(Not Verified)

Oxidative stress is thought to be the critical effectors in hypobaric hypoxia induced cognitive dysfunctions. 12/15-Lipoxygenase(12/15-Lox) which converts arachidonic acid into bioactive lipid derivatives, has recently been described as potent mediator of oxidative stress and reported to be closely associated with cognitive decline in neurodegenerative diseases. However, the involvement of 12/15-LOX in hypobaric hypoxia induced neuronal damage and cognitive deficits largely remain obscure. The current study was designed to investigate the underlying role of 12/15-LOX on hypobaric hypoxia induced memory impairment and neuronal damage. Male Balb/c mice subjected to simulated hypobaric hypoxia showed working memory impairment which was significantly attenuated along with decrease in neuronal damage and oxidative stress following treatment with baicalein, specific inhibitor of 12/15-LOX. Our results demonstrated increased expression of 12/15-LOX in neurons and microglia along with elevated levels of 12-HETE in hippocampus and plasma of hypobaric hypoxia exposed mice. 12/15-LOX inhibition reduced hypoxia mediated upregulation of HIF-1α, which promotes the expression of NOS isoforms and COX-2 genes. Further 12/15-LOX inhibition significantly decreased NO level in homogenate by downregulating the expression iNOS, nNOS but not eNOS. 12/15-LOX inhibition could effectively modulate central cholinergic system during hypobaric hypoxia in hippocampus. Further our analysis revealed that CA3 region of hippocampus undergoes apoptosis characterized by elevated activity of caspase-3, 9 & 8 and baicalein administration in hypoxia exposed group significantly attenuated caspase dependent cell death. 12/15-LOX co-localization with mitochondria suggests that 12/15-LOX may directly damage mitochondria triggering release of cytochrome C in cytosol. We observed significantly elevated level of cytochrome C in cytosol along with increase in BAX/BCL-2 ratio and 12/15 LOX inhibition successfully restores the cytochrome C level and attenuates caspase dependent BAX/Bcl-2 mediated in hippocampus. We propose 12/15 LOX as valid target for treatment of neurological deficit induced by hypobaric hypoxia.

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03. D. NAGARJUNA
Molecular Characterization of Virulence Genes in Pathogenic *Escherichia* coli Causing Nosocomial Infections.
Supervisor: Dr. Manisha Yadav
Th 22779

Abstract
(Not Verified)

Nosocomial infections are a major public health concern throughout the world. Patients admitted in intensive care unit (ICU) are at a higher risk of developing bacteremia and septicemia. E.coli exhibits huge genetic diversity that includes commensals and pathogens. Studies on phylogenetic analyses give a better understanding about acquisition of virulence genes by pathogenic E.coli strains. Both the host and bacterial factors direct the propensity of disease among which susceptibility of the host is very crucial factor. We investigated various pathogenicity-associated features among the E.coli isolates recovered from blood and fecal of patients admitted in ICU. We hypothesize that in ICU patients, fecal E.coli has the potential to cause the disease via endogenous infection. We investigated the phylogenetic groups and virulence factors, pathotypes, adherence patterns among the E.coli isolates from sepsis and non-sepsis patients. Also the intra-species relationship among the isolates was investigated by ERIC-PCR. The antibiotic susceptibility patterns, ST131 clone and its sub groups were investigated and compared between the fecal and blood isolates. A significant proportion of pathogenic phylogroups (B2, D) was found among the fecal isolates and overall fecal isolates showed significant virulence repertoire. In this study, ETEC

were predominantly observed in blood isolates which correlated with the clinical outcome that is sepsis. More than 90% of the isolates were fluoroquinolone resistant. High prevalence of resistance to all the Cephalosporins among the fecal isolates was observed which is alarming and fecal isolates were found to be reservoir of the ESBL genes that confer resistance. A specific identification studies on E.coli from patients will give an idea about the prevalence of these infections and population at risk and their potential sources of transmission. This information may lead to implement medical care and prevention strategies at right point of time to avoid the situation leading to severe form of sepsis.

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04. LUBNA WASIM

To Investigate Molecular Mechanism of Anticancer Effects of Histone Deacetylase Inhibitors Alone and in Combination with Topoisomerase Inhibitors Against Cervical Cancer.

Supervisor: Dr. Madhu Chopra

Th 23085

Abstract
(Verified)

Cervical cancer is the fourth major cause of cancer-related deaths in women worldwide and is the most common cancer in developing countries. Studies suggest that the histone deacetylases (HDACs) play a causative role in both tumorigenesis and metastasis. Therefore, treatment modalities using molecular targeted drugs have shown potential. We investigated the interacting partners of various HDACs and their categorization to delineate the important functions in which HDACs are involved. The present study also investigated the effect of pan histone deacetylase inhibitor, 'panobinostat', on cervical cancer cells alone and in combination with topoisomerase inhibitors (topotecan/etoposide). We assessed the effect of panobinostat on two cervical cancer cell lines, HeLa and SiHa. The results indicate that panobinostat reduces the viability of cervical cancer cells in a dose- and time-dependent manner. Panobinostat induced apoptosis through an increase in the ROS production and the disruption of mitochondrial membrane potential. Concomitantly the expression of anti-apoptotic gene Bcl-xL was reduced, while levels of CDK inhibitor p21 and caspase-9 were increased. In addition, panobinostat also showed synergistic effect with topoisomerase inhibitors mediated by increased activation of caspase-3/7 activity compared to that in cells treated with panobinostat and topotecan/etoposide alone. The combination treatment synergistically enhanced the apoptosis in cervical cancer cells. The combination treatment inhibits the PI3K/AKT/NF-κB prosurvival pathway and activates the p-ERK to induce apoptosis by down regulating the Bcl-2 and Bxl-xL antiapoptitic proteins. The combination of another pan-HDACi vorionostat and etoposide encapsulated in nanogel formulation was also synergistic in enhancing the apoptotic cell killing of HeLa cells in comparison to the free dug combination. Thus, indicating the potential to use nanogels for drug delivery. These results suggest a combination therapy using inhibitors of histone deacetylase and topoisomerase together could hold the promise for an effective targeted therapeutic strategy against cervical cancer.

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05. MAINI (Jayant)
Characterization of Human PRE-PIK3C2B, a Regulatory Element with Dual Function and Long-Range Interactions.
Supervisor: Prof. Vani Brahmachari
Th 23212

Abstract
(Verified)

The transcriptional layout established by gene specific regulators during early development is taken over by maintenance machinery, which comprises of the Polycomb and Trithorax proteins interacting with the cis-elements PREs or TREs (Polycomb/Trithorax Responsive Element), constituting the cellular memory modules. We identified a human PRE, PRE-PIK3C2B, which maps to the intronic region of PIK3C2B gene (Bengani et al. 2013). In the present thesis, using DNA affinity purification followed by mass spectrometry, Trithorax group members such MLL, MLL4, TH-POK (human homolog for GAGA factor) and SET1 were identified as proteins interacting with hPRE-PIK3C2B. A total of 14 proteins were identified, which have been assigned binding activity (Chromatin binding/ Transcription factor binding) or catalytic activity (Acetyl-trasnferase/de-acetylase) based on molecular function. The members of the Polycomb group were identified albiet with low confidence. We further validated the binding of these activating group members, using chromatin immunoprecipitation experiments. We observed that binding of MLL, MLL4 and Th-POK to hPRE-PIK3C2B is dependent on the cellular concentration of YY1, a Polycomb member. We further demonstrated that binding of the PcG group members such as YY1 and EZH2 is dependent on the dosage of the MLL and Th-POK. Thus, the binding of activating or repressor proteins is dependent on the level of the respective protein(s) in the cell. Using transgenic Drosophila (PI-17), carrying hPRE-PIK3C2B upstream of miniwhite promoter driven miniwhite gene, we demonstrated that the hPRE-PIK3C2B interacts with a number ofcomponents of regulatory complexes. These results strengthen the dual role of hPRE-PIK3C2B, as a repressor as well as an activator. We further investigated the role of hPRE-PIK3C2B in long-range interactions using 4C (Capturing Circular Chromosome Conformation)-sequencing and identified a network of intra- as well as inter-chromosomal interactions.

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06. MANRAL (Apra)
Synthesis and Biological Evaluation of Diallyl Disulfide Analogs as Multi-Functional Agents for the Treatment of Alzheimer's Disease.
Supervisor: Dr. Manisha Tiwari
Th 22780

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07. MITTAL (Shilpi)
Deciphering the Role of p53 and Sin3B Under Mitotic Stress Conditions Induced by Colchicine.
Supervisor: Prof. Daman Saluja
Th 22781

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1. Introduction 2. Review of literature 3. Objectives 4. Material and methods 5. Results. 6. Discussion 7. Summary. References. Appendix. List of publications.

08. NEETIKA LAL

Molecular Machanism of Apoptosis and G2/M Cell Cycle Arrest Induced by Demethoxycurcumin in Glioblastoma Model.

Supervisor: Dr. Pratibha Mehta Luthra

Th 23213

Abstract
(Not Verified)

Glioblastoma (GBM) remains a fatal disease with an average survival time of 14.6 months following diagnosis. Present, therapy consisting of surgical resection , radiotherapy and chemotherapy with temozolomide (TMZ). Demethoxycurcmin (DMC), a pleiotropic compound shows anti-oxidative, anti-inflammatory and antitumor activity in cellular and in vivo models. However, the molecular mechanism of DMC-mediated cell death and G2/M cell cycle arrest in human glioma cells has not been yet divulged. In this thesis, a significant understanding of the molecular events contributing to the DMC-induced cell death and G2/M cell arrest in human glioma U87 MG cells has been revealed. Chapter 1.The current knowledge on the anticancer potential of Curcumin (Cur) in vitro and preclinical animal models of GBM has been illustrated critically. Based on the literature survey for the curcumin, the objectives for the DMC in the thesis have been designed. Chapter 2. An understanding of the molecular events involved in DMC-induced apoptosis and G2/M cell arrest in human glioma U87 MG cells has been exposed. The anticancer potential of DMC was also studied in GBM mice model. The $O2^{\cdot-}$ generated by DMC in U87 MG glioma cells regulate the DMC induced apoptosis and G2/M arrest. It was observed that blocking the Mn-SOD in mitochondria decreased the DMC-mediated superoxide anion generation, thus altering the MMP and release of cytochrome c to trigger the apoptotic event in U87 MG cells. In addition, these events lead to reduce protein expression of Cdc-25C, Cyclin B1, p-Cdc-2 (161) and degradation, ubiquitination of cyclin B1 leads to G2/M cell arrest. Moreover, the initial in vivo GBM mice model study demonstrated that DMC is more potent than Cur and TMZ and possesses significant potential in the anticancer therapy. Chapter 3. The details of material and its source of procurement have been disclosed. The methods has been shortly discussed

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1. Introduction and Review of Literature 2. Study the molecular mechanism of apoptosis and G2/M cell cycle arrest induced by demethoxycurcumin in glioblastoma model 3. Materials and Methods. List of publications.

09. RAI (Shweta)

Mechanistic Insights into Immunopathogenesis of Murine Cerebral Malaria: Comparison of C57BL/6J and BALB/c MICE.

Supervisor: Dr. Anju Katyal

Th 23214

Abstract
(Not Verified)

ABSTRACT The pathophysiological features of cerebral malaria (CM) in children have disparity and poor prognosis compared to the disease in adults. The adult C57BL/6J mice are considered to mimic some of the features of cerebral malaria and widely used to understand the pathogenic mechanisms in comparison to relatively less prone BALB/c mice. The age and immune status of the host as in young children can influence the disease sequelae and cerebral manifestations. Therefore, the present study

was designed to dissect and differentiate the immunopathology of cerebral malaria in young BALB/c and C57BL/6J mice infected with Plasmodium berghei ANKA (PbA) parasites. A multipronged approach including the longitudinal progression of parasitaemia, relative gene expression of MSP1 parasite protein, histopathology, expression of TNFα, IFNɤ, and IL-1β , IL-4, IL-10, TGF β1, and molecules involved in IgE-CD23 pathway was studied in cortex of brain of PbA infected mice groups. Our results demonstrate a significant increase in the expression of TNFα, IFNɤ, IL-1β, IL-4, IL-10 with increasing parasitemia in PbA infected Balb/c mice compared C57bl/6J. Additionally, progressive increase in IL-4, iNOS, and CD23 in brain resident cells during colocalization studies in PbA infected Balb/c mice demonstrate a reciprocating relationship between astrocyte, microglia and neuronal cells. Further we identified and characterized IgE specific cytosolic P. berghei ANKA proteins which may be associated with IgE mediated pathologies. The discrepancy of cytokine balance and generation of IgE against parasite antigens resulted in worsening of disease manifestation in Balb/c mice model similar to pediatric CM and propose that the BALB/c mouse infected with PbA could be a promising animal model for cerebral malaria.

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10. SAINI (Vikas)

An Evaluation of the Anticancer Activity of Novel Analogs of Diallyl Disulfide, an Active Principle of Garlic.

Supervisor: Dr. Manisha Tiwari

Th 23084

Abstract
(Not Verified)

Diallyl disulfide (DADS), a principal organosulfur component of garlic, is known for its medicinal properties including anti-cancer activity. In the current study, we have evaluated a series of 21 novel diallyl disulfide derivatives as potential anticancer agents. The preliminary in silico findings showed the antagonizing activity of DADS derivatives against anti-apoptotic protein Bcl-2, suggesting the possibility of promoting apoptosis. In addition, evaluation of ADMET profile of these derivatives indicated that the compounds possess appropriate physicochemical properties with minimum toxic effects. Also, the results from cytotoxicity studies showed that most of the designed compounds have potential anti-proliferative activities against three different human cancer cell lines and showed good safety index against normal HEK-293 cells. The compound bis[3-(3-fluorophenyl)prop-2-ene]disulphide (5b) displayed most potent activity against all the cancer cell lines. However, the potential mechanisms of the cytotoxic activity of the promising compound 5b was further investigated on the most sensitive cell line MIA PaCa-2. Furthermore, the mechanistic studies highlighted that the underlying mechanism of cytotoxic effect of compound 5b could be attributed to its ability to induce reactive oxygen species (ROS) generation. This was accompanied by cell cycle arrest in the G2/M phase and apoptosis in MIA PaCa-2 cells. The western blot analysis showed that the compound 5b induces G2/M phase arrest by ROS mediated DNA-damage, which causes activation of Chk1 mediated phosphorylation/inactivation of key cell cycle proteins. Also, the altered levels of ROS triggers intrinsic apoptotic cascade, marked by dissipated mitochondrial membrane potential (ψ), decrease in Bcl-2/Bax ratio, cytochrome c release and cleavage of procaspase-3. Overall, the results of this study highlight the potential of DADS analogs as good lead-candidate for further optimization to develop into new potent antitumor agents.

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11. SEEMA

Study of Circulatory microRNAs as Biomarkers for Diagnosis, Prognosis and Progression of Breast Cancer.

Supervisor: Dr. Ajay Kumar Yadav

Th 22782

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1. Introduction 2. Review of literature 3. Materials and Methods 4.Results. 5. Discussion 6. Summary 7. Conclusions 8. References. Annexures. List of Publications

12. TAUHEED HASAN

Investigating the Role of pH and Divalent Cations on the Structure and Stability of PAH domains of Human Sin3B.

Supervisor: Prof. Laishram Rajendrakumar Singh

Th 22783

Contents

1. Review of Literature 2. Effect of pH on the structure and thermodynamic stability of PAH domains of hSin3B 3. Effect of divalent salts on the structure and thermodynamic stability of PAH domains of hSin3B. Summary. References. Appendix. List of publications.