NATIONAL INSTITUTE OF PATHOLOGY
NEW DELHI

Highlight 2014-2015

Tumor Biology

1. Study on gene expression and methylation profiles in early onset breast cancer

Present study had been undertaken to study gene expression and methylation profiles in early onset breast cancer to understand molecular pathogenesis of early onset breast cancer. Twenty cases of early onset and twenty cases of late onset breast cancer have been studied by using Illumina microarray. The data had been analyzed using bioinformatics approach to identify unique expression and methylation profiles of early and late aged females. Early onset breast cancer had 466 differentially expressed while 1,545 genes were differential in late onset breast cancer. Pathways analysis revealed pathways like ABC transporters, Axon guidance, Cytokine cytokine receptor interaction pathway coming out differentially significant in early onset breast cancer while Fanconi anemia pathway, DNA Replication pathway ,p53 signalling pathway were significantly deregulated in late breast cancer. Methylation profiling of breast cancer patients identified 672 and 689 differentially methylated genes in early and late breast cancer patients respectively. Genes like RASSF1, WT1 were commonly methylated in early and late breast cancer, while CD40, CD86, HOX genes were methylated in early cancer while GSTP1, CCND1 were found uniquely methylated in late breast cancer. Presently validation of genes is undergoing by Realtime PCR and Methylation specific PCR. The data was also merged to gain knowledge on epigenetic regulation of breast cancer by identifying certain genes which were showing good correlation between gene expression and methylation.

2. Study on Micro RNA Signatures Associated with Breast Cancer Stem like Cells (CSCs) and their role in Drug Response

Recent studies have shown existence of small fraction of cancer stem cells (CSCs) or tumour-initiating cells in various solid tumors such as breast, brain, prostate, pancreas and colon cancers, which play important role in cancer establishment, progression, and resistance to therapy. Traditional cancer therapies are effective in debulking the tumors but often fail to completely eradicate CSCs and thus, fail to stop recurrence or metastasis to distant organs. This study has been initiated to understand the molecular characteristics of CSCs by studying unique miRNA, gene expression profiles associated with CSCs compared to bulk tumor cells. Isolation of breast cancer stem (CD44+/CD24-)cells using CD44 and CD24 markers in 5 breast cancer cell lines, MCF7, MDA-MB-231, ZR-75-1, T47D, MDA-MB-468 has been done followed by expression profiling of miRNAs and gene expression profiling. Currently we are doing *in silico*analysis of the data to identify the differentially expressed miRNAs and transcripts in cancer stem cells.

3. Targeted sequencing of breast cancer specific genes in early-onset breast carcinoma

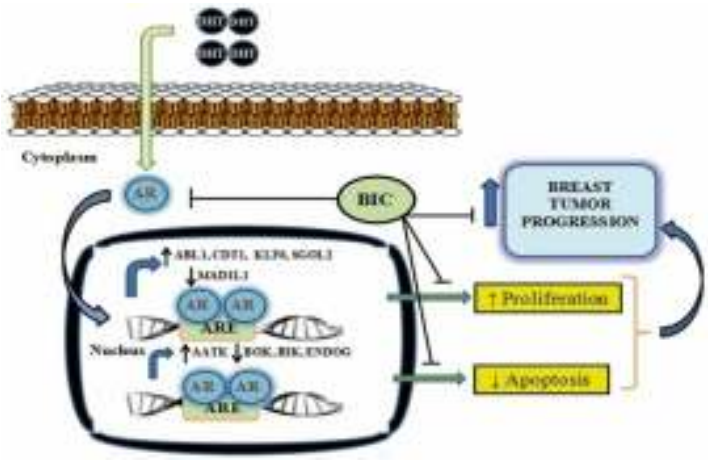
This study had been under taken to identify genetic variations unique to early onset breast tumors (≤40 years of age) with validation of these genetic variations in breast tumors of different subsets (ER, PR, and ErbB2).

Exome sequencing has revealed presence of 46174 genetic variations in early onset tumors and 116224 variations in late onset tumors, belonging to various groups, when compared to human genome

reference sequence, GRCh38.76 on ENSEMBL. About 85889 variants were found on average in each sample, out of which about 75839 are known while, 10050 are novel variants. In early onset tumors we found 42717 SNPs, 1732 insertions, and 1725 deletions; in the late onset tumors. We have analyzed the data further to identify unique genetic variants found in ealry and late onset tumors. In the first step, to remove population specific variants we have removed the variants present in controls also. Further we have identified genetic variants which are existing in all the tumors, to identify commonly occurring genetic variants. We found 2562 genes that are commonly mutated in all early onset tumor and 2689 genes that are mutated in all late onset tumors. Among the two groups we found 474 genes which are commonly mutated.104531SNPs, 5903 insertions and 5790 deletions were found. The data is at present being analyzed by using various bioinformatics tools.

4. Understanding the role of androgen receptor signaling in breast cancer

AR is a member of the family of intracellular steroid hormone receptor that function as ligand dependent transcription factor. Androgens are steroid hormones that control male development and reproductive function and are key regulators in the development of secondary sex characteristics, spermatogenesis andcarcinogenesis. Recently AR has been implicated in the development and progression of breast and prostate cancers. Although some of the functions of the AR are known but the mechanistic details of these divergent processes are still not clear. Aim of the present study was to identify novel genes up regulated by AR, upon DHT stimulation in breast cancer cell line MDA-MB-453. Identification of the new AR targets in breast cancer will reveal potential targets for targeting of breast cancer for the purpose of therapeutic intervention. Herein, using MDA-MB-453 as the model for studying triple negative breast cancer cell line positive for AR expression, we have shown that DHT (AR agonist) stimulation leads to proliferation of the breast cancer cells and this effect was abrogated by the addition of bicalutamide (AR inhibitor). Moreover, we have identified 10 novel targets which are upregulated by AR in DHT-dependent manner. Taken together these data suggest that AR binds and directly up regulates the cell cycle genes and causes the cell proliferation in MDA-MB-453 cells. Moreover, treatment with bicalutamide reverses the AR binding and abrogates the effects of AR on cell proliferation. **Taken together the data suggests that AR mediated effect can be reversed by AR antagonist like bicalutamide and therefore we propose to use bicalutamide for the treatment of AR positive, triple negative breast cancer.**



Proposed model showing AR regulation of the breast tumour progression.
Androgen receptor (AR) on DHT stimulation translocates into the nucleus and bind to its cognate androgen response elements (AREs). Inside the nucleus, AR up regulates the expression of ABL1, CDT1, KLF6 and SGOL2, while it represses MAD1L1 expression to induce cell proliferation. At the same it induces AATK expression and down regulates the expression of BOK, BIK and ENDOG to decrease apoptosis and promotes breast cancer progression. Bicalutamide (BIC) reverses the effect of AR on cell cycle and apoptosis by binding and preventing its activation. Due to its ability to negate the effects of AR, bicalutamide can be used to block breast cancer progression.

5. Expression of aldo-ketoreductase family 1B10 (AKR1B10) gene in Breast carcinoma: The effects on drug and tobacco exposure

In our previous study, strong association of breast cancer risk with betel quid chewing in Northeast Indian population has been reported. Copy number analysis using microarray on breast cancer patients portrayed AKR1B10 as one of the key markers for betel quid associated breast cancer.To further validate its potential role in breast cancer, AKR1B10 protein expression was analyzed in breast tumor samples by immunohistochemistry. AKR1B10 positivity was found in 50% of the samples with both cytoplasmic and nuclear localization. Fourteen to fifteen percent samples had strong and weak positivity respectively whereas moderate positivity was seen in 20% of samples. Expression of AKR1B10 was not associated with ER, PR and Her2neu expression however it significantly correlated with patients with history of tobacco use. To further elucidate AKR1B10role its expression was analyzed in five breast cancer cell lines (MCF-7, MB MDA 231, MB MDA 435, T47D, ZR75-1) and a normal breast cell line HBL100 by immunocytochemistry and RT-PCR. MCF-7, ZR 75-1 and MDAMB 435 breast cancer cell line were positive for AKR1B10 expression both at RNA and protein level. To identify the tobacco effects on expression of AKR1B10 gene MCF-7 and ZR75-1 cell lines were exposed to NNK (4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone) (10µg) and smokeless tobacco extract (STE)(20µg/ml). STE was able to further induce AKR1B10 expression in ZR75-1 at 24 and 48hrs (cytoplasmic expression) of exposure as compared to MCF-7 at 24hrs and 48hrs (nuclear AKR1B10 expression) implicating that components of tobacco have a positive regulatory effect on AKR1B10 expression.

6. Study on characterization of TMPRSS2: ERG and PCA3 as prostate cancer Biomarkers in Indian patients

TMPRSS2:ERG (T2:ERG) and prostate cancer antigen 3 (PCA3) are the most advanced prostate cancer (PCa) early detection biomarkers. The recent identification of gene fusions of the 5′-untranslated region of TMPRSS2 (21q22.3) with the ETS transcription factor family members, like ERG (21q22.2), suggests a mechanism for overexpression of the ETS genes in the majority of prostate cancers. Study has been undertaken with aim to evaluate molecular biomarkers TMPRSS2-ERG gene fusion and PCA3 in patients with prostate cancer, and to analyze their clinical relevance as a prognostic/diagnostic tool. The study showed the T2:ERG rearrangements in 58% of prostate cancer biopsy samples. **Analyses of matched urine samples to assess the potential of T2:ERG as a non-invasive marker showed a concordance level of 95.2% between tissue and urine sample results.Sensitivity of TMPRSS2: ERG fusion marker was 58 % whereas specificity was 100% as none of BPH sample was found to have the fusion transcript.** The positive predictive value of fusion marker is 100% and negative predictive value of the marker is 71.25%. Comparison of expression of PCA3 gene between T2:ERG fusion positive, fusion negative and BPH patients, expression of PCA3 gene was found to be 54.63 fold unregulated in fusion negative patients group as compared to 23.06 fold up regulation in fusion positive patient group (p=0.05) when BPH group was taken as control group.

7. Differential protein profile for identification of markers in recurrent urothelial cancer

The objective of this study is to identify cancer-specific tumour proteins secreted into the urine in Urothelial cancer patients in order to identify biomarkers for surveillance of recurrence. The discovery phase of the work is already completed with identification of differentially expressed tumor proteins in urothelial bladder cancer by iTRAQ LC-MS/MS. The data obtained is at present being analyzed by bioinformatics tools to identify cancer specific proteins which can be used as non invasive biomarkers followed by validation in urine samples.

8. Epigenetic Studies in Esophageal Cancer in High Risk Region of Northeast India

Esophageal cancer incidence is reported in high frequency in northeast India. The etiology is different from other population at India due to wide variations in dietary habits or nutritional factors, tobacco/betel quid chewing and alcohol habits. Since DNA methylation, histone modification and miRNA-mediated epigenetic processes alter the gene expression, this study has been undertaken to find out epigenetic markers of esophageal squamous cell carcinoma (ESCC) in northeast Indian population.

Study of chromatin modification enzymes in ESCC showed higher expression of enzymes regulating methylation (DOT1L and PRMT1) and acetylation (KAT7, KAT8, KAT2A and KAT6A) of histone was found associated with ESCC risk. Tissue microarray study suggests the association of PRMT1 and KAT8 with esophageal cancer risk and their involvement in the transition process of low to high grade tumor formation. Differential methylation profiling of Tumor Suppressor Genes (TSGs) in tumor and corresponding normal esophageal tissue showed hypermethylation of OPCML, NEUROG1, TERT and WT1 and hypomethylation of SCGB3A1, CDH1, THBS1 and VEGFA gene. The immunohistochemical expression of OPCML protein displayed no significant change in control and ESCC, however, differential OPCML protein expression was found among different grades of tumor. Integration of methylation data with microarray expression data published earlier by our group was also done to prepare a network of genes displaying enriched pathways together with list of genes exhibiting promoter hypermethylation or hypomethylation with inversely correlated expression. The study resulted in 23 Integrome network enriched genes having relevance to tumor progression. These includes 4 genes with promoter hypermethylation and down regulation and 19 genes with promoter hypomethylation and up regulation. Top 5 gene with highest Methylation Efficiency Index (MEI) were COL1A1, TAC3, SERPINA4, TNFSF13B and IL22RA2. **The methylation and expression status of circulatory proteins involved in immunoregulation (IL22RA2 and TNFSF13B), extra cellular matrix remodeling (SERPINA4) and contraction of the circular muscle of human esophagous (TAC3) could be further explored as non-invasive biomarker for esophageal cancer.**

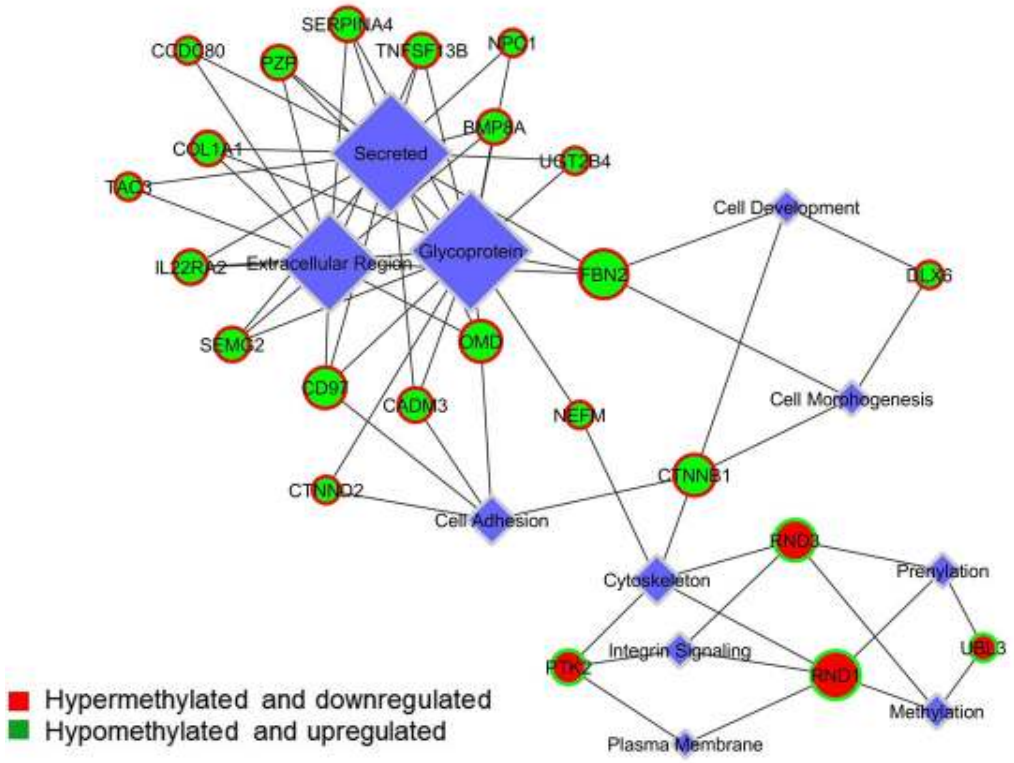


Figure: Results of Integrome network analysis. It resolved 23 genes that correlated methylation status with gene expression status. **N**etwork encompasses biological categories, differentially expressed and methylated genes that were significantly enriched. Green and red color circles representing upregulated genes with hypomethylation and downregulated genes with hypermethylation respectively.

9. Functional characterization of putative molecular marker in esophageal cancer patients from North east region of India

This study is focused to search putative molecular signatures and their functional characterization in esophageal cancer by analyzing data from the studies of copy number and gene expression profiling of familial and non-familialpatients in north east populations of India.I*n silico*analysis of data from these studies identified three putative molecular signatures from amplified region (*CCR1*, *ENAH* and *FGF12*), eight from deleted region genes (*MAPT, FGF18, UBE2E1, PARD3, SCP2, FGF14, PPP2CB, IL12A*). Gene expression studies was able to identify two molecular marker i.e. *STAG1, FGF12*. These makers further analyzed among the 22 type of cancer to know the mRNA level of these genes to find out suitable and appropriate esophageal cancer gene. FGF12 wasthe only putative marker showingupregulated expression in esophageal cancer in oncomine and Cancer Cell Line Encyclopedia (CCLE) databases. With these leads we did functional characterization of FGF12 by knockdown studies in ESCC cell line KYSE410. Further investigations of functional role of FGF12 were established by defined functional assays like proliferation, colony formation, and wound healing assays. This assay showed, more than 50% of cancerous cells were inhibited after knockdown. We are in process to examine the mechanism of action of this signature. Validation of these proteins would be done in clinical samples at protein level.

10. Contribution of susceptibility locus at HLA class I region and environmental factors to occurrence of nasopharyngeal cancer in Northeast India

In India elevated frequency of NPC has been reported from population based cancer registries in north east India with highest age-adjusted incidence rate (AAR) of NPC 19.4/100 000 at Kohima district in Nagaland state, followed by Imphal district in Manipur State with an AAR of 7.4/100 000.The current study had been undertaken with the aim to investigate the contribution of environmental risk factors and genetic variation of HLA region for the high incidence of NPC in NE India. The study is planned to identify the allelic variation in microsatellite markers present on HLA region in patients with nasopharyngeal carcinoma as compared to the controls, to identify genetic alterations in HLA region among patients with nasopharyngeal carcinoma and corresponding controls by employing sequencing approach and to correlate presence of Epstein Barr viral sequences in the tumor tissue of patients with nasopharyngeal carcinoma in NE India. The results of previous study has demarcated a section on HLA class I region and EBV RNA sequences with susceptibility for NPC. Further to study additional genetic alterations the entire HLA super locus (3.8 Mb regions) using next-generation sequencing (NGS) technology was studied. The study was carried out in 4 NPC cases and 4 corresponding age and sex matched controls. Analysis of results showed a high association of five SNP located in HLA region with the Nasopharyngeal cancer. Of these five SNPs two SNPs were novel present in genes namely COL11A2and MUC22 whereas three SNPs present in genes HLA DRB5, HLA-DPA1 and TAP2 were already known. Further the validation of these SNPs is undergoing in large sample size.

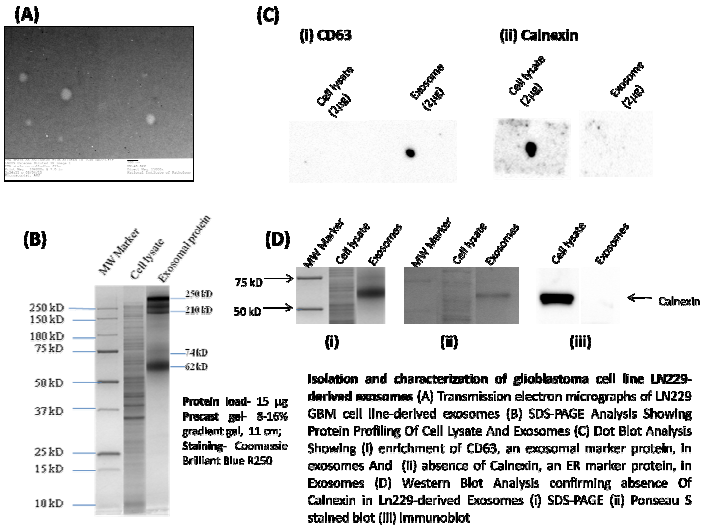
11. Molecular Mechanism of drug resistance in acute myeloid leukemia (AML): Role of ATP-binding cassette (ABC) transporters

Resistance of tumor cells to drug treatment is the major impediment in curing leukemia. Despite the best efforts and advances in the field of treatment, acute myeloid leukemia still remains largely an incurable and ultimately fatal disease. In the present study, we studied the expression of 44 ABC transporter genes in acute myeloid leukemia (AML) samples using low density ABC transporter array and identify the differentially expressed genes involved in the mechanism of resistance mediated by ABC transporter genes. Results showed high expression (>1.5 fold) for genes ABCA1, ABCA3, ABCB5, ABCC6, ABCE1, ABCF1 and ABCG1. Further, expression of ABCA1, ABCC2, ABCD4, ABCG1 and ABCF1 genes were found to be significantly up regulated in non-responder patient of AML compared to responders. In accordance with the results of the arrays, all the three genes were overexpressed in leukemic samples compared with the healthy controls. In vitro experiment showed the drugs Cytosine arabinoside (Ara-C) led to an increase of ABCF1 and ABCG1 expression in THP-1 and K-562 cells. This suggests that ABCF1 and ABCG1 might be involved in drug resistance. The cytotoxicity assay in K562 silenced cell further indicates that ABCF1 contributes to drug resistance. The study is currently identifying the proteins involved in the resistance mediated by ABCF1 protein.

12. Isolation and Characterization of Human Glioblastoma Cell Line LN229-Derived Exosomes

Glioblastoma (GBM) are highly malignant brain tumours constituting 80% of all primary brain tumors arising in brain and spinal cord. Till date, the detection and monitoring of tumor during treatment is mainly based on CT-scan and MRI imaging techniques. However, the need for discovery of clinically important plasma based tumor markers for development of non invasive assays is widely recommended. Exosomes, secreted membrane vesicles ranging from 40-100 nm in size, being remarkably stable in bodily fluids have shown great promise for its use in prognosis, therapy, and biomarkers for cancer. In the present study, we have optimized the cell culture conditions for

isolation of exosomes followed by isolation and characterization of GBM LN229 cell line derived exosomes. Exosomes were isolated by differential centrifugation method and characterized based on transmission electron microscopy, and dot blot and/or western blot analysis for CD63, an exosomal marker protein and calnexin, an ER marker protein. Cell viability assay showed minimal cell death (≥95% cell vialbility) at 24 h after replenishing the media with exo-free FBS at 70-80% confluency. TEM analysis showed exosomes with size range of 30-100 nm. Dot blot analysis showed enrichment of CD63 in exosomes. Dot blot and Western blot analysis of calnexin showed absence of calnexin in exosomes. These results suggest enrichment of exosomes with the method used in the study. Finally, the exosomal proteins were subject to in-solution trypsin digestion for the sample preaparation for mass spectrometric analysis to identify the exosomal protein and to further explore their potential as diagnostic biomarkers for the GBM.



Isolation and characterization of glioblastoma cell line LN229-derived exosomes (A) Transmission electron micrographs of LN229 GBM cell line-derived exosomes (B) SDS-PAGE Analysis Showing Protein Profiling Of Cell Lysate And Exosomes (C) Dot Blot Analysis Showing (i) enrichment of CD63, an exosomal marker protein, in exosomes And (ii) absence of Calnexin, an ER marker protein, in Exosomes (D) Western Blot Analysis confirming absence Of Calnexin in Ln229-derived Exosomes (i) SDS-PAGE (ii) Ponseau S stained blot (iii) Immunoblot

INFECTIOUS DISEASES

LEISHMANIASIS

1. Studies on miltefosine resistance in visceral leishmaniasis:

Increasing incidence of relapse in VL cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard efficacy. We have approached genomic microarray tools to study transcriptome profiling in clinical isolates of *Leishmania donovani* from pretreatment and relapse group. The study revealed important differences in gene expression pattern between relapse group and pre-treatment isolates. Approximately 1800 genes comprising ~20% of total Leishmania genome were found to be differentially modulated of which 7.4% genes were up regulated while 12.4% were down regulated. Further we validated the rxpression of selected genes by Q-PCR which correlated well with the microarray results.

Data analysis using BLAST2GO, AmiGO and KEGG pathway led to classification of modulated genes into various functional categories including metabolic pathways, transporters, signal transduction pathway, nucleotide binding and cellular components. Transporters comprised the major category following unclassified proteins which include hypothetical proteins (proteins with unknown function).

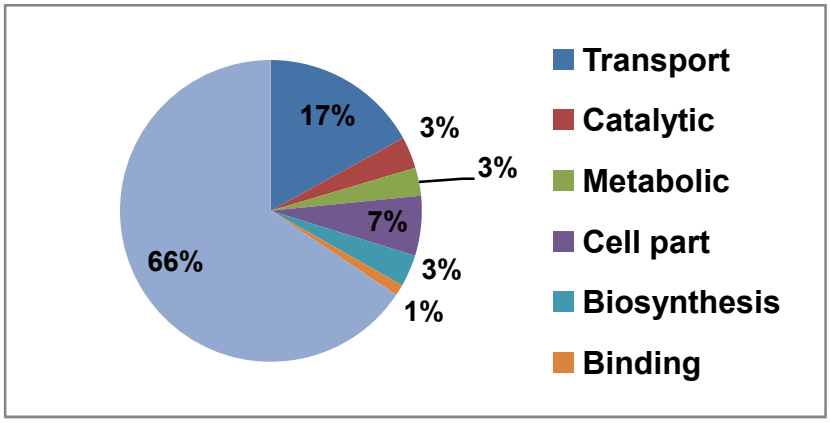


Fig: Distribution of differentially expressed genes in relapse group according to Gene Ontology (GO) function categories

The Lipase precursor like gene, involved in lipid metabolic pathway, was consistently up regulated in parasite from relapse group as well as in experimental miltefosine resistant parasites. To investigate its role in establishing unresponsiveness towards miltefosine we transfected this gene into miltefosine sensitive *L. donovani* parasites and assessed growth kinetics as well as miltefosine susceptibility in transfected parasites. Parasites overexpressing lipase precursor (*LdLip*$^{++}$) showed better tolerance towards miltefosine pressure as compared to the sensitive parasite. There was a significant decrease in susceptibility towards miltefosine in *LdLip*$^{++}$ both at promastigote and intracellular amastigote stages. *LdLip*$^{++}$ showed more than 3 fold higher IC_{50} value than the wild type parasite.

2. **Mechanism of resistance towards paromomycin in *Leishmania donovani***

Paromomycin (PMM) is a new treatment option registered for the treatment of VL in India. Although no clinical resistance has yet been reported, it is crucial to understand the mechanism resistance towards PMM to ensure its long term effectiveness. In the present study, we induced PMM tolerance in three different field isolates to establish the mechanism of drug resistance in line with our earlier studies. All the three paromomycin resistant (PMM-R) isolates showed 6-7 fold reduced susceptibility towards the drug. In order to validate the role of ABC transporters in paromomycin resistance, we compared the susceptibility of the PMM resistant and wild type isolates towards paromomycin in presence of inhibitors of ABC transporters. There was a significant increase (2 fold) in susceptibility towards PMM in PMM-R isolates in presence of verapamil, which is an inhibitor of MDR1 and approx. 6 fold increase in presence of amlodipine, which is an ABCG2 inhibitor. A partial reversion of resistant property of PMM-R isolates in presence of verapamil and amlodipine established the role of ABC transporters in paromomycin resistance.

3. Role of CD8+ T cells in protection against *Leishmania donovani* infection in healed Visceral Leishmaniasis individuals

Majority of individuals with history of visceral leishmaniasis (VL) exhibit strong immunity to re-infection, however, the mechanism of resistance is poorly understood. It is unclear whether $CD8^+$ T cells contribute to protection against *Leishmania donovani* infection through cytotoxic activity. The present

study aims to evaluate immunological mechanism associated with resistance to the disease in healed VL (HVL) individuals and further, the contribution of $CD8^+$ T cells in the protective immunity. Peripheral blood mononuclear cells (PBMCs) from VL, HVL and naive groups were exposed *in vitro* to total soluble Leishmania antigen (TSLA). We observed significantly higher lymphoproliferation, cytokines and granzyme B levels in HVL group compared to naive or VL group. More strikingly, we found a strong association (r_s = 0.895, P < 0.0001) between proliferation index (PI) and granzyme B level, with a significant proportion of activated $CD8^+$ T cells in HVL group. *Leishmania* immune group (HVL) exhibited durable and strong cellular immune response to TSLA in terms of lymphoproliferation as well as production of Th1 cytokines and granzyme B. Additionally, the elevated level of activated $CD8^+$ T cells and stimulation of cytotoxic activity through granzyme B production, indicated a possible role of $CD8^+$ T cells in resistance to *L. donovani* infection in the HVL group (*BMC Infect Dis* 2014, 14:653).

4. Application of new LAMP assay for diagnosis of VL and PKDL

The LAMP assay was applied to clinical samples for diagnosis of VL and PKDL. The assay gave high sensitivity of 96.4% for VL and 96.8% for PKDL with 100% specificity. Further, the assay was subjected to third party validation at RMRI, Patna, where it showed sensitivity of 98% and specificity of 97%.

TUBERCULOSIS

1. Signature Sequences': Novel Genetic Markers for Diagnosis of Tuberculosis (TB)

Tuberculosis (TB) remains a major global health problem taking one human life every 15-20 seconds globally and ranks as the leading cause of death from any bacterial infectious diseases. Conventional diagnostic methods include examination of sputum smear under a microscope for acid-fast *Mycobacteria* and an x-ray of lungs. However, in large number of cases the sputum smear examination is negative for *Mycobacteria* in early stages of infection and lung changes are not obvious on an x-ray until several months following infection. A rapid test capable of reliably detecting the presence of *Mycobacterium tuberculosis* is vital for early detection and treatment of this disease. Therefore, need of the hour is to: a) identify more effective targets; b) targets which are unique to *Mycobacterium tuberculosis*, and c) use more than one target to increase detection accuracy. Our probes will not only permit rapid, cost-effective, high specificity and high sensitivity detection of bacillus in regular TB patients but these SS probes may be able to detect the bacterium from sputum/blood, also in cases where these could not be detected by GenExpert or other tests currently in the market.

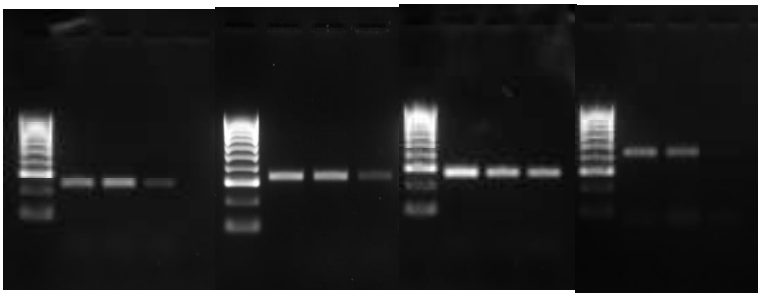


Fig: PCR amplification of 4 different signature sequences probes and its sensitivity assessment.

2. Understanding the Underlying Mechanism of Macrophage Immune Modulation: Cross-talk between Inflammation and Cellular stress

TLRs are evolutionarily conserved pattern recognition receptors (PRR) and represent primary triggers of the innate immunity. They are responsible for sensing and responding to pathogen-associated molecular patterns (PAMP) of diverse invading organisms.Resistin, a member of the adipokine family, is known to be involved in the modulation of immune responses including inflammatory activity. We have establish the role of resistin in innate immunity by way of its interaction with TLR2 and activating/inactivating the downstream players leading to inflammation and anti-apoptotic pathway.
Resistin treatment induces both anti-inflammatory IL-10 **(Fig 1A**) and the pro-inflammatory cytokines TNF-α**(Fig 1B)** and IL-6 **(Fig 1C**). This reveals a complex network of signaling events in response to resistin**.**
Considering earlier report on the role of resistin in increasing the expression of TLR2 in human adipocytes **(Kusminski et al, 2007),** computational docking was performed to identify the possible interacting domain/s of resistin and TLR2

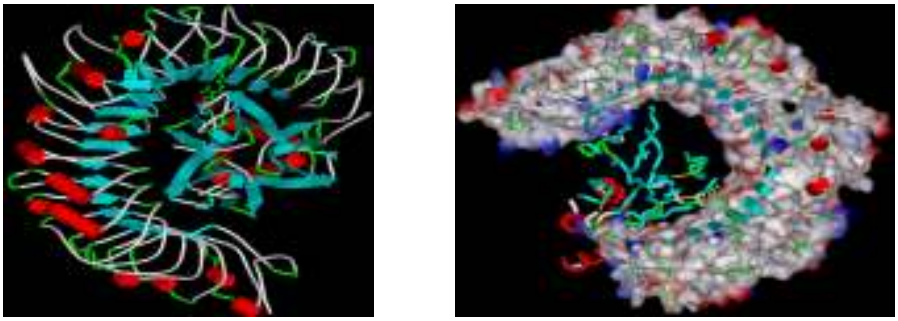
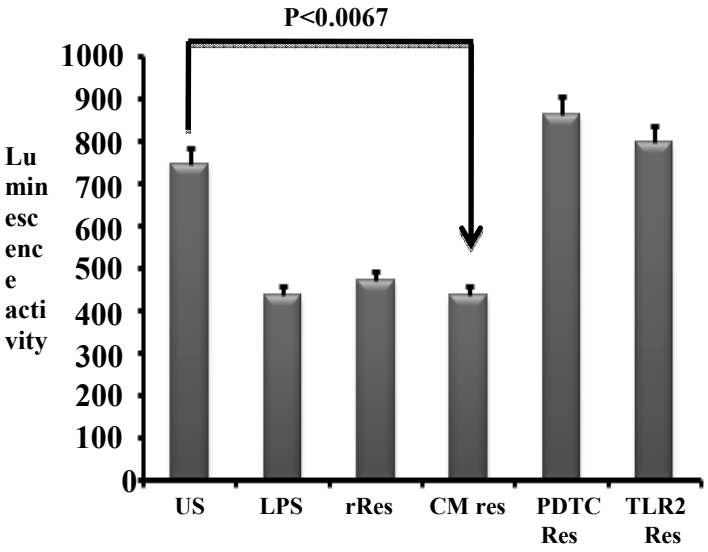


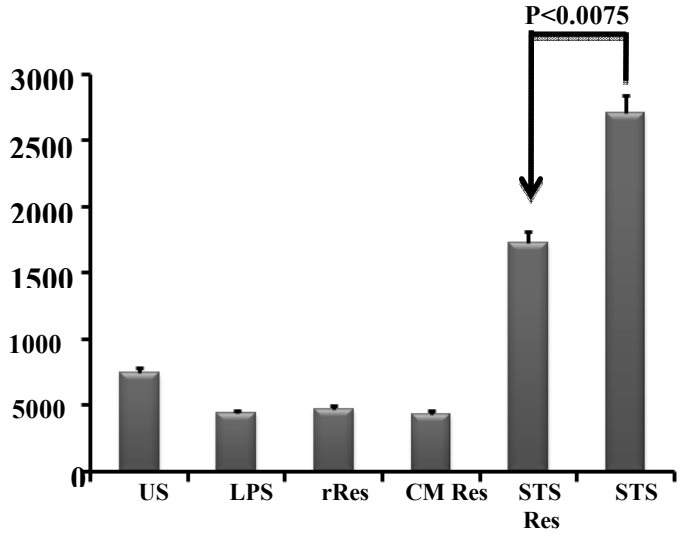
Fig: **Computational analysis predicts TLR2 as a strong interacting partner for human resistin.**Schematic diagram showingTLR2 as molecular surface coloured with electrostatic potential and human resistinas ball and tube model. Position of interacting residueson the binding interface of resistin-TLR2 complex.

Corroborating above finding of resistin interaction with TLR2, the effect of resistin on the expression of TLR2 in human macrophage cells *in vitro were investigated*. FACS analysis shows a significant and proportionate increase in the expression of TLR2 on macrophage cell surface as compared to untreated cells. Interestingly, neutralization of cells with anti-resistin antibody or anti-TLR2 antibody abrogate the

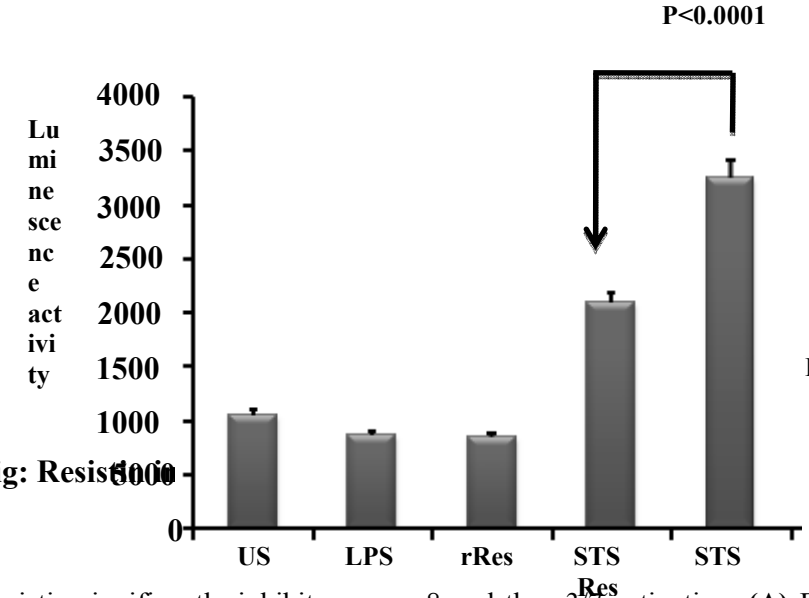
effect of resistin on TLR2 expression suggesting that the observed effect was indeed due to resistin.Given the role of TNF-α and TLR2 in inducing apoptosis in THP1 differentiated macrophage cellsthe downstream signaling effect of resistin on Caspase8 and 3/7 activation was elucidated. Resistin dramatically suppressed both Caspase8 and 3/7 activation; levels similar to that observed for the cells treated with *E. coli* LPS, (Serotype-O128:B12) a known inhibitor of caspases and thus apoptosis. However, neutralizing the cells with anti-TLR2 antibody apparently abrogate the inhibitory effect which suggests the involvement of TLR2 in down regulating the caspase activation **(Fig 5A)**. This antagonistic response of resistin is observed to be upstream of NF-κB as inactivation of NF-κB using PDTC fails to eliminate the inhibitory effect of resistin**(Fig 5B)**. Thesedata hence satisfactorily demonstrates that resistin signals inactivation of Caspase8 and therefore Caspase3/7 and favours an anti-apoptotic state in the host cell via TLR2.



Resistin inhibits Caspase 8 activation in TLR2 dependent manner



Resistin inhibits Caspase 8 activation



Resistin inhibits Caspase 3/7 activation

Fig: Resistin

Resistin significantly inhibits caspase8 and thus 3/7 activation. **(A)** Bar diagram represents the Caspase 8 inhibition in human macrophage cells; levels are equal to *E. coli* LPS (Serotype O128:B12)

which is used as positive control. This inhibitory effect of resistin is TLR2 dependent as immunoneutralization of cells with anti-TLR2 antibody prior to resistin treatment abrogate the same; levels become equal to cells without resistin treatment. **(B)**Resistin also lowers the caspase activity stimulated by staurosporine. However, treating the cells with NF-kB inhibitor (PDTC) does not alter resistin mediated caspase inhibition. **(C)** Bar diagram showing the inhibitory effect resistin on caspase 3/7 activation.

CHLAMYDIASIS

To understand the pathogenesis of *Chlamydia trachomatis* in Reactive Arthritis (ReA)/ Undifferentiated Spondyloarthropathy (uSpA) patients, Th1/ Th2/ Th17 cytokines were estimated in Synovial Fluid (SF) as well as in serum. IFN-gamma was significantly higher ('p' = 0.0003 *versus* control rheumatoid arthritis) in the SF of *C. trachomatis*-positive ReA/ uSpA patients in comparison to uninfected patients while IL-4 was downregulated in both SF as well as in serum. IL-17A was comparable in *C. trachomatis*-infected *versus* non-infected ReA/ uSpA patients, however, it was significantly higher ('p' = 0.0001 *versus* OA) than the control osteoarthritis patients. In SF, IL-6 was positively correlated to IFN-gamma (r = 0.72, 95% CI 0.23-0.91, 'p' = 0.007).

Furthermore, level of IFN-gamma ('p' = 0.04) and IL-17 ('p' = 0.0001) cytokines were significantly upregulated than IL-6 in chlamydial heat shock protein 60-positive ReA/ uSpA patients; while IL-17 was also significantly higher ('p' = 0.01) than IFN-gamma in these patients. Comparison of cytokines and hsCRP in *C. trachomatis*-infected and HLA B27-positive *versus C. trachomatis*-negative and HLA B27-positive patients showed that hsCRP was significantly higher ('p' = 0.007) in the former group of patients while there was a decrease in IFN-gamma among HLA B27-positive patients ('p' > 0.05).

Present study showed Pro-inflammatory cytokine response was observed in patients with *C. trachomatis*-induced ReA with pleiotropic cytokine, IL-6 appearing as the key player.

ADULT STEM CELL BIOLOGY

1. Pre-clinical trial groundwork towards Cultured Epithelial Autograft application studies in burns patients. 2014-16

A cost-effective method of growing cultured epidermis for application in burns has been standardized at our laboratory comprising of in vitro cultivation of epidermal sheets using commercially available human epidermal keratinocytes and SWISS 3T3 cells as feeders after growth arresting the feeders with low concentrations of Mitomycin C (Chugh et al 2015) and a Prototype has been prepared (Yerneni and Chug 2014). Now, the technique needs to be translated into application in burns patients. It is therefore, necessary to simulate large-scale production of Cultured Epithelial Autografts (CEA) from the human skin biopsy as the start up material employing the in-house technique. It is further proposed to perform Quality Control and Quality assurance issues.

We have completed estimation of traces of mitomycin C in the final product by HPLC-MS and found the traces only at earlier steps of feeder processing which reduced to undetectable levels in the qualified feeders and also in the cultured epidermis. The chromosomal stability in the cultured

keratinocytes of 3rd and 4th passages by G-banding suggested normal chromosomal structure and number. Although, we have proven that the cultured keratinocytes were devoid of anchorage independent growth in the *in vitro* tumorigenesis assay, additional *in vivo* tumorigenesis assessment in Nude mice is being planned.

2. Technology to grow non-xenogeneic CEA through the use of human dermal fibroblasts as feeders.

This project is aimed at using the human neonatal dermal fibroblasts as feeders on similar lines of the established technique of cultured epithelial autografts using Swiss 3T3. Therefore, the present proposal is aimed at achieving optimal feeder functionality through our exposure cell density based derivation of mitomycin C dosing followed by volumetric titrations on a safe cell density. The primary objective of dose derivation has been completed. The subsequent volume titrations have indicated differential feeder extinctions with concentration of lower mitomycin C which is unreported so far. Further fine-tuning experiments are necessary to pin point accurate conditions for co-culture with keratinocytes. The preliminary experiments were conducted to isolate the keratinocytes from co-cultures. However, strategies of using EDTA as known with mouse feeders have not given satisfactory isolation and newer strategies are being probed.

ENVIRONMENTAL TOXICOLOGY

1. Human environmental biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and intrauterine growth restriction (IUGR)

This study has been designed to examine the association between IUGR and PAH exposure in expectant women. During the year under report samples of maternal and cord blood, placenta and urine were collected from additional 55 IUGR and 10 AGA deliveries in labour rooms of Safdarjung Hospital, New Delhi. Samples were processed for PAHs exposure analysis. After optimization of the analytical conditions, the extracts obtained from placental tissue and bloods as per the standardized protocol were analyzed for presence of the PAHs residues by HPLC using PDA Detector. Acenaphthylene Phenanthrene and Pyrene have been detected in significant quantity in IUGR cases in comparison to control. The data is being analysed.

2. Biomedical Informatics Centre's of ICMR (Phase-II) at NIP, New Delhi

Biomedical informatics Centre at National Institute of Pathology has initiated research and training facility for biomedical scientists, research scholars and students to promote and support informatics in medical research.

The following initiatives have been taken to achieve primary objectives of the Biomedical Informatics Centre.

- Integrated catalogue on genome wide association studies and drug targets of psoriasis.

- *In silico* screening of TNF-alpha, PDE4 and JAK-2 inhibitors.
- Computational design of Interleukin – 6 antibodies.
- Putative drug target identification against zoonotic pathogens.
- Developed Perl scripts for predicting presence of toxic compound in patient samples.
- Bioinformatics centre web portal to in-house integrated catalogue and databases.
- Organized one National workshop to render Bioinformatics training to 30 Medical and Biomedical scientists/researchers.
- Six M.Sc. students were given training to complete dissertations.
- Developing NIP patient sample electronic record is undergoing.

Publications

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ACCEPTED PUBLICATIONS

1. MeenaLakhanpal, LaishramChandreshwor Singh, TashninRahman, Jagnnath Sharma, M. Madhumangal Singh, Amal Chandra Kataki, SaurabhVerma , SanthiLathaPandrangi, Y. Mohan Singh, SaimaWajid, SujalaKapur, SunitaSaxena "**Study of single nucleotide**

polymorphisms of Tumour necrosis factors and HSP genes in nasopharyngeal carcinoma in North East India." Tumor Biology (Accepted).

2. Nitu Kumari, Pawan Vasudeva, Anup Kumar, **Usha Agrawal**. Adenocarcinoma of urinary bladder: A report of two patients. Journal of Cancer Research and therapeutics (Accepted)

3. Sharma I**, Singh A**, Mishra AK, Singh LC, Ramesh V, Saxena S.
Is CXCL10/CXCR3 axis a better indicator of leprosy type 1 reaction than inducible nitric oxide synthase**?** *Indian J Med Res 2015 (In press)*

4. Mallya V, Siraj F, Sharma KC, **Singh A**. Giant cell glioblastoma with calcification and long-term survival. *Indian J Cancer 2015 (In Press)*

MANUSCRIPT

BOOK CHAPTER

1. Book chapter entitled "Human pathogenic fungi and their survival mechanisms in the host". Authors- Tapish Dogra, Lakshna Mahajan, Santosh K. Upadhyay, Poonam Gautam in Book on "Applied Microbiology: Microbes in Action" Editors- Neelam Garg and Abhinav Aeron and Publisher**- Nova Science. (Accepted, in Press)**

Awards, patents obtained/filed during the year

1. Dr Poonam Salotra was elected Fellow of the National Academy of Medical Sciences, India (FAMS) in Sep 2014.

2. Dr Poonam Salotra was elected Fellow of The World Academy of Sciences (FTWAS) in Sep 2014.

3. Dr. Sunita Saxena received "Dr. PN Wahi award of ICMR.

4. LK Yerneni and RM Chugh (2014). A method for processing of feeder cells suitable for adult stem cell proliferation. File Number 3115/DEL/2014, Filing date 30/10/2014.

Future Program

- Third party validation and release of kit of Monoclonal antibody for diagnosis of *c. trochomatis*
- Third party validation and release of kit of DOT-BLOT assay for diagnosis of sequalae of *C. trochomatis* using cHSP60
- Commercialization of **LAMP assay** for detection of L. donovani in clinical samples.
- Development of Live attenuated vaccine for Leishmaniasis
- Establishment of cGMP facility at NIPto grow cultured epithelial autografts for auto transplantation in burns patients.
- To establish tumor tissue bank at NIP
- To get Detailed Project Report prepared for submission before cabinet for getting University status for ICMR
- To monitor activities at MRHRU, Bhunga, Hoshiarpur like construction of building, purchase of equipments and initiation of research projects.
- Renovation of Labs and administrative block.

WORKSHOPS/SEMINARS ORGANIZED AT
National Institute of Pathology

1. Organized a Hands on workshop on "In-situ Hybridization" from 2nd-4th April 2014.

2. Organized 14th Smt. Pushpa Sriramachari Foundation Day Oration delivered by Dr. Chandrima Saha, Director, National Institute of Immunology, New Delhi on "Decision to live or die-a cellular view" on 7th May 2014.

3. Organized workshop on Hands on training in "Basic Flow Cytometry and its application" from 28th -29th August 2014.

4. Celebrated Rashtriya Ekta Diwas on 31st October 2014. The message of DG, ICMR was broadcasted through video conferencing in all the institutes.

5. Organized Scientific Advisory Committee at NIP on 18th Nov. 2014.

6. Vigilance awareness week was celebrated from 27th Oct. - 1st Nov. 2014.

7. Organized workshop on "Tissue Microarray" from 18th – 19th Dec. 2014.

8. Organized Foundation workshop in clinical and laboratory medicine research for Member countries of South Asian Forum for health research (SAFHeR) from 9th – 12th Feb. 2015.

9. Organized a Hands on workshop on " In-situ hybridization" from 12th – 13th Jan. 2015.

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10. Organized National workshop on “Bio-informatics tools for Biomedical Research” from 24th -27th March 2015.

11. Hands on workshop on “Molecular cloning and expression of recombinant proteins”. From 18th - 20th March 2015.