ANNUAL REPORT

2013 - 2014

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)
PO Box 4909, Safdarjang Hospital Campus, New Delhi 110 029
Website : www.instpath.gov.in Email : info@instpath.gov.in

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CONTENTS

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

Executive Summary

The scientists at National Institute of Pathology, New Delhi have continued their pursuit for cutting edge research in the thrust areas of research mainly tumor biology, infectious diseases including *leishmaniasis, tuberculosis, leprosy* and *chlamydiasis*, stem cell biology and environmental toxicology. The scientists conduct both basic as well as translational research leading to development of **Vaccines** for prevention and **Biomarkers** for screening, diagnosis, prognosis and prediction of drug response/resistance for various diseases **with mission to bring lab to bed**. The annual report provides the panoramic view of progress, achievements and other activities undertaken during the year under report.

Under translational research at NIP we have developed monoclonal antibody to *C.trachomatis,* DOT –BLOT assay for diagnosis of sequalae to *Chlamydia trachomatis* infection using cHSP60, constructed tissue microarray for various cancers viz breast, prostate, urinary bladder, gliaomas, mennigiomas and oesophageal cancer. Efforts are going on for development of LAMP assay for detection of *L. donovani* in clinical samples; Live attenuated Leishmania Vaccine and a cost-effective & improved technology to grow cultured epithelial autografts (CEA) for burns patients.

Studies in Tumor biology are focused on breast, prostate and North east cancers. Study of gene expression in early onset (<40 years) and late onset (>55) breast cancer cases have identified discriminating signatures associated with early onset cancers, ER/PR +ve, HER2+ve, TNBC and TPBC cancer subtypes. Also patterns specific to Stage 2 and Stage 3 of breast cancer were also identified. Amongst the ER/PR +ve, HER2+ve groups 108 gene were getting commonly upregulated while 387, 137, 412, 115 were uniquely associated and upregulated with ERPR+ve, HERNU+ve, TPBC, TNBC groups respectively. In these groups 300 genes were commonly downregulated while 308, 86, 322, 61 genes were individually

downregulated amongst groups ERPR+ve, HERNU+ve, TPBC,TNBC respectively. Whole exome sequencing of 14 breast tumors and 3 control samples was carried out which showed presence of 55 thousand SNPs, about 2500 insertions and 2300 deletions in the breast tumors.

Isolation of breast cancer stem cells had been done using $CD44^{+}$ and $CD24^{-}$ markers from MDA-MB-231 (triple negative), T47D (triple positive) and MCF7 (triple positive), NIPBC1 (triple negative) and NIPBC2 (triple negative) cell lines. The cancer stem cells ($CD44^{+}/CD24^{-}$) were found 96% MDA-MB 231 cell line and 0.5 % in MCF7 cell line. In T47D cell line cancer stem cells were 0.1%. In the cell lines we have developed in our laboratory earlier, NIPBC-1 and NIPBC2, found 0.2% and 0.1% of CD44+/CD24- cells respectively. The isolated CD44+/CD24- cells from these cell lines showed mammosphere formation in non adherent conditions with features of self renewability. To understand the role of androgen receptor signaling in breast cancer bioinformatics tools were used to down select 10 novel AR regulated genes in breast cancer cell-line MDA-MB-453. AR was found to up regulate the cell cycle genes and down regulate the apoptotic genes. DHT (Androgen receptor agonist) stimulation was found to increase cell growth and this effect can be blocked by drugs like bicalutamide. Collectively the data enhances our understanding of AR function in breast cancer and it provides novel targets for the therapeutic intervention. Study on association of environmental risk factors and polymorphisms in DNA repair and cell cycle genes (TP53-72Arg>Pro, RAD51-135G>C, BRCA2, and CCND1-G870A) with breast cancer risk in Northeast Indian population showed betel quid chewing conferring an elevated risk for breast cancer attributable to betel quid carcinogens and minor roles of BRCA2 mutation and C allele of RAD51. Gain of 7q33 in betel quid chewers harboring the AKR1B10 gene was seen and the enhanced expression of AKR1B10 in tobacco associated breast cancers was found.

A prospective case control study to evaluate the diagnostic and prognostic value of TMPRSS2-ERG gene fusion and PCA3 molecules for prostate cancer showed that both PCA3 and TMPRSS2-ERG have significant additional predictive value for risk calculation. TMPRSS2:ERG fusion marker is highly specific (100%) for prostate

cancer as none of BPH sample was positive for fusion transcript. This fusion marker showed significant association with serum PSA (p=0.01), DRE (p=0.01), prostate volume (p=0.008), Gleason's score (p=0.016) and number of cores positive for cancer in 12 core TRUS biopsy (p=0.021).

Genome-wide analysis of genetic alterations in patients with esophageal cancer from NE India using single nucleotide polymorphism arrays identified FGF12 as a potential biomarker. The functional role of FGF12 was validated after knockdown of this gene with inhibition of more than 50% of cancerous cells. Hypermethylation of tumor suppressor genes CDH1, OPCML, NEUROG1, TERT and WT1, and hypomethylation of SCGB3A1, THBS1 and VEGFA genes were found in oesophageal cancer patients of NE India. Higher expression of enzymes regulating methylation (DOT1L and PRMT1) and acetylation (KAT7, KAT8 and KAT2A) of histone proteins was found in tumor.

Nasopharyngeal carcinoma (NPC) in North east region showed high association of HLA class I region and expression of EBV. *TNF* β (+252A>G) and *HSP 70* (+2437 T>C) genes polymorphisms also showed significant association with occurrence of NPC. The association with HLA Class III sub region in NPC pathogenesis is important since it is known to play an important role in the escape of tumor cells from host immune surveillance and may be responsible for decreased recognition and killing of cancer cells.

In Acute Myeloid Leukemia cases, seven ATP-binding cassette (ABC) transporters were found to express more than 1.5 fold expression (ABCA1, ABCA3, ABCB5, ABCC6, ABCE1, ABCF1 and ABCG1). The over expression of three genes (ABCA1, ABCF1 and ABCG1) was further verified by real-time PCR in 30 patients of adult AML and in 5 samples of healthy person. Incubation of cell line THP-1 and K-562 with chemotherapeutic drugs induced consistent up regulation of ABCF1 and ABCG1. These two transporters may play significant role in drug resistance in AML.

Increasing incidence of relapse in visceral leishmaniasis (VL) cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard

efficacy. The transcriptome profiling of clinical isolates of *Leishmania donovani* from pretreatment and relapse group revealed approximately 1800 genes comprising ~20% of total Leishmania genome differentially modulated and falling 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). 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. Transfection of this gene into miltefosine sensitive *L. donovani* parasites 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.

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 of resistance towards PMM to ensure its long term effectiveness. Tolerance to PMM was induced in three different field isolates which showed 6-7 fold reduced susceptibility towards the drug. To validate the role of ABC transporters in paromomycin resistance, the susceptibility of the PMM resistant and wild type isolates towards paromomycin was compared 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.

Although majority of individuals with history of visceral leishmaniasis (VL) exhibit strong immunity to re-infection, the mechanism of resistance is poorly understood. Evaluation of immunological mechanism associated with resistance to the disease in healed VL (HVL) individuals showed significantly higher lymphoproliferation,

cytokines and granzyme B levels in HVL group compared to naive or VL group with 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

Genital *Chlamydia trachomatis* infection causes serious disease sequelae, one of which is Reactive Arthritis (ReA). Study on association between HLA-B27 and genitourinary-induced ReA showed that among 23.3% *C. trachomatis*-positive ReA/ uSpA patients, 57.1% were positive for HLA-B27 gene also. All infected patients positive for HLA-B27 showed moderate to severe effusion with multiple joint involvement and disease duration <6 months. Results suggest that the presence of HLA-B27 gene probably causes moderate to severe disease in the *C. trachomatis*-positive ReA/ uSpA patients and screening for HLA-B27 should be considered in such patients. Identification of antigenic components of *C. trachomatis* which elicit T-cell mediated responses showed that CD4+ T-cells play a major part in controlling chlamydial infection, probably through the production of IFN-gamma. Another T-cell derived cytokine relevant to joint destruction, cartilage breakdown and bone erosion might be IL-17. High level of IFN-gamma and IL-6 were found in the synovial fluid (SF) of *C. trachomatis*-positive ReA/ uSpA patients in comparison to uninfected control RA/ Osteoarthritis (OA) patients. IFN-gamma was also significantly elevated in SF of infected ReA/ uSpA patients in comparison to uninfected ReA/ uSpA patients. IL-17 in SF/ serum was significantly enhanced in comparison to non-inflammatory control patients, while serum IL-17 was comparable in *C. trachomatis*-positive ReA/ uSpA, *C. trachomatis*-negative ReA/ uSpA and RA patients.

The complete genome sequences of the 44 Mycobacterial species available in the public domain had been undertaken. The *Mycobacterium sp. MOTT36Y* (MMM, 5613626bp) represents the opportunistic pathogens (OP) group of mycobacteria

closest to *MIP* (5589007 bp), in terms of genome size. Amongst the OP group of mycobacteria, those closest to *Mycobacterium intracellulare* (5402402<->5501090 bp) are *Mycobacterium sp. MOTT36Y* (MMM, 5613626 bp), *Mycobacterium avium 104* (MYCA1, 5475491 bp) and *Mycobacterium abscessus ATCC 19977* (MYCAB, 5090491 bp). It is an interesting observation that based on the genome size the MYCA1 genome - an OP, fits between *Mycobacterium intracellulare MOTT-64* (MIR, 5501090 bp) strain and *Mycobacterium intracellulare MOTT-02.* Although the genome size of opportunistic and true pathogens are reduced compared to the genome size of nonpathogen (NP), the genome of true pathogens (TP) have acquired few enzyme-coding genes. Therefore, they may have a likely association between these acquired enzymes and the virulence of these OPs and TPs. One of these encodesferredoxin-dependent sulfite reductase (encoded by the*nirA*gene), is active during the dormant phase and has been reported to be a potential drug target for *Mycobacterium tuberculosis.* Four signature sequences specific to *M. tb* have been identified as improvement on already available diagnostic tests for tuberculosis treatment which are being validated on a large scale in patients

Investigations have been carried to use of SWISS 3T3 cells to develop a culture system for growing Cultured Epithelial Autografts, by adopting a cost effective strategy of employing mitomycin C (MMC) at a reduced concentration and to characterize the epidermal sheets produced thereof. The results indicated that it was the stimulation of colony initiation rather than increase in mitosis per colony that turned out to be the advantageous consequences of fine-tuning of MMC treatment by including both concentration and dose per cell as compared to γ-Irr feeders. Studies had been also under taken to identify a cost-effective non-xenogeneic product suitable for resurfacing burn wounds.

To investigate on the association between Intra Uterine Growth Retardation (IUGR) and PAH exposure in pregnant women residing in and around Delhi, analysis has been done for various PAH extraction in placental tissue, maternal and cord blood by HPLC which showed presence of Naphthalene, Pyrene, Acenaphthylene, Phenanthrene, Chrysene, Benzo(a) anthracene, Benzo(g,h,i)perylene, Benzo(k) fluranthrene, Benzo(a)pyrene and acenaphthene. On the basis of the preliminary

results, Naphthalene is highly detected in 39% IUGR and 8% AGA cases. Pregnant women may be exposed through indoor air pollution, moth balls and tobacco smoke. Second highest PAH, detected is pyrene in 32% of IUGR cases while 5% control also showed the presence of pyrene who may be exposed through dietary sources (grilled and smoked food) and second hand cigarette smoke.

To increase visibility in state medical colleges and to transfer recent technologies to medical and biomedical students various interactive workshops, special training programs have been organized throughout the year. The academic activities have been continued for training PhD and DNB students with vigour. Dr Poonam Salotra was appointed Member of Regional Technical Advisory Group, WHO and member of the WHO Advisory Panel on Parasitic Diseases (Leishmaniasis). One **p**atent had been granted for "Development of DOT–BLOT assay for diagnosis of sequalae to *Chlamydia trachomatis* infection using cHSP60 and another patent had been filed by ICMR for "Loop mediated isothermal amplification (LAMP) assay for a reliable and rapid diagnosis of *Leishmania* infection". Overall 2013-14 year had been productive through significant contributions in areas of basic, translational and clinical research. I take this opportunity to convey my thanks to Dr. V. M. Katoch , Director General, ICMR and Secretary, Department of Health Research for his support for infrastructure development and encouragement for scientific and academic programs. I acknowledge my sincere thanks to my scientific, technical and administrative staff for their contribution towards enhancement of infrastructure and scientific activities.

DR.SUNITA SAXENA

LIST OF ONGOING PROJECTS

1. Study on gene expression and hypermethylation profiles in early onset breast cancer.
2. Study on micro RNA signatures associated with breast cancer stem like cells (CSCs) and their role in drug response.
3. Targeted sequencing of breast cancer specific genes in early-onset breast carcinoma.
4. Understanding the role of androgen receptor signalling in breast cancer.
5. Induction of autophagy by drug-resistant breast cancer cells: functional involvement and mechanism of action.
6. Association of DNA Repair and Cell Cycle Gene Variations with Breast Cancer Risk in Northeast Indian Population.
7. Expression of aldo-ketoreductase family 1B10 (AKR1B10) gene in Breast carcinoma: The effects on drug and tobacco exposure.
8. Study on characterization of TMPRSS2: ERG and PCA3 as prostate cancer Biomarkers in Indian patients
9. Immunogenetic profile of nasopharyngeal cancer in a high-prevalence region of North-east India.
10. Epigenetic studies in esophageal cancer in high risk region of Northeast India.
11. Genome-wide analysis of genetic alterations in patients with esophageal cancer from north-east India using single nucleotide polymorphism arrays.
12. Molecular mechanism of drug resistance in acute myeloid leukemia (AML): Role of ATP-binding cassette (ABC) transporters.

13. Dynamic regulation of lymphocyte signaling of acute leukemia.

14. Mechanism of resistance to Miltefosine (MIL) in *Leishmania donovani*.

15. Studies on mechanism of resistance towards paromomycin in *Leishmania donovani* parasite.

16. Protective immunogenicity of Centrin knock-out live attenuated *Leishmania* parasite in the animal models and in human cells.

17. Development of new live attenuated vaccine candidates for Kala-azar.

18. Ubiquitin related modifier 1: A post translational modification machinery in *Leishmania donovani*.

19. Development of Loop-mediated isothermal amplification (LAMP) assay for diagnosis of Leishmania infection.

20. Understanding the underlying mechanism of macrophage immune modulation: Role of Resistin.

21. Comparative analyses of non-pathogenic, opportunistic and totally pathogenic mycobacteria reveal genomic and biochemical variability and highlight the survival attributes of *Mycobacterium tuberculosis.*

22. Immunopathogenesis of reactive arthritis induced by *Chlamydia trachomatis*.

23. A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture

24. Technology to grow non-xenogeneic CEA using human dermal fibroblasts as feeders.

25. Human environmental biomonitoring of polynuclear aromatic hydrocarbons (PAHs) in urban megalopolisof NCR Delhi and investigate the association between PAH exposure and intrauterine growth restriction.


RESEARCH
ACTIVITIES


TUMOR
BIOLOGY

TUMOR BIOLOGY

BREAST CANCER

1. Study on Gene Expression and Hypermethylation Profiles in Early Onset Breast Cancer

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Dr. Sujala Kapur, Ms. Shreshtha Malvia

In collaboration with : Dr. Chintamani, Dr. R. S. Mohil, Department of Surgery, Safdarjang Hospital, New Delhi
Dr. A. Bhatnagar, Department of Cancer Surgery, Safdarjang Hospital, New Delhi

Duration : 2008-14

Aims, Objectives & Background:

Breast cancer diagnosed at young age exhibits aggressive features such as large tumor size, high histologic grade, positive lymph nodes, triple negative phenotype and high S-phase fraction. In the present study, we aimed to elucidate genetic and epigenetic factors associated with early onset breast cancer in Indian women, with objectives to understand molecular pathogenesis and identify genetic risk factors.

Work done during the year:

Study of gene expression in early onset (<40 years) and late onset (>55) breast cancer cases have identified discriminating signatures associated with early onset cancers, ER/PR +ve, HER2+ve, TNBC and TPBC cancer subtypes. Also patterns specific to Stage 2 and Stage 3 of breast cancer were identified. Amongst the ER/PR +ve, HER2+ve groups 108 gene were getting commonly

upregulated while 387, 137, 412, 115 were uniquely associated and upregulated with ERPR+ve, HERNU+ve, TPBC,TNBC groups respectively. In these groups 300 genes were commonly downregulated while 308, 86, 322, 61 genes were individually downregulated amongst groups ERPR+ve, HERNU+ve, TPBC,TNBC respectively (Fig.1a).

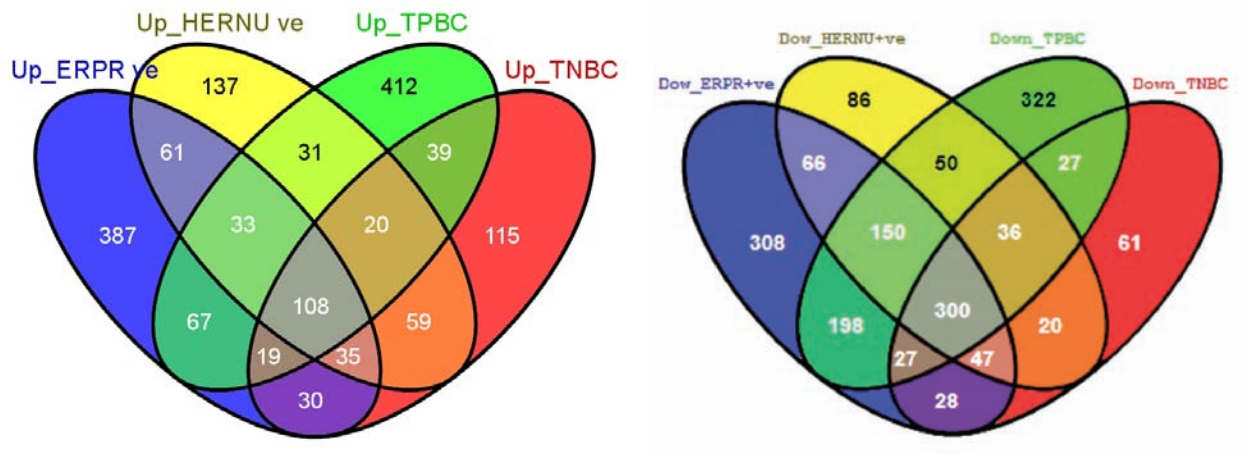


Fig.1a: Venn diagram for common genes identification

When we compared different stages, 256 genes were commonly upregulated in stage 2 vs stage 3 (more aggressive stages) and 621 genes downregulated. While 151 and 223 genes were uniquely upregulated in stage2 vs stage 3 and 260 and 186 were uniquely downregulated amongst them (Fig.1b).

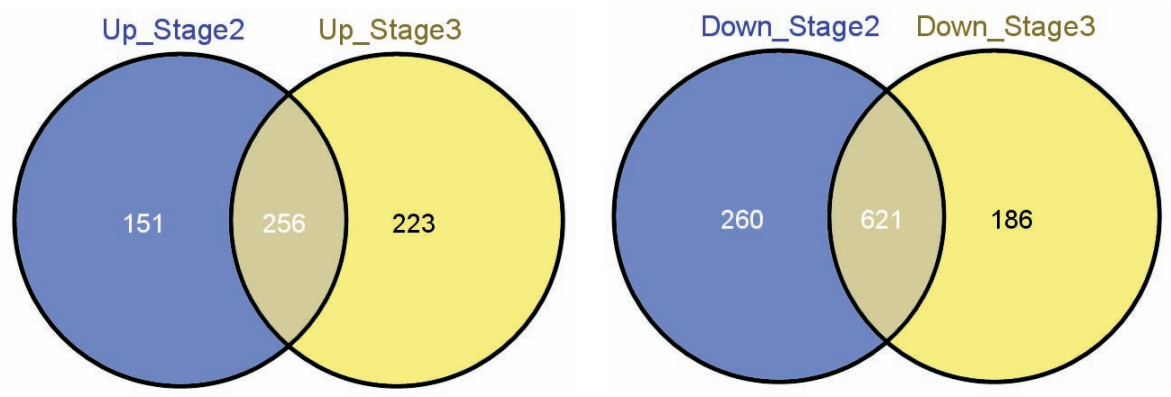


Fig. 1b: Venn diagram for common gene identification

To gain further insight into regulatory modeling, Gene Regulatory Network analysis of these genes was performed and various differential genes amongst the groups were identified. When comparing stage 2 vs stage 3, we identified FBLN1, FBLN7, PLAU, TWIST1, CDH1, KIF25 significantly differentially regulated and receptor wise TPBC and TNBC showed SDC1, COL1A1, CCBE1, COMP, ITGA11 to be differentially regulated amongst the groups.

Methylation analysis showed 672 cpg sites differentially methylated in early onset and 689 in late onset breast cancers, hypermethylation of 246 genes being unique to early onset and 347 genes to late onset cancers. Merging of gene expression and methylation data identified various significant genes where the hypermethylation is associated with suppression in gene expression and hypomethylation associated with overexpression. Major pathways identified using KEGG are; cAMP signaling pathway, cell adhesion molecules, cGMP-PKG signaling pathway, cytokine-cytokine receptor interaction, neuroactive ligand receptor interaction were coming significant when late gene expression was merged with late methylation, while cell cycle, apoptosis, TNF signalling pathway, transcriptional misregulation in cancer were major differential pathways in early onset breast cancer (Fig. 2).

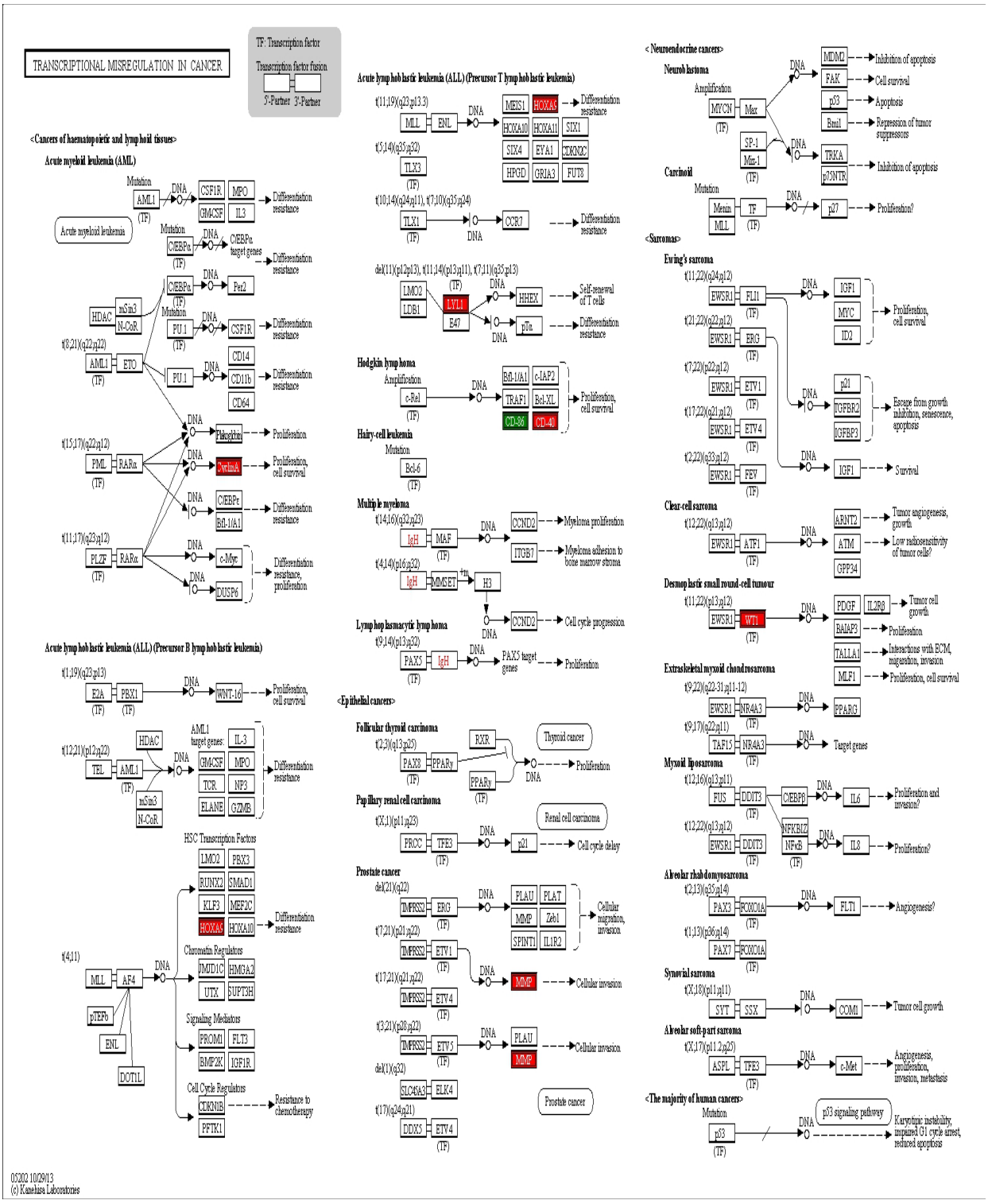


Fig. 2: Transcriptional Misregulation in Cancer Pathway

2. Targeted Sequencing of Breast Cancer Specific Genes in Early-Onset Breast Carcinoma

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Ms. Shreshtha Malvia

In collaboration with : Dr. Chintamani, Dr. R. S. Mohil, Department of Surgery, Safdarjang Hospital, New Delhi
Dr. A. Bhatnagar, Department of Cancer Surgery, Safdarjang Hospital, New Delhi
Dr. Deepshikha Arora, Dr. Ramesh Sarin, Indraprastha Apollo Hospital, New Delhi

Duration : 2015-15

Aims, Objectives & Background:

This study has been undertaken to identify sequence variations and chromosomal rearrangements of deregulated genes in early onset breast cancer along with validation of the genetic variations in different subsets of tumor viz., ER, PR and ErbB2 status, stages, etc

Work done during the year:

During the year under report, the whole exome sequencing of 14 breast tumors and 3 control samples was carried out. We have analyzed 7 early (≤ 40 years) and 7 late (≥55 years) breast cancer cases. The cases fall mainly into 3 groups, Triple negative (4), Triple Positive (4), ErbB2 positive (4). Targeted sequencing has shown presence of 55 thousand SNPs, about 2500 insertions and 2300 deletions in the breast tumors (Fig.1a). When the mutation distribution was analyzed for their distribution among various portions of genes, we found majority of them in introns followed by exons, UTRs and splice sites in descending order (Fig. 1b).

Currently the analysis of the data is underway.

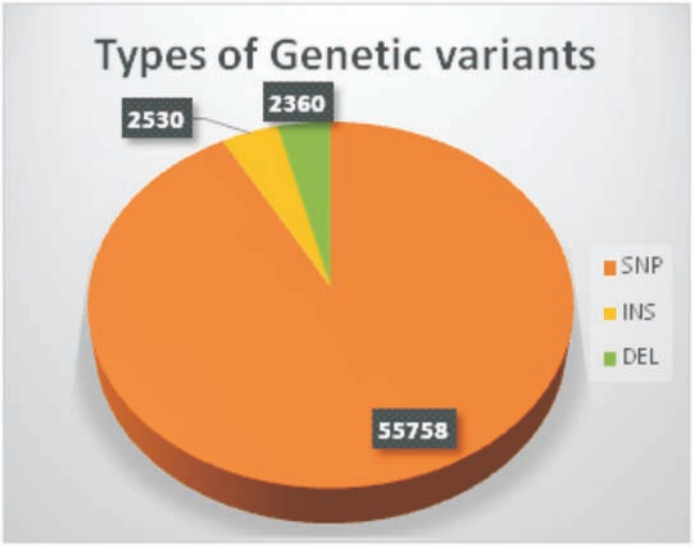


Fig. 1a: Showing the types of genetic changes

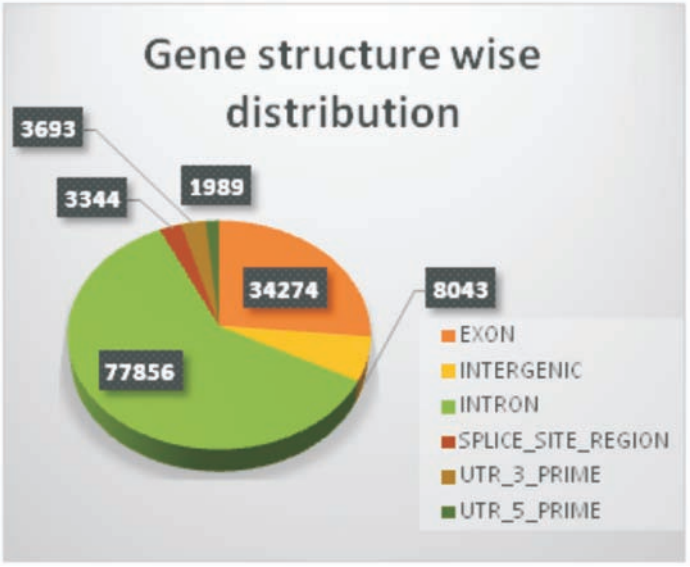


Fig. 1b: Showing the number genetic changes that are attributed to various parts of genes in breast tumors

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

Scientific staff : Dr. Sunita Saxena, Dr. S.A. Raju Bagadi, Dr. Saurabh Varma, Ms. Renu Yadav

Duration : 2013-15

Aims, Objectives & Background:

This study is aimed to identify miRNA signatures assoicated with breast cancer stem cells and to understand their contribution for the response of cancer cells to chemotherapeutic agents, resistance to therapy and recurrence of tumor.

The study proposes the estimation of the proportion of CSCs present in various breast cancer cell lines and their correlation with their phenotype (ER+/- and triple negative), identification of miRNA, gene expression and methylation profiles associated with CSCs compared to bulk tumor cells and relevant pathways responsible for stem cell characteristics of CSCs by functional characterization of miRNAs and their association with drug response.

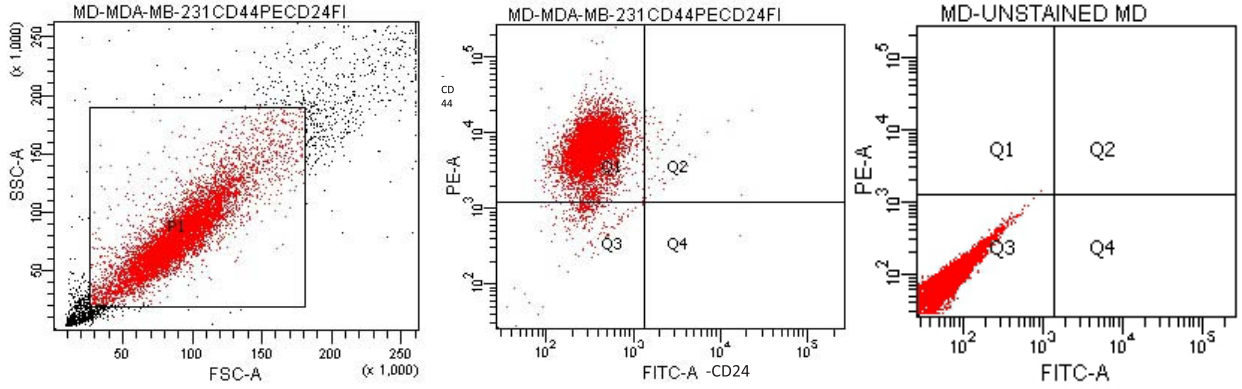
Work done during the year:

Isolation of Cancer stem cells:

Isolation of breast cancer stem cells had been done using $CD44^+$ and $CD24^-$ markers for stem cells by FACS Aria cell sorter. $CD44^+/CD24^-$ cells are widely accepted markers for cancer stem cells. Cancer stem cells were isolated from MDA-MB-231(triple negative), T47D (triple positive) and MCF7 (triple positive), NIPBC1 (triple negative) and NIPBC2 (triple negative) cell lines. The cancer stem cells ($CD44^+/CD24^-$) were found 96% MDA-MB 231 cell line and 0.5 % in MCF7 cell line. In T47D cell line cancer stem cells were 0.1% (Fig. 1c). In the cell lines

we have developed in our laboratory earlier, NIPBC-1 and NIPBC2, found 0.2% and 0.1% of CD44+/CD24- cells respectively (Fig.1).

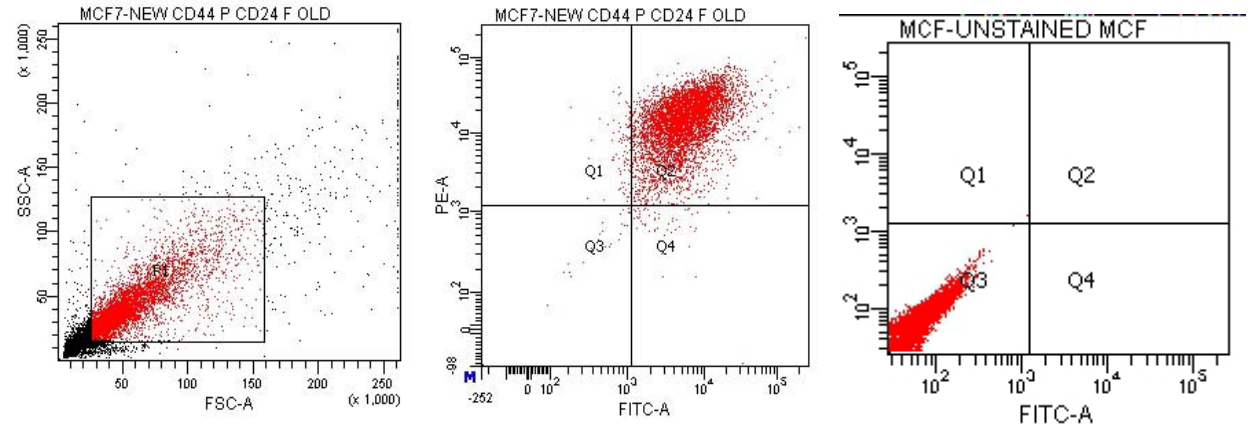
Fig. 1: Isolation of CD44+/CD24- cells from breast cancer cell lines



Tube: MDA-MB-231CD44PECD24FI

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	8,095	81.0	81.0
Q1	7,750	95.7	77.5
Q2	66	0.8	0.7
Q3	276	3.4	2.8
Q4	3	0.0	0.0

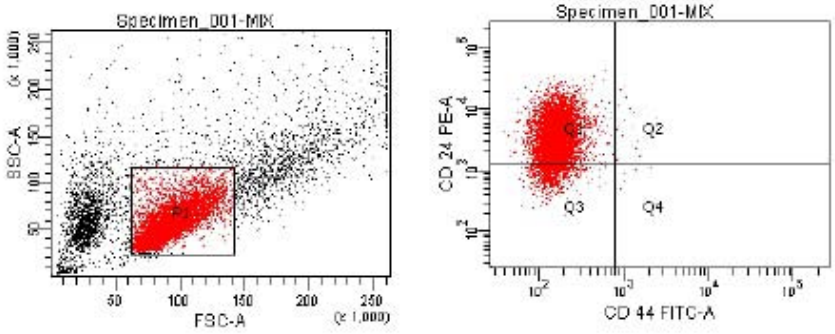
Fig. 1a: MDA-MB231



Tube: NEW CD44 P CD24 F OLD

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	5,770	57.7	57.7
Q1	81	1.4	0.8
Q2	5,592	96.9	55.9
Q3	28	0.5	0.3
Q4	69	1.2	0.7

Fig.1b: MCF7



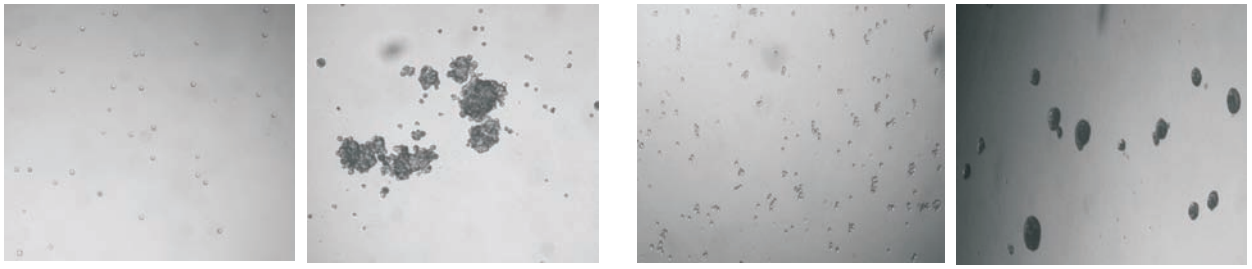
Tube: MIX

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	6,590	65.9	65.9
Q1	5,648	85.7	56.5
Q2	19	0.3	0.2
Q3	916	13.9	9.2
Q4	7	0.1	0.1

Fig. 1c: T47D

Since formation of spheres in non-adherent conditions is associated with stem cell properties of the cells; we tested the ability of the $CD44^{+}/24^{-}$ cells separated from the cell lines to form mammospheres. The $CD44^{+}$ and $CD24^{-}$ population sorted flow cytometer was seeded onto non-adherent plates and were grown for 7-10 days. We have optimized seeding densities by testing various dilutions, 100-5000 cells/well in a 24 well plate. When, 1000 cells per well were seeded, MDA-MB-231 has formed several rough edged colonies (4-8 colonies/field) but smaller (~ 60-70uM) diameter on non-adherent conditions, while MCF7 has formed fewer smooth edged colonies (2-4 colonies/field) compared to MDA-MB231, but larger colonies compared to MDA-MB-231, upon 5 days culture (Figure 2, Table 2). T47D which is a triple positive cell line (similar to MCF7), has formed smooth edged colonies (2-4 colonies/field). NIPBC-1 and NIPBC2 have also formed smooth edged colonies (2-4, 1-3 colonies/ field respectively). These mammospheres were digested and disrupted to test their ability to form mammospheres for two more generations to test their self-renewal ability. The mammospheres obtained in P2 were used for isolation of RNA and DNA for miRNA profiling, as at passage 2 we found maximum number of spheres in both cell lines. Similarly, bulk cells remained after separation of CSCs ($CD44^{+}/CD24^{+}$ & $CD44^{-}/CD24^{+}$ cells) were collected used for isolation of RNA and DNA for molecular profiling.

a. Mammosphere formation 1st generation



b. Mammosphere formation 2nd generation

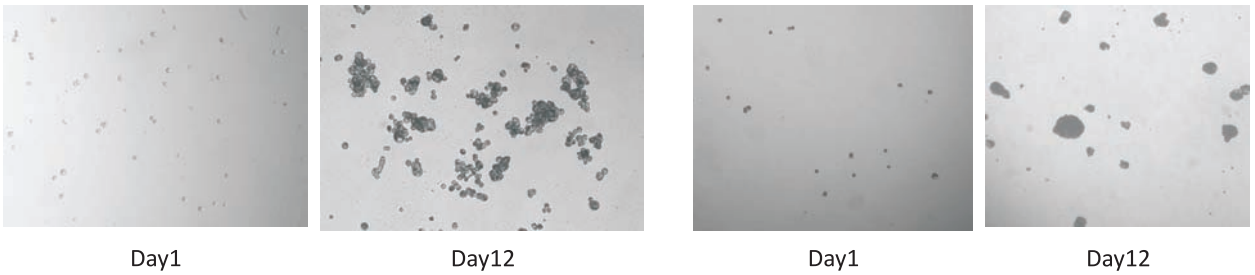


Fig. 2: Showing mammosphere formation in MDA-MB231 and T47D

4. Understanding the Role of Androgen Receptor Signaling in Breast Cancer

Scientific staff : Dr. Sunita Saxena, Dr. Jatin Mehta

Project Duration : 2013-14.

Aims, Objectives & Background:

Androgen Receptor (AR) is an essential transcription factor for the development of secondary sex characteristics, spermatogenesis and carcinogenesis. 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 is still not clear. Breast cancer

is the most prevalent malignancy and primary cause of cancer death in women worldwide. Breast cancer is a heterogeneous disease that encompasses a range of phenotypically distinct tumour types and accounts for 1.38 million new cases of breast cancer worldwide, with a mortality rate of more than 458,000 cases. Traditionally, estrogen receptor (ER) and progesterone receptor (PR) are known to be the prominent players in the progression and development of breast cancer but recent evidences suggest an important role of AR in breast cancer progression. Aim of the present study was 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.

Work done during the year:

A total of 576 candidate genes having prominent role in cell cycle, apoptosis and cellular metabolism were randomly sourced from the cell cycle gene database (www.cyclebase.org, www.itb.cnr.it/cellcycle/), Apoptosis gene database (www.deathbase.org) and metabolic gene database (www.humancyc.org). 576 genes were scanned for the presence of an AR site and it was observed that a total 75 novel genes were having AR binding sites. All the novel genes were found out using the bioinformatics tool TF search (www. http://mbs.cbrc.jp/research/db/TFSEARCHJ). Apart from the scores of an AR binding site in the target gene other factors which were given importance were relationship of the target to AR functions, distance of the AR binding site from the Transcription start site for downsizing the 75 genes to 10 final genes. It was observed that on characterization of the function of the 75 genes, out of the total 75 putative AR targeted genes, approximately 60% had a role in cell cycle, 27% in apoptosis and 13% were found to be involved in the process of metabolism Finally, characterization of 10 putative AR targets was done using real time qPCR to show the genomic actions of these genes. Gene expression profiling of the AR targets was done in MDA-MB-453 breast cancer cell line in presence of DHT for 24 hrs and vehicle control. It was observed that the genes involved in cell cycle

were up regulated, whereas all the genes, involved in the process of apoptosis were down regulated.

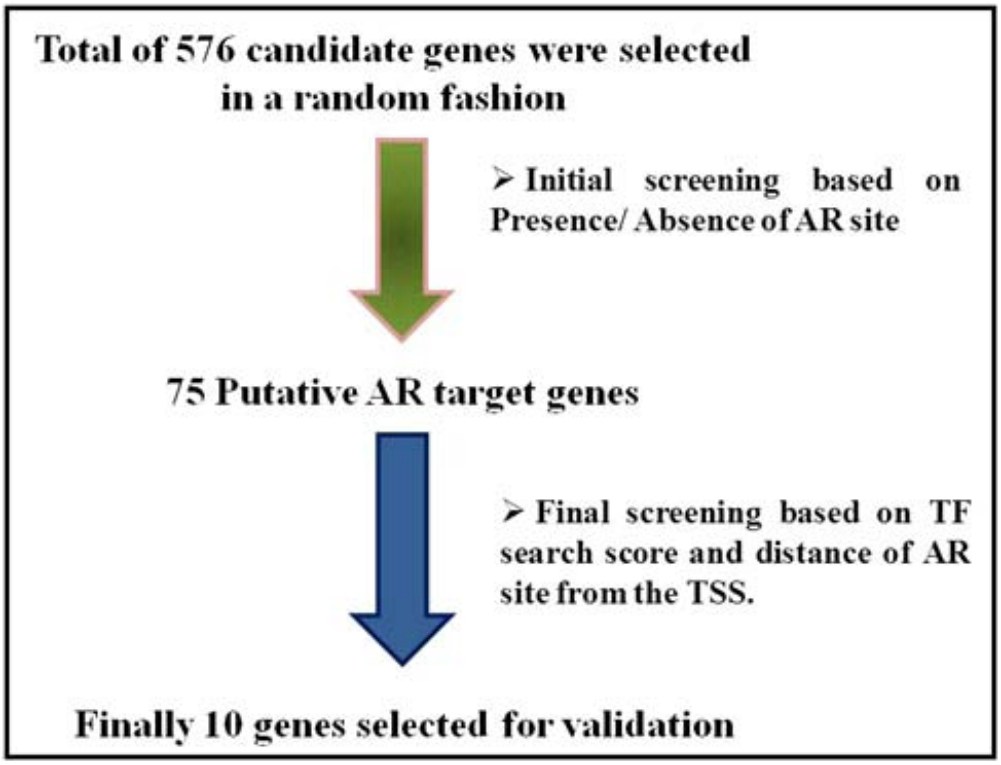


Fig. 1: Strategy for the identification of the novel androgen eceptor (AR) regulated genes. Flow chart of the methodology followed in finding novel AR targets.

5. Induction of Autophagy by Drug-resistant Breast Cancer Cells: Functional Involvement and Mechanism of Action

Scientific staff : Dr. Sunita Saxena, Dr. Avtar Singh Meena

Duration : 2013-15

Aims, Objectives & Background:

Autophagy is a catabolic degradation pathway in which damaged proteins (unfolded or aggregated) or organelles are cleared and digested by lysosomal

enzymes to maintain cellular metabolism. In cancer cells with defect in apoptosis, autophagy allows prolonged survival. Ironically, defects in autophagy are correlated with poor prognosis and increased tumorigenesis; however, mechanism behind this is not studied yet. Chemotherapy or radiotherapy intervention and treatment is crucial and essential for the treatment of breast cancer and achieving favorable prognosis. However, therapeutic outcome of chemotherapy is generally poor due to inherent resistance of cancer cells to the treatment or due to development of acquired resistance. Studies were conducted worldwide to overcome the obstacles associated during the treatment of breast cancer cells, mainly targeting cells which have survived post-treatment. It is speculated that autophagy is involved in mechanisms of cell survival and eventually relapse of tumor after long-term cytotoxic treatment. Therefore, identification and better understanding of novel molecules or exploring the pathways associated might be helpful to overcome resistance.

This study has been under taken to delineate the pathways which are responsible in the development of drug resistance with respect to autophagy.

Work done during the year:

Comparative chemosensitivity profiles in MCF-7 and MDA-MB-231

The aim of the study is to investigate the possible reasons that contribute to lower success rates of cancer chemotherapeutic drugs and the factors that contribute towards inability of cells to positively respond to chemotherapy. In this direction, we are developing drug resistant cells (MCF-7 and MDA-MB-231) in response to various chemotherapeutic drugs including paclitaxel, Trichostatin A (TSA), Phenyl-ethyl-isothiocynate (PEITC), Tamoxifen, Carboplatin and 5-Fluorouracil (5-FU). To study the kinetics of cell death, MCF-7 and MDA-MB-231 cells were treated with increasing concentration of chemotherapeutic drugs for 48 h and cell survival evaluated by MTT assay (Figure 1). The IC50 for chemotherapeutic drugs towards MCF-7 and MDA-MB-231 is summarized in Table 1.

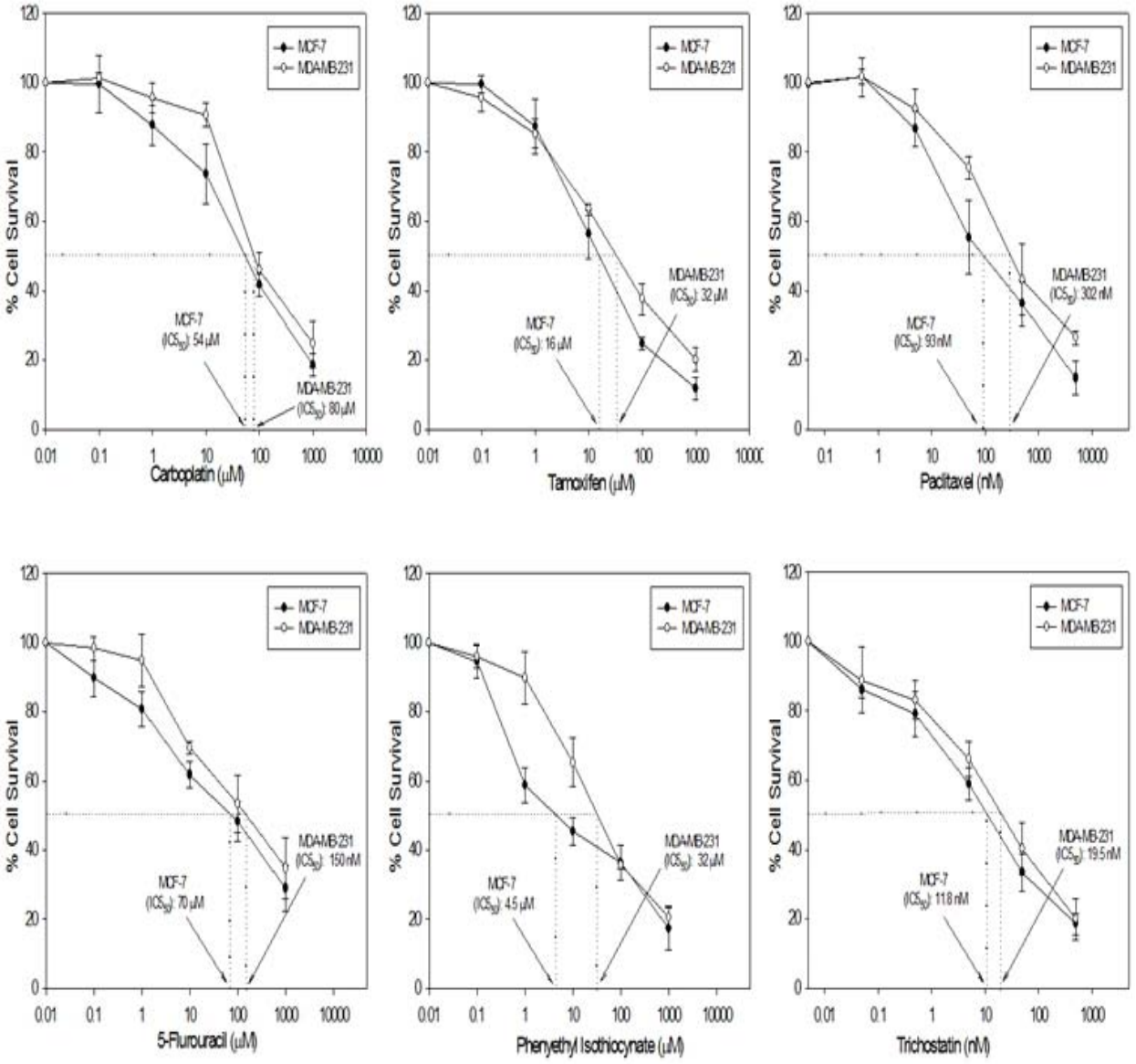


Fig. 1: Dose response curves for human breast cancer cell lines exposed to chemotherapeutic drugs

Table 1: Chemotherapeutic drugs IC_{50} for MCF-7 and MDA-MB-231 breast cancer cell lines

Drugs → / ↓ Cells	Carboplatin	Tamoxifen	Paclitaxel	5-FU	PEITC	TSA
MCF-7	54 µM	16 µM	93 nM	70 µM	4.5 µM	11.8 nM
MDA-MB-231	80 µM	32 µM	302 nM	150 µM	32 µM	19.5 nM

For development of acquired drug resistant cells, MCF-7 and MDA-MB-231 cells will be exposed to increasing concentration of chemotherapeutic drugs. Briefly, cells will be seeded in 35 mm petri plates and allowed to grow. After 24 hrs incubation, drugs (IC50) will be added for another 48 hrs. Subsequently, drug containing medium will be replaced with fresh drug free medium and allow to grow. When confluency is reached, cells will be trypsizined and will be re-exposed to double the dose of drug. This process will be repeated until drug resistant clones are developed. Work on development of drug resistant cells to various chemotherapeutic drugs is undergoing.

6. Association of DNA Repair and Cell Cycle Gene Variations with Breast Cancer Risk in Northeast Indian Population

Scientific Staff : Dr. Mishi Wasson, Dr Sunita Saxena

In collaboration with : Dr. Jagannath D Sharma, Deptt. of Pathology, BBCI, Guwahati
Dr. Amal Chandra Kataki, BBCI, Guwahati
Dr. Eric Zomawia, Civil Hospital, Aizwal

Duration : 2012-13

Aims, Objectives & Background:

Northeast Indian population reportedly has distinct culture and food habits with extensive tobacco consumption. A high incidence of esophageal, gastric and oral cancers associated with both smoking and smokeless tobacco has been documented in this region. Strong association of breast cancer risk with betel quid consumption and its potential in causing chromosomal damage and genetic alterations have been reported in Northeast Indian population.

Polymorphisms in DNA repair and cell cycle genes contribute to increased breast cancer risk. Their association and interaction in relation to betel quid and tobacco chewing habits needs exhaustive multi-analytical investigation to explain Breast Cancer predisposition due to DNA damage.

Work done during the year:

Polymorphism in TP53-72Arg>Pro, RAD51-135G>C, BRCA2, and CCND1-G870A were examined in 204 BC cases and 217 controls from Northeast Indian population. Multifaceted analytic approaches were used to explore relationships between polymorphisms, tobacco history and BC susceptibility. Betel quid chewing was identified as the predominant risk factor. CCND-AA and dominant model showed protection towards BC in BQC (betel-quid chewer) and NBQC (non betel-quid chewer). TP53-Pro/Pro genotype showed protection towards BC in NBQC. RAD51-C allele was associated with BC risk in BQC. Two BQC cases had BRCA2 8415G >T:K2729N mutation in Exon18.

The hierarchical interaction graph showed (figure 1), large independent effect of betel quid chewing (9.38%) among environmental factors for total sample set. A strong interaction (1.32%) was seen between RAD51 and TP53. MDR analysis showed best 4 locus model in NBQC. Interaction diagram concurred the interactions between TP53 and RAD51 (1.32%) with independent effect (1.89%) of CCND1in NBQC (Figure 2). In CART analysis, BQC with CCND1 GG genotype were at risk followed by combination of BQC, CCND1, No-Smk, Alc . Risk was also observed in BQC, CCND1, No-Smk, Non-Alc, TP53 combination and BQC, CCND1, No-Smk, Non-Alc, TP53 (Figure 3). NBQC group showed risk with combination of NBQC and TP53.

These data indicate that common genetic variations in DNA repair and cell cycle genes contribute towards breast cancer risk. In addition, unparallel predisposition was observed amongst BQC and NBQC breast cancer patients rendering dissimilar susceptibility towards breast cancer. BQC might be at an elevated risk for breast cancer attributable to betel quid carcinogens and minor roles of *BRCA2* mutation and C allele of *RAD51.* Whereas NBQC could be at slightly lower risk for breast cancer due to the protection offered by the Pro/Pro-TP53 form. CCND1 polymorphism conferred protection irrespective of the betel quid chewing status.

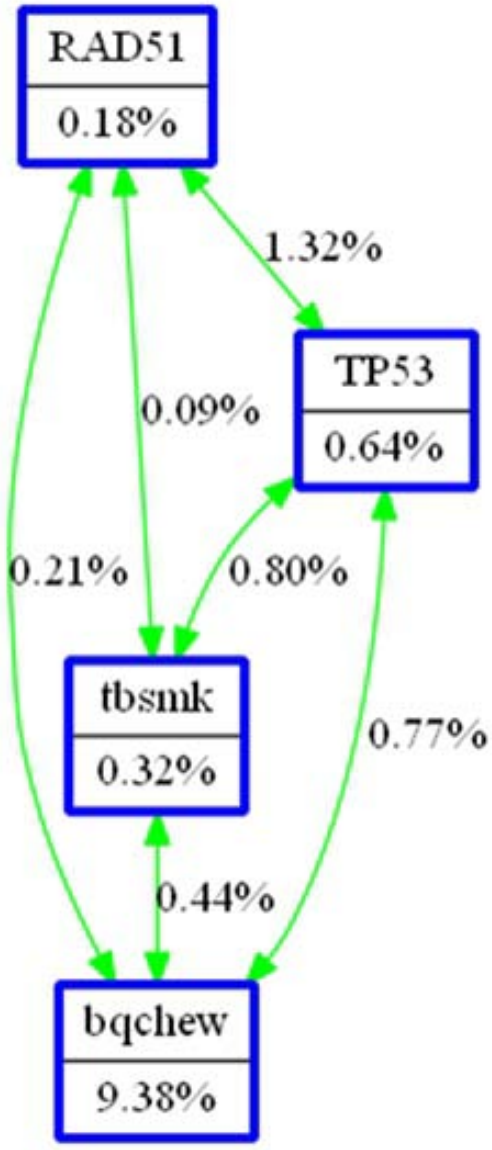


Fig. 1: Interaction dendogram using orange software for the total data set

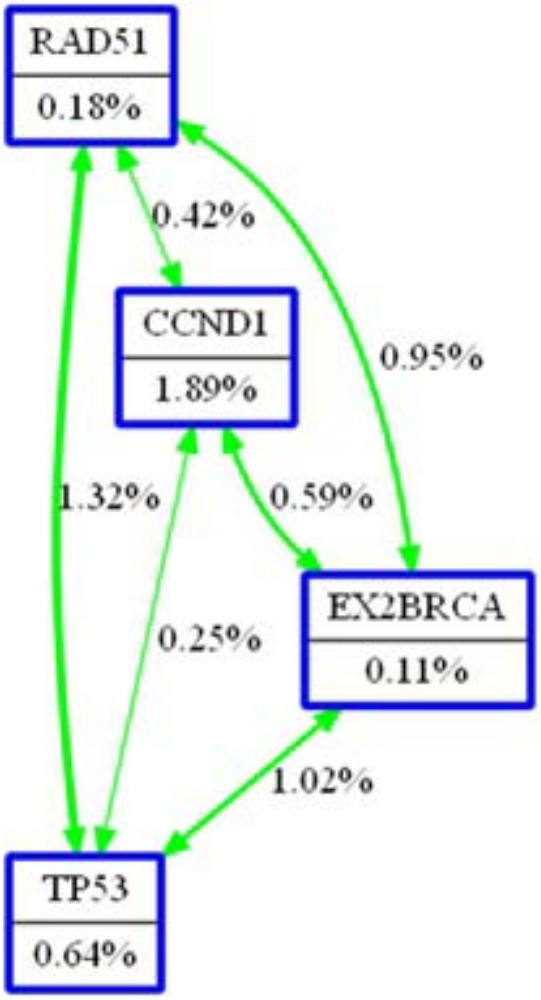


Fig. 2: Interaction dendogram using orange software for the NBQC data set

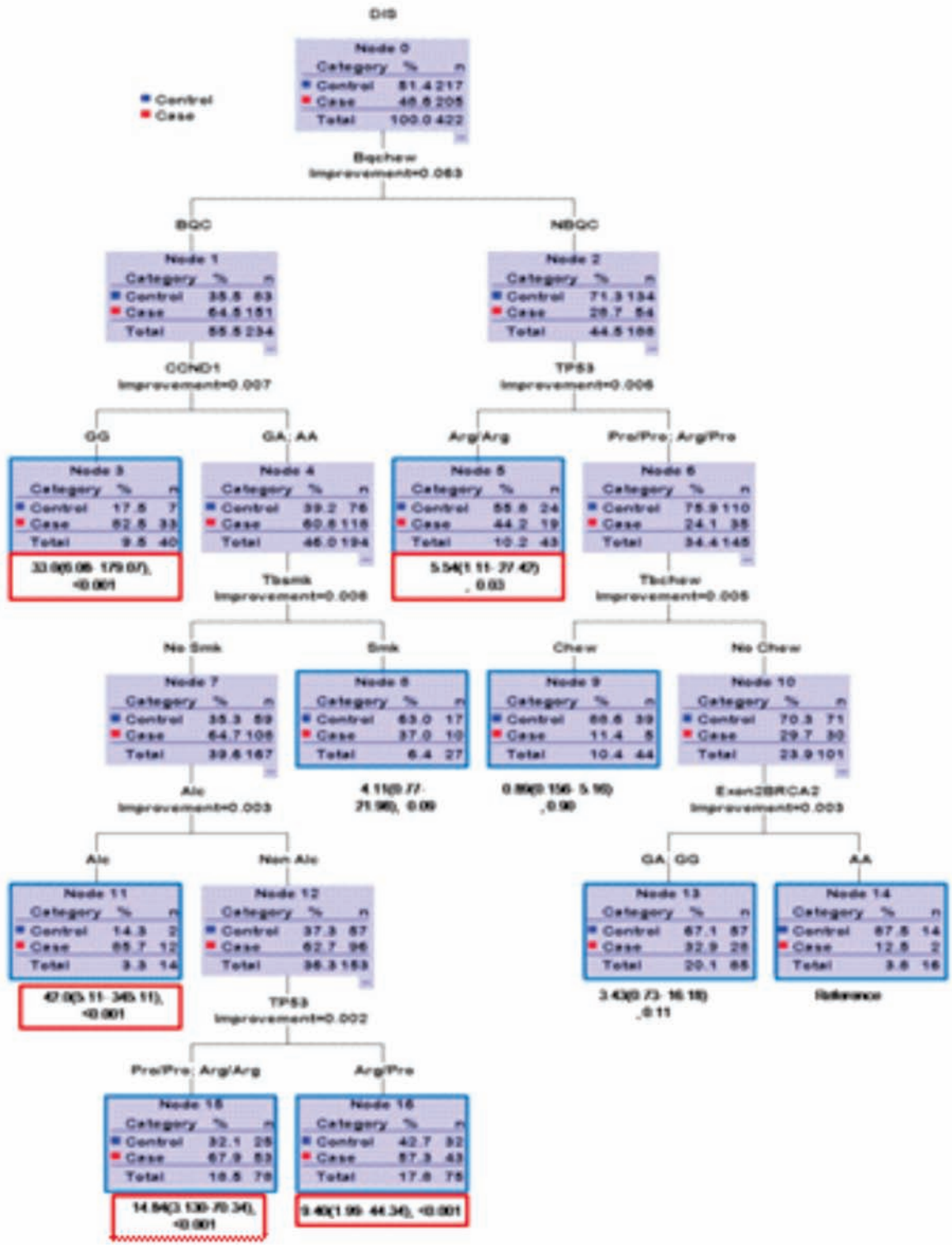


Fig. 3: Classification and regression tree (CART) analysis for the DNA repair and cell cycle genes and environmental risk factors. Terminal nodes are bordered thick blue. Red bordered odd ratio boxes values are <0.05.

7. Expression of Aldo-ketoreductase Family 1B10 (AKR1B10) Gene in Breast Carcinoma: The Effects on Drug and Tobacco Exposure

Scientific Staff : Dr. Mishi Wasson, Dr. Sunita Saxena

Duration : 2013-15

Aims, Objectives & Background:

We have previously demonstrated that breast cancer in women having betel quid chewing habit had significantly higher total number of genomic alterations, as compared to those with non betel quid chewer habit. Data analysis revealed the 7q33 locus to be significantly amplified in the betel-quid chewers which harbors the AKR1B10 gene.

AKR1B10 belong to the Aldo-ketoreductases (AKRs) gene superfamily is a Phase I drug-metabolizing enzyme. They play a central role in the metabolism of drugs, carcinogens and reactive aldehydes and hence has been implicated in the metabolism of cancer chemotherapeutics.AKR1B10 has also been implicated in the regulation of retinoid metabolism regulating retinoic acid homeostasis which may contribute to carcinogenesis.

A single report analyzing AKR1B10 expression in breast cancer shows positive correlation with tumor size and lymph node metastasis but inversely with disease-related survival in breast cancer. Silencing of AKR1B10 in BT-20 human breast cancer cells inhibited cell growth in culture and tumorigenesis in female nude mice. Moreover, AKR1B10 levels in serum were correlated with its expression in tumors suggesting that AKR1B10 over expression may be a novel prognostic factor and serum marker for breast cancer.

There is a dearth of studies depicting role of AKR1B10 in breast cancer. AKR1B10 expression in breast cancer cell lines due to environmental

carcinogens, stress and drugs needs to be explored to delineate its role in breast carcinogenesis. Therefore this study was put forward to investigate the significance of AKR1B10 as a potent biomarker for breast cancer and its role in tobacco associated breast carcinogenesis.

Work done during the year:

Thirty samples of primary breast carcinoma were analyzed for AKR1B10 protein expression by immunohistochemistry.All the thirty cases were of infiltrating ductal carcinoma. Tissue spots with appreciable histological quality were evaluated for AKR1B10 expression by at least a researcher and a pathologist. AKR1B10 expression levels were scored on the staining intensities from ''0'' to ''3'', representing no (''0''), low (''1''), intermediate (''2'') or highexpression (''3''), respectively.

The cases ranged from 23 to 80 years with mean age 49.05 ±14.09. AKR1B10 was seen to be overexpressed in 33.3% (10/30) breast tumors scored at ''1–3''in staining strengths (Table 1). The expression was seen to be cytoplasmic as well as nuclear or both. Fig. 4–5 shows representatives of AKR1B10expression in primary malignant breast tissues showing nuclear cytoplasmic and cytoplasmic AKR1B10 expression.

Table 1: AKR1B10 expression in breast tumors

	AKR1B10 expression level (%)				
Variables	**3**	**2**	**1**	**0**	**Subtotal**
Subtotal	1 (3%)	6 (20%)	3 (10%)	20 (66.6)	30

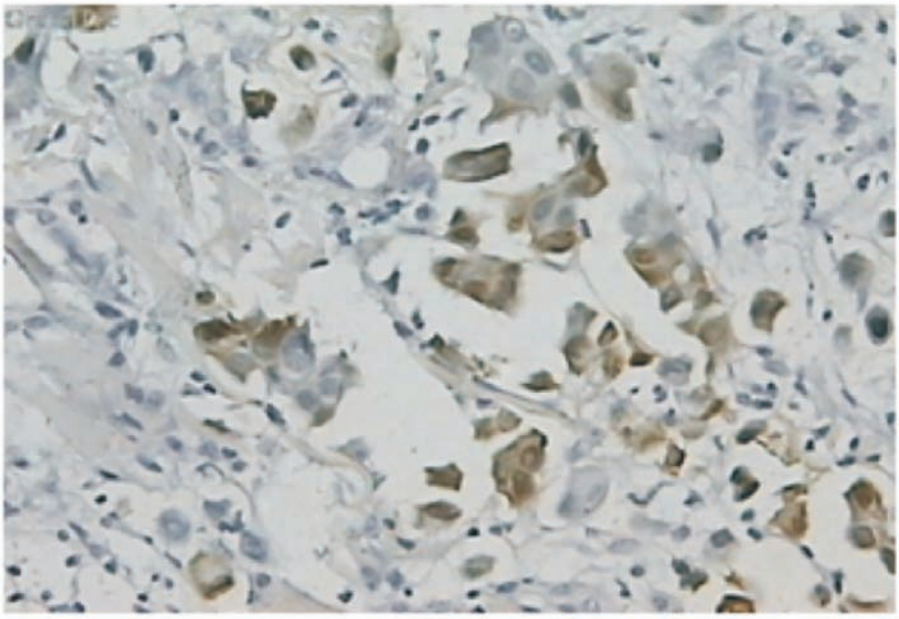


Fig. 4: Nuclear and cytoplasmic expression 40X

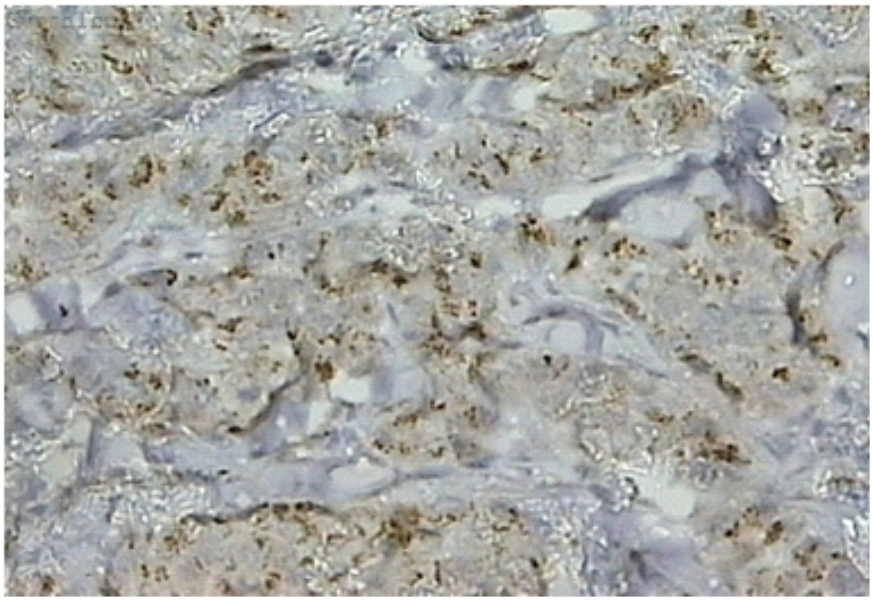


Fig. 5: Cytoplasmic expression 40X

PROSTATE CANCER

1. Study on Characterization of TMPRSS2: ERG and PCA3 as Prostate Cancer Biomarkers in Indian patients

Scientific Staff : Dr Sunita Saxena, Dr Anju Bansal, Dr Shalu Jain

In collaboration with : Dr. Anup Kumar, Department of Urology, Safdarjang Hospital, New Delhi.

Duration : 2013-15

Aims, Objectives & Background:

Prostate cancer (PCa) is one of the most common malignancies among men over the age of 55 and is responsible for about 6% of all male cancer deaths. The availability of a highly accessible blood test for prostate-specific antigen (PSA) has revolutionized the diagnosis of prostate cancer over the past three decades. But PSA test have low specificity to detect PCa with only a 25% to 40% positive predictive value within the PSA gray zone range of between 4.0 and 10.0 ng/ml, resulting in almost a 75% negative biopsy rate. Therefore, the identification of new biomarkers as useful tools in the diagnosis and clinical management of PCa is important. The recent discovery of fusion of the transmembrane-serine protease gene (*TMPRSS2*) with erythroblast transformation-specific (*ETS*) family members (*TMPRSS2-ETS*) and their possible involvement in the clinical management of patients with PCa makes fusion genes as specific markers for prostate tumor diagnosis and prognosis. The other gene which shows very high specificity to PCa is PCA3 (prostate cancer gene 3). Aim of the present study is 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.

Work done during the year:

Patients who were pre diagnosed by serum PSA and digital rectal examination, scheduled for diagnostic needle core prostate biopsy for prostate enlargement at Dept of Urology, Safdurjung Hospital campus were recruited for this study. The pathology of each biopsy with 12 needle cores was reviewed by expert pathologist at National Institute of Pathology, New Delhi. All included slides were assigned a grade according to the modified Gleason grading system. RNA extraction was done in total 54 prostate biopsy tissue samples using Qiagen RNAeasy Mini Kit. The presence of the TMPRSS2: ERG fusion transcript and PCA3 gene expression was assayed by Quantitative reverse transcription-PCR (qRT-PCR) by using Taqman chemistry. Quantitative PCR was performed using Applied Biosystems inventoried Taqman assays (20x Primer Probe mix) corresponding to PCA3 (Assay ID Hs01371939_g1), TMPRSS2: ERG (Assay ID Hs03063375_ft) and GAPDH (Assay ID Hs00266705_g1). The amount of each target gene relative to the housekeeping gene GAPDH was determined using the comparative threshold cycle (Ct) method. All assays were performed in triplicate.

Total 54 tissue samples were analysed for TMPRSS2: ERG Fusion. Histo-pathologically reports have confirmed 28 samples as adenocarcinoma prostate (taken as case group) and 26 samples as Benign Prostate Hyperplasia (taken as control group). Among 28 PCa cases analysed, 18 (64.2%) were found to be positive for TMPRSS2: ERG Fusion and 10 (35.8%) were fusion negative. Only one out of the BPH control was positive for TMPRSS2: ERG Fusion.

Table 1: Frequency of TMPRSS2: ERG fusion in prostate cancer and BPH patients

	Prostate Cancer	BPH	Total
Fusion Positive	**18 (64.2%)**	**1**	**19**
Fusion Negative	**10(35.8%)**	**25**	**35**
Total	**28**	**26**	**54**

The table-1 shows that TMPRSS2: ERG fusion marker is highly specific (96.1%) for prostate cancer as only one of BPH sample was positive for fusion transcript. Sensitivity of TMPRSS2: ERG fusion marker is 64.2%. The positive predictive value of fusion marker is 94.7%, whereas the negative predictive value of the marker is 71.4%.

Clinical Parameters were compared between fusion positive PCa cases and fusion negative PCa cases. Significant difference was found in Gleason's score and no. of positive cores between fusion positive PCa cases and fusion negative PCa cases.

PCA3 Expression analysis: So far analysis of PCA3 levels has been done in12 cases and 12 controls. The levels of PCA3 were compared between PCa and BPH cDNA samples with GAPDH as a control housekeeping gene. This much of samples were not showing any significant difference of PCA3 expression between cases and controls.

We plan to study Fusion marker in prebiopsy urine samples along with the biopsy tissue samples. If the TMPRSS2: ERG fusion results in urine samples correlates with fusion results in biopsy tissue samples and finally to the confirmed diagnosis by histopathology report, this marker can be developed as a potential diagnostic marker for prostate cancer, which will be much more specific than serum PSA and being a non-invasive marker it can reduce the number of unnecessary prostate biopsies.

NORTH-EAST CANCERS

1. Genome-Wide Analysis of Genetic Alterations in Patients with Esophageal Cancer from Northeast India using Single Nucleotide Polymorphism Arrays

Scientific Staff : Dr Sunita Saxena, Dr Sujala Kapur, Ashish Bhushan

In collaboration with : Dr Jagannath D Sharma; Deptt. of Pathology, BBCI, Guwahati
Dr Avadesh Rai, Research Scientist, BBCI, Guwahati
Dr B B Barthakar; Dept. of Surgical Oncology, BBCI, Guwahati
Dr. Jagdish Mahanta; Director. RMRC, Dibrugarh
Dr R K Phukan; RMRC, Dibrugarh, Assam.

Duration : 2011-14

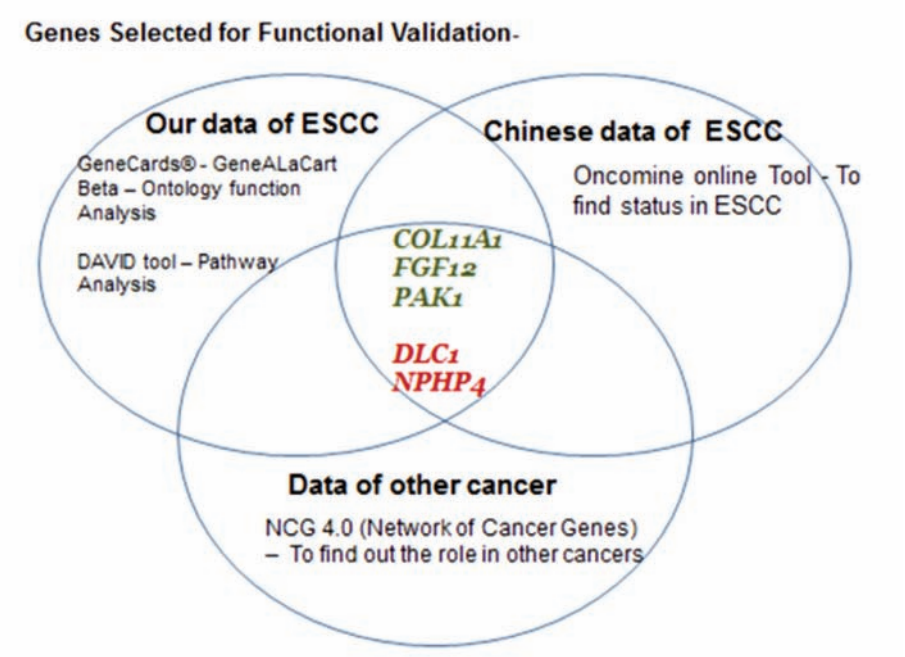
Aims, Objectives & Background:

Esophageal cancer is the second leading cancer in males and third leading cancer in women in North-East population. The development of cancer in this population has been reported with or without chewing of fermented areca nut. In combination with exposure to environmental factors (tobacco paste and tobacco water), genetic susceptiblity and familial aggregation of esophageal cancer has also been reported in Assam region of North-East.

In the current study we have investigated the genetic variations including chromosomal changes, LOH and copy number alterations through Affymetrix® Genome-Wide Human SNP Array in esophageal cancer patients in NE region of India showing high consumption of tobacco and familial clustering with aim to identifying suitable biomarkers for preclinical screening, early diagnosis in familial cases and target for biological intervention. 15 tumor tissue and blood samples (germ line DNA) from the same patients, had been processed for Affymetrix®

Genome-Wide Human SNP Array 6.0. Thirteen of 43 genes in amplified regions and 18 of 50 genes in deleted regions were found significantly associated with cancer associated pathways by David tool annotation. Of these 13 genes in amplified regions, six genes (*NTRK2, TPO, PLA2G5, PAK1, MAPK10, FGF12* and *FGF4)* were found to be significantly associated with MAPK signaling pathway. Of 18 genes found in deleted regions, many genes were found significantly involved in MAPK signaling pathway (*MAPT, CACNA1D, TGFBR2, PPP3CC, CACNB2* and *FGF14*), Cytokine-cytokine receptor interaction (*IL22, IL26, CACNA1D, PPP3CC* and *FLT1*) and Wnt signaling pathway (*PPP2CB, WNT7A, PPP3CC* and *NFATC1*).

To know the functional role of 93 genes biological GO analysis was performed by **GeneCards® - GeneALaCart Beta**. This software recorded different biological role like cell adhesion, cell differentiation cell apoptosis, cell proliferation, cell migration and cellular morphogenesis and cell signalling which is significantly associated with esophageal cancer. Six genes (*WNT7A, TNC, FGF14, TNR, MAPK10,* and *FGF12*) were recognized as candidate genes which were involved in common function showing high associations with Signal transduction. We also compared our data with Chinese datasets of esophageal cancer by **Oncomine online tool** and other 23 cancer type by **NCG 4.0 (Network of Cancer Genes).**



Based on this analysis five cancer candidate genes (amplified region genes *COL11A1, FGF12, PAK1, and* deleted region genes *DLC1, NPHP4*) have been selected for functional validation.

For functional validation we selected *FGF12* from our dataset. In addition to genomic alterations found in the current study, we have also reported *FGF12* to be significantly upregulated in our earlier study *(Chattopadhyay et al; 2007).* This gene belongs to the MAPK signaling pathway (hsa04010) and Pathways in cancer (hsa05200). Both these pathways play a major role in tumorigenesis and cell signalling.

To know the expression of FGF12 gene in ESCC cell lines we blast against all ESCC cell lines with differentially expressed datasets in cell line encyclopedia (http://www.broadinstitute.org/ccle/home), where we found FGF 12 is highly expressed with 7.6 fold value in KYSE410 cell lines. (Table 1).

Table 1: In silico expression in top 10 ESCC cell lines

ESCC Cell Lines	Fold Value
KYSE410	7.621754
TE5	6.012266
TT	5.337422
TE14	5.207637
TE8	4.951249
TE9	4.933152
KYSE30	4.87811
TE4	4.850669
KYSE520	4.589666
TE6	4.534348

The KYSE410 esophageal cancer cell line has been selected for siRNA knockdown experiments for FGF12. This cell line is being maintained in a medium containing RPMI 1640 + Ham's F12 (1:1) with 2% Fetal Bovine Serum (FBS) at 37°C

with 5% CO2. Doubling time of the cell line is on an average 30 - 35 hours. KYSE 410 cell line was transfected using Lipofectamine® RNAiMAX Reagent (Life Technologies™) followed by Proliferation assay using Vybrant MTT Cell Proliferation Assay Kit (Life technologies-Invitrogen). GAPDH siRNA were used as a positive control. This assay showed more than 50% of cancerous cells were inhibited after 48 hours of transfection, but no significant inhibition were found at 72 hour due to transient transfection (Fig.1).

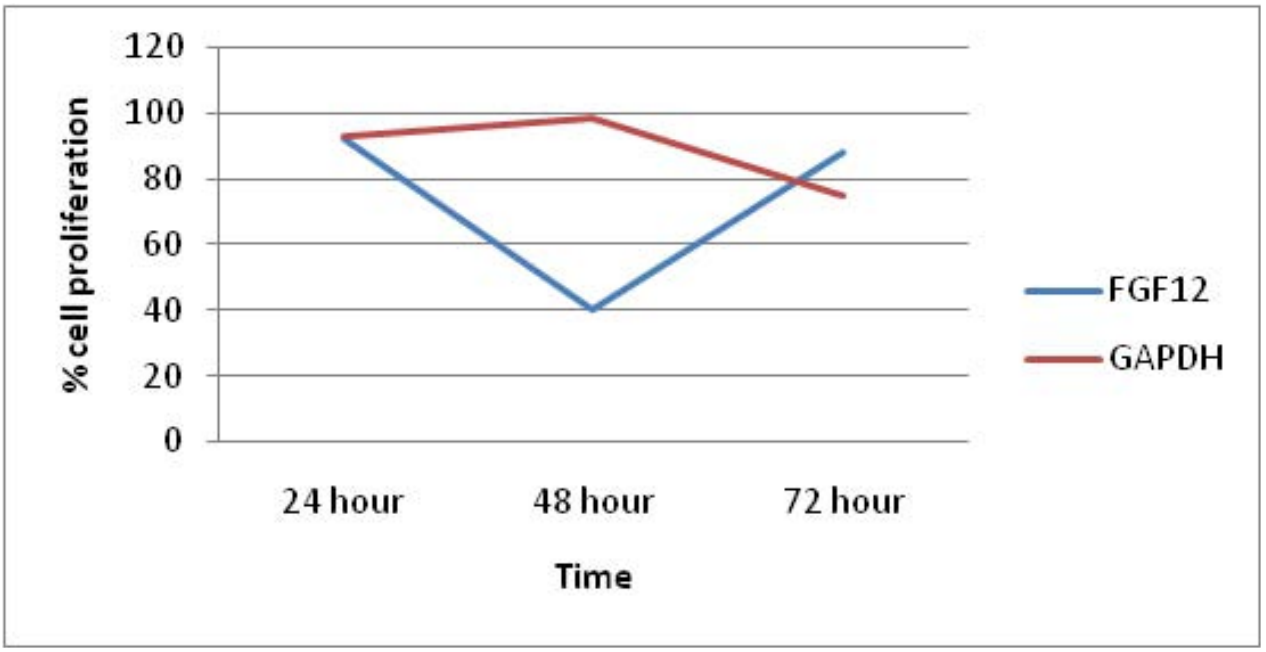


Fig. 1: Cell proliferation Assay

2. Epigenetic Studies in Oesophageal Cancer in High Risk Region of Northeast India

Scientific staff : Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. L.C. Singh, Dr. Virendra Singh

In collaboration with : Dr. Amal Chandra Kataki, Director, Dr. B. Borooah Cancer Institute, Guwahati, Assam
Dr. J. Mahanta, Director, Regional Medical Research Centre, Dibrugadh

Duration : 2011-14

Aims, Objectives & Background:

The etiology of esophageal cancer in Northeast Indian population is different from other parts in India due to wide variations in dietary habits, tobacco/betel quid chewing and alcohol habits. Although classical genetic analysis provide important information, the study of epigenetic processes viz DNA methylation, histone modifications, RNA interference which do not change the sequence of the DNA itself, but modify the way genes are expressed during development provide significant insight in molecular carcinogenesis. The DNA methylation and changes in chromatin proteins due to modifications in histone tails can be inherited with chromosomes. Diets or exposure to chemicals that interfere with the DNA methylating enzymes involved may have major effects both on normal physiology and on the manifestation of diseases such as cancers. In addition, tobacco and pesticide related carcinogens have long been suspected to act as co-factors to activate genes associated with certain cancers.

DNA methylation and expression of histone modification enzymes was analyzed in patients with esophageal cancer using commercially available real-time PCR array profiler. The results will provide insights into the molecular mechanisms and biological pathways in the etiopathogenesis of esophageal cancer in a high-prevelance region of NE India.

Work done during the year:

Samples of peripheral blood, tumor and normal tissue were collected from 70 histopathologically confirmed esophageal cancer patients at BBCI, Guwahati. Quantitative differential mRNA expression profiling of 84 epigenetic chromatin modification enzymes in normal and tumor tissue samples was done in 24 tumor and 24 normal tissues of esophageal squamous cell carcinoma (ESCC). The results indicate that higher expression of enzymes regulating methylation (DOT1L and PRMT1) and acetylation (KAT7, KAT8, KAT2A and KAT6A) of histone was found associated with ESCC risk. Nuclear protein expression of KAT8 and PRMT1 was analyzed by tissue microarray (TMA) having 15 cores of control (non-neoplastic esophageal epithelium) and 70 cores of ESCC. Results of TMA showed higher nuclear protein expression of KAT8 and PRMT1 in tumor similar to mRNA expression (Fig. 1). Earlier we have done promoter methylation profiling by PCR array that showed hypermethylation of tumor suppressor genes CDH1, OPCML, NEUROG1, TERT and WT1 and hypomethylation of SCGB3A1, THBS1 and VEGFA in tumor tissue compared to normal. Thrombospondin 1 (THBS1) promoter methylation result was validated by Methylation Specific PCR (MSP) in 18 tumor and 18 normal tissue samples (Fig. 2). Among 18 tumor samples 12 were detected as unmethylated and 6 was methylated. Among 18 normal tissue samples 5 were found unmethylated and 13 were methylated.

We plan to analyze KAT2A histone modification enzyme expression status at protein level by TAM based immunohistochemistry. As well as promoter methylated status of the genes will be validated by real time PCR based in large number of samples.

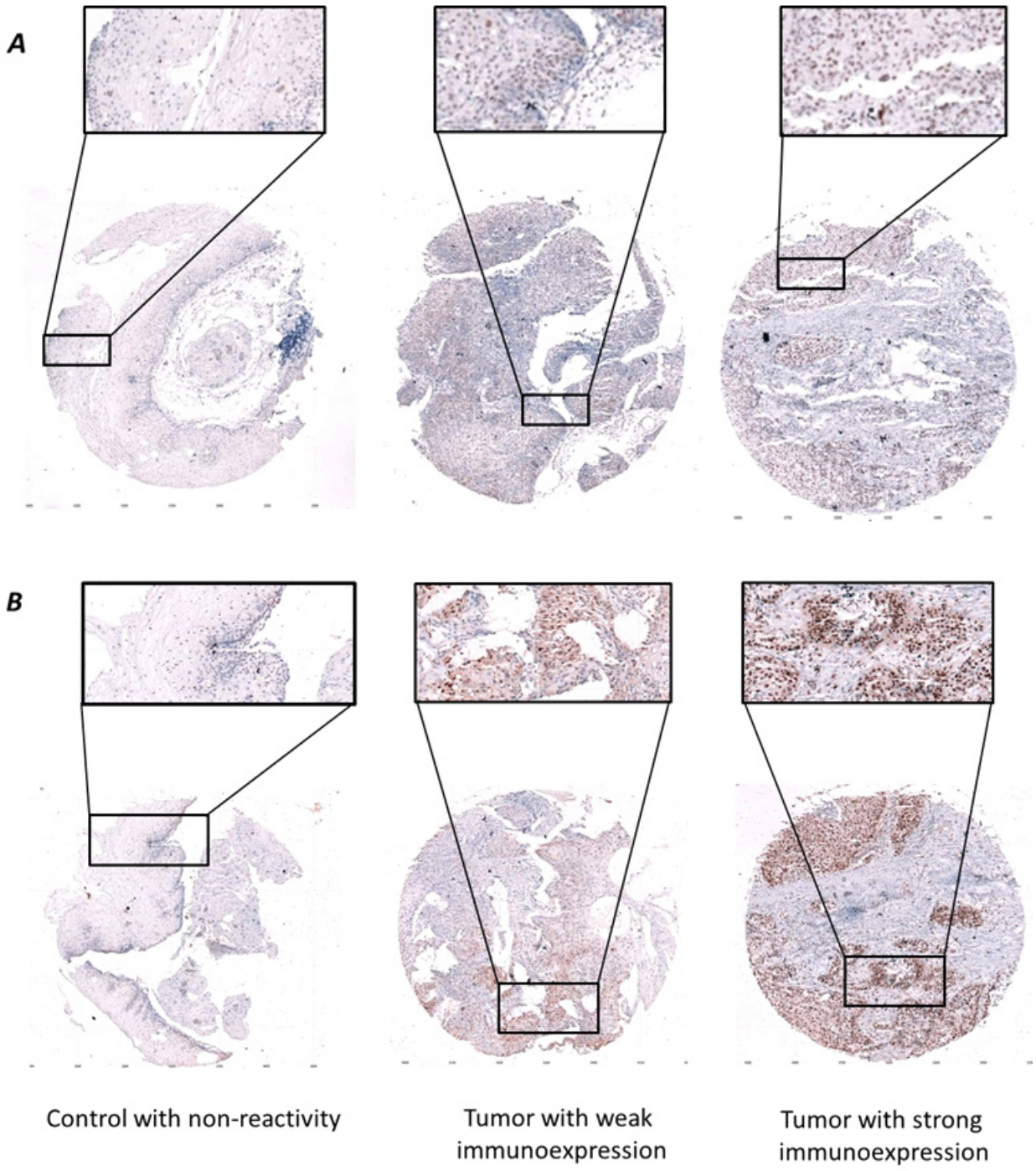


Fig. 1: Tissue microarray images of (A) KAT8 and (B) PRMT1 showing no nuclear reactivity in control epithelium whereas weak and strong nuclear expression respectively in ESCC. Zoom image of selected region from the core (inset)

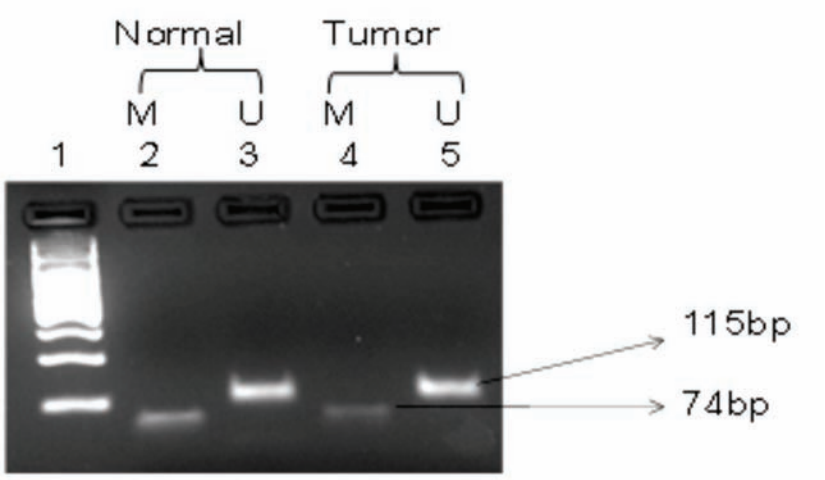


Fig. 2: Agarose gel electrophoresis (2%) of MSP products of THBS1 gene in normal and tumor tissue. Lane 1, 2, 3, 4, and 5 represent 100bp DNA ladder, methylated product (normal), unmethylated product (normal), methylated product (tumor) and unmethylated product (tumor). Both the samples were partially methylated as PCR amplification occurred with both the methylated and unmethylated set of primers. However the intensity of methylated band was higher in case of normal compared to tumor tissue showing higher methylation level in normal tissue.115bp and 74bp bands represent unmethylated and methylated target genomic DNA.

3. Immunogenetic Profile of Nasopharyngeal Cancer in a High-Prevalence Region of Northeast India

Scientific Staff : Dr. Sujala Kapur, Dr. Sunita Saxena, Dr.L.C.Singh, Dr. Saurabh Verma, Dr. A.K. Mishra, Ms Meena Lakhanpal

In collaboration with : Dr. A.C.Kataki, B. Barooah Cancer Institute, Guwahati, Assam

Dr. Y. Mohan Singh, RIMS, Imphal, Manipur

Duration : 2010-14

Aims, Objectives & Background:

Nasopharyngeal carcinoma (NPC) is an epithelial tumor with a low incidence rate in the western countries (with the age-standardized incidence rate (ASR) \1/100,000), but a high incidence in south east Asia (ASR = 9–12/100,000) and

Southern China (ASR = 30–50/100,000). NPC has complex etiology with significant interactions of genetic, viral, environmental and dietary factors. Although in areas where NPC is endemic, the EBV infection is found nearly ubiquitous but only few people develop the disease suggesting that EBV infection alone is not responsible to induce cancer, and other co-factors may have an important role in its pathogenesis. The co-factors include both exogenous and the host's genetic susceptibility factors, such as chromosomal aberrations, polymorphisms in human leukocyte antigen (HLA) class I and II alleles, glutathione S-transferase M1 (GSTM1), X-ray repair cross complementing group 1 (XRCC1). Among different genetic factors reported only human leukocyte antigen (HLA) present at chromosome 6 had shown strong association with NPC and has been linked to outcomes of NPC. Specific human leukocyte antigens (HLA) and alleles have been associated with NPC in several populations including Asian and North African populations. Studies have shown that many HLA-classes I and II alleles and other candidate genes which may lie within or nearby the HLA may be implicated in NPC susceptibility. The HLA-linked TNF-α and heat shock protein 70 (HSP70) genes are of particular interest because of their involvement in tumor immunity and cancer pathogenesis. With this the aim of current study is to analyze if Epstein Barr viral sequences in the tumor tissue along with host imunogenetic factors can explain the high prevalence of nasopharyngeal carcinoma in different ethnic groups of Northeastern States.

During the year under report we have studied variations at the genetic level in HLA class III region that contains genes encoding the pro-inflammatory cytokines (TNFα and TNFβ) and heat shock proteins HSP, gp 70.

Work done during the year:

A total of 120 blood samples from newly diagnosed cases of NPC have been collected. Of these 56 are from Guwahati and 64 from Imphal. 35 tissue samples from these 91 cases have also been collected. Genomic DNA was extracted from blood samples from patients and contros (Table 1).

Table 1: Blood and tissue samples received from two centers of Northeast India.

Centre	Blood Samples received		Tissue Samples received	
	Cases	Controls	Cases	Controls
RIMS, Imphal	64	45	07	1
BBCI, Guwahati	56	55	28	-
Total	**120**	**100**	35	1

To study the variation at the genetic level in HLA class III region that contains genes encoding the pro-inflammatory cytokines (TNF α and TNF β) and heat shock proteins HSP, gp 70, allelic discrimination experiment was performed using an ABI 7000 Sequence Detection System (Applied Biosystems, Foster, CA, USA) for detection of SNPs with DNA as template using TaqMan probe Assay. Cases in each cancer group were individually matched with control samples on the basis of age (±5 years), sex, and ethnicity. The association of TNF and HSP genotypes with disease outcome was assessed by χ^2 or Fisher exact test. Further, the associations of these genotypes with NPC were evaluated by multivariate conditional logistic regression. Estimates of cancer risk imparted by different genotypes and their interaction with other environmental covariates were determined by deriving the odds ratio (OR) and corresponding 95% confidence intervals using univariate (unadjusted) and multivariate (adjusted) conditional logistic regression models. For multivariate conditional logistic regression models, the adjusted estimates for the specific phenotypic variables and genotypic variables were adjusted for all other phenotypic and genotypic variables under consideration. A true risk estimate of the gene was measured after accounting for the other environmental factors. If the risk associated with the gene remained significant even after adjusting for other confounder variables, it showed a true association with the variable being analyzed. For all the tests, a two-sided $P < 0.05$ was considered statistically significant. The data analysis was performed using STATA 12.0 software.

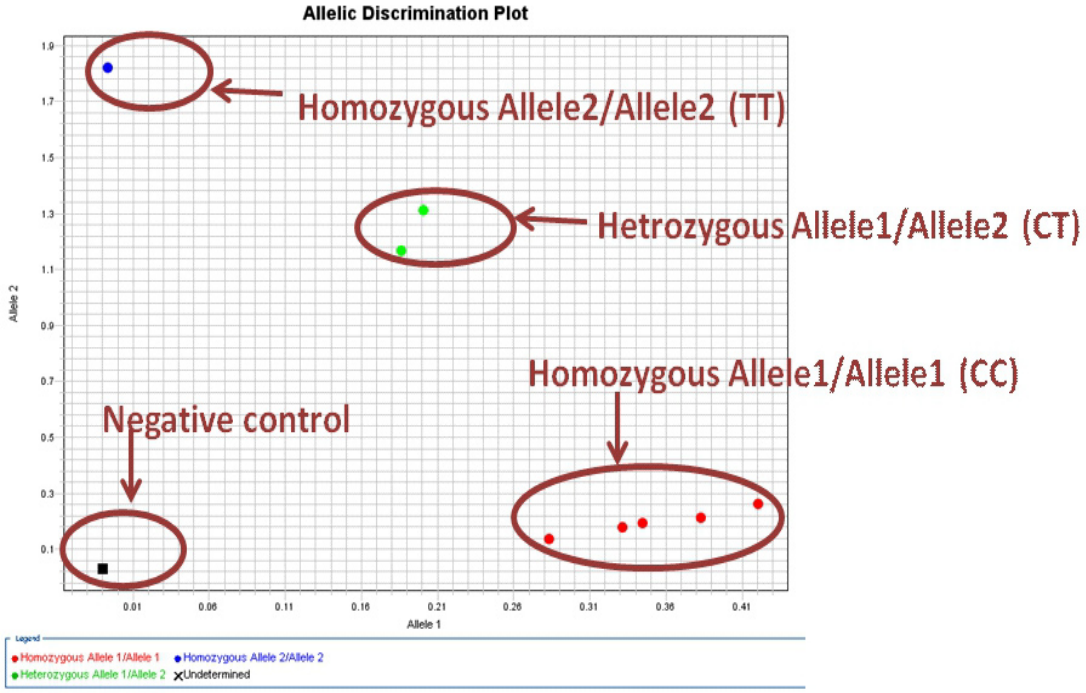


Fig.: Showing Allelic 8 discrimination plot of different samples

The results of the study showed association of TNF beta and HSP 70 gene polymorphism with risk of NPC. The frequency distribution of TNF β genotypes AA, AG and GG was 11.67%, 49.17% and 39.17% in cases and 39%, 24% and 37% in controls. Conditional logistic regression analysis showed significant higher risk associated with AG (OR2=1.97, 95%CI=1.019-3.83,P=0.04) genotype whereas the GG genotype (OR2=0.27, 95%CI=0.125-0.611,P=0.001) appeared to be significantly protective. The results of conditional logistic regression analysis of SNP (+2437 T>C) of HSP 70 showed significant protective effect of CC genotype (OR2= 0.17, 95%CI=0.0430.69, P=0.013). No significant association of TNF α (-308 C>T) and HSP 70 +190 genotypes were observed with NPC.

Thus the association with HLA Class III sub region in NPC pathogenesis is important since it is known to play an important role in the escape of tumour cells from host immune surveillance and may be responsible for decreased recognition and killing of cancer cells. The TNF β gene could conceivably be attractive candidate genes for being genetic host markers in evaluating individual susceptibility to development of different cancers. Further HSPs are the major

stress-inducible proteins that function as key components in regulating cellular homeostasis, probably through their molecular chaperone activities to inhibit accumulation of protein aggregates or through their cytoprotective properties to protect against DNA damage, thus promoting cell survival under stressful stimuli. Therefore it can be concluded that the high incidence of NPC in NE India may be a cumulative outcome of interaction of environmental factors with genetic variations in HLA region.

4. Molecular Mechanism of Drug Resistance in Acute Myeloid Leukemia (AML): Role of ATP-binding Cassette (ABC) Transporters

Scientific Staff : Dr Sujala Kapur, Dr. Sunita Saxena, Dr Pradeep Singh Chauhan

Duration : 2013-14

Aims, Objectives & Background:

Resistance of tumor cells to drug treatment is the major impediment to curing cancer. Despite our best efforts and advances in the field of cancer treatment, cancer remains largely an incurable and ultimately fatal disease. Various cellular mechanisms can give rise to drug resistance (MDR), but best studied is the transmembrane protein-mediated efflux of cytotoxic compounds which leads to a decreased intracellular drug accumulation and toxicity. Among these transporters identified to date, the ATP binding cassette (ABC) super family of active transporters, including multidrug resistance gene product 1 (MDR1/ABCB1), breast cancer resistance protein (BCRP/ABCG2), and the multidrug resistance-associated proteins (MRPs/ABCC1), are probably the most significant in terms of drug transport, being efficient transporters of a wide variety of xenobiotics/endobiotics. Many ABC transporters were initially described as drug efflux pumps, conferring resistance to cancer cells and rescuing these cells from drug-induced apoptosis.

However, significantly less attention has been paid to the mechanism by which these transporters act. More recently ABC transporters have been implicated in cell protection from various pro-apoptotic injuries induced by endogenous molecules such as cytokines and hypoxic damage. Despite profound research, the clinical relevance of cellular resistance, molecular mechanism mediated by other ABC transport proteins, remains unclear. The objectives of the study were to study the expression pattern of ABC transporter genes in acute myeloid leukemia (AML) samples using low density ABC transporter array and to identify the differentially expressed proteins involved in the mechanism of resistance mediated by ABC transporter genes.

Work done during the year:

Peripheral blood samples from 20 patients of acute myeloid leukemia (AML) (>15 years age) admitted to the Division of Hematology, Safdarjung Hospital New Delhi for induction chemotherapy, were collected during the current year. RNA was isolated from AML cells using TRIZOL reagent and complementary DNA (cDNA) was generated using high capacity cDNA archive kit (Applied Biosystems). Integrity of cDNA was checked by amplification of DHFR gene. Moreover, Quality control of cDNA for real-time array, was done through relative quantization of expression level of the 18s rRNA endogenous control gene using TaqMan assay by real time RT-PCR ABI Prism 7000. The TaqMan® Array Human ABC Transporters 96-well plate containing 44 assays for ABC transporter associated genes and 4 assays for candidate endogenous control genes was used for a focused analysis to investigate differential ABC transporter gene expression in drug resistant and drug sensitive clinical samples in response to induction chemotherapy in 10 samples (Eight AML and 2 Healthy control).

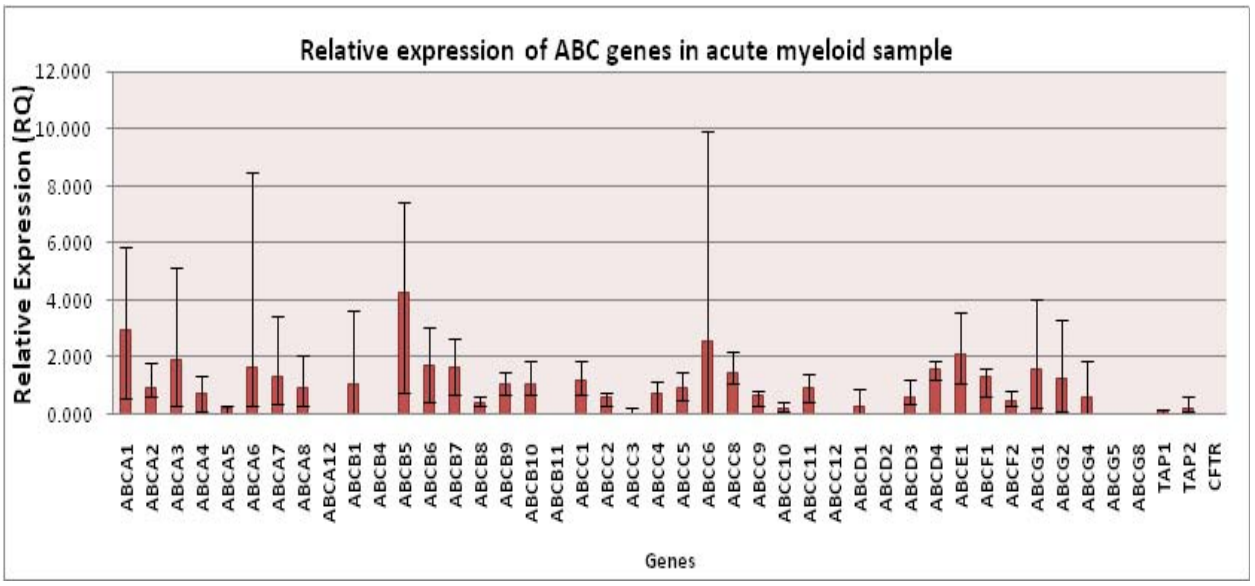


Fig. 1: Relative Expression of ABC transporters in AML Samples compared to normal PBMC cells

No expression was found for genes such as ABCA12, ABCB11, ABCC12, ABCG5 and ABCG8 genes. High Expression level (>1.5 fold) was found for genes ABCA1, ABCA3, ABCB5, ABCC6, ABCE1 and ABCG1 (Fig.1).

Correlation with Induction chemotherapy

Response to the chemotherapy was determined at the end of completion of induction chemotherapy (4 CR and 4 NCR). Expression of ABCA1, ABCC2, ABCD4 and ABCF1 genes were found to be significantly up regulated in non-responder patient of AML compared to responders (Fig.2).

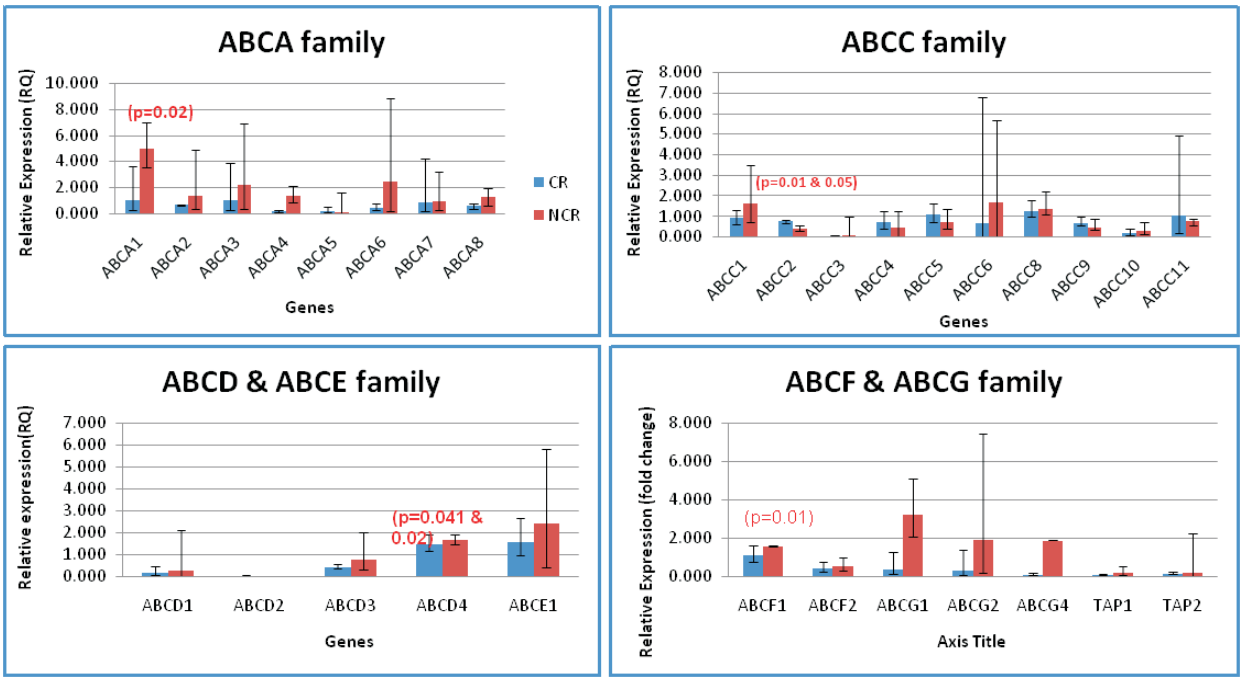


Fig. 2: Expression of ABC transporters in AML samples responded (CR ■) and non-responded (NCR ■) to induction chemotherapy compared to normal PBMC.

5. Dynamic Regulation of Lymphocyte Signaling of Acute Leukemia

Scientific staff : Dr. Anand Kumar Verma, Dr. Sunita Saxena

In collaboration with : Dr. S.P.Kataria, Safdarjang Hospital

Duration : 2013-16

Aims, Objectives & Background:

Leukemia is a progressive, neoplastic disease of the hematopoietic system characterized by unregulated proliferation of uncommitted or partially committed stem cells. It includes a heterogeneous group of neoplasm that differ with respect to aggressiveness, cell of origin, clinical features, and response to therapy. Leukemias are divided into two broad categories that are based on the cell involved (myeloid or lymphoid) and disease aggressiveness (either acute or

chronic). AL is broadly divided into acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) categories based on cell of origin. Natural killer (NK) cells, cells that secrete cytokines and kill other cells, are one cell type that has been studied extensively. It was once thought that all NK cell functions decline with age, but this theory was based primarily on mouse studies and on human studies performed without using the SENIEUR protocol. In studies where the SENIEUR protocol was employed, it was found that NK cell activity in human's changes very little over time, although one contradictory study suggests that even though the cytotoxic (cell-killing) function of NK cells is maintained, the cytokine secretory activity is impaired. Natural killer (NK) cells have been implicated in defense against malignancies, especially leukemia. Because patients with leukemia and preleukemic disorders manifest low NK activity, it is possible that NK cell impairment may contribute to leukemogenesis. In view of this possibility, it is important to characterize the NK cell defect of leukemic patients and to design new approaches for its correction. NK cells could play an important role in the innate immune surveillance of leukemic cells. NK cells participate in the innate immunity against oncogenic virus-infected cells with abnormal expression of HLA class I molecules. It has been reported that leukemic cells can have downregulated expression of HLA class I molecules and in some situations show a complete loss of a HLA class I allele. The aim of study to understand the characteristic of NK cell in acute leukemia and explore the possibility of NK cell mediated cell therapy of acute leukemia. The goal is to find out dynamically regulated intracellular molecule and if these intracellular molecules can be used for targeted therapy of acute leukemia

Work done during the year:

To analyze the impact of cellular redox state on NK cell, PBMC from patient's samples (n=30) and age and sex matched controls (n=30) were cultured in conditioned media for 72 hours and induced with optimized concentration of hydrogen peroxide. Three color flowcytometry was used to analyze the expression of dynamically modulated NCR (NKp44, NKP46, NKp30) and reactive

oxygen species in gated 7-AAD negative live CD56 +ve cell. On analysis of flowcytometry data, expression of NKp46 highly modulated in AML NK cells as compared ALL NK cells (Fig. 1). Expression NKp30 was not found higher than NKp46 in both AML and ALL cells but it did not show significant dynamic change. Though AML NK cells showed relative modulation of NKp30 on induction (Fig. 2), the expression of NKp44 was dim in AML NK cells and ALL NK cells and it was found that ROS mediated modulation present in AML NK cell not in ALL NK cells . Thus it can be concluded that ROS mediated NK cell therapy would have higher prospect to success in AML.

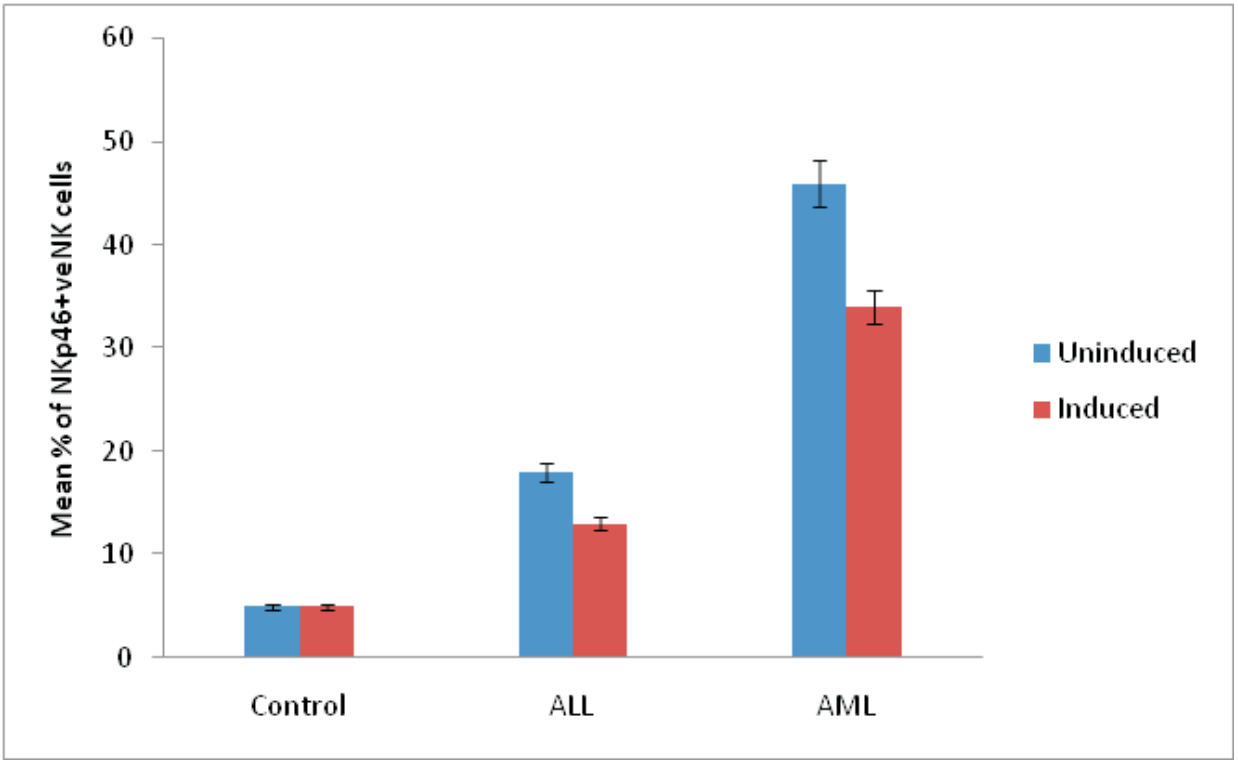


Fig. 1: NKp46 expression modulation on CD56+ve cell (n=30) : AML NK cell highly sensitive to oxidative stress that lead to significant modulation of NCR NKp46 in comparison to ALL NK cells.

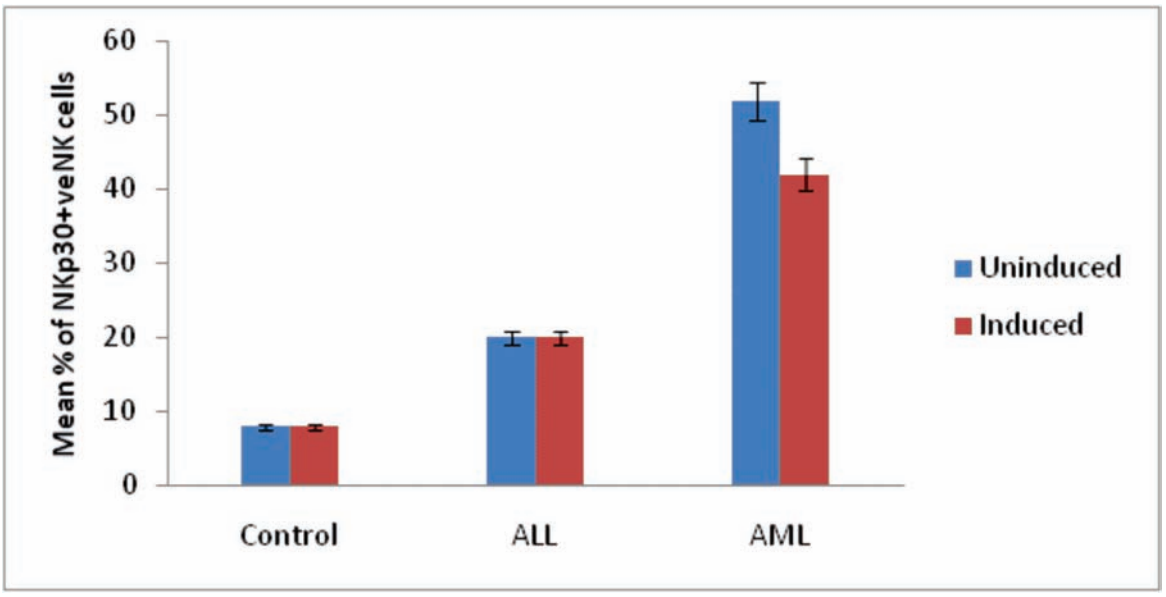


Fig. 2: NKp30 expression modulation on CD56+ve cells: AML NK cells highly sensitive to oxidative stress that lead to significant modulation of NCR NKp30 in comparison to ALL cells while ALL NK cells insensitive to oxidative stress.

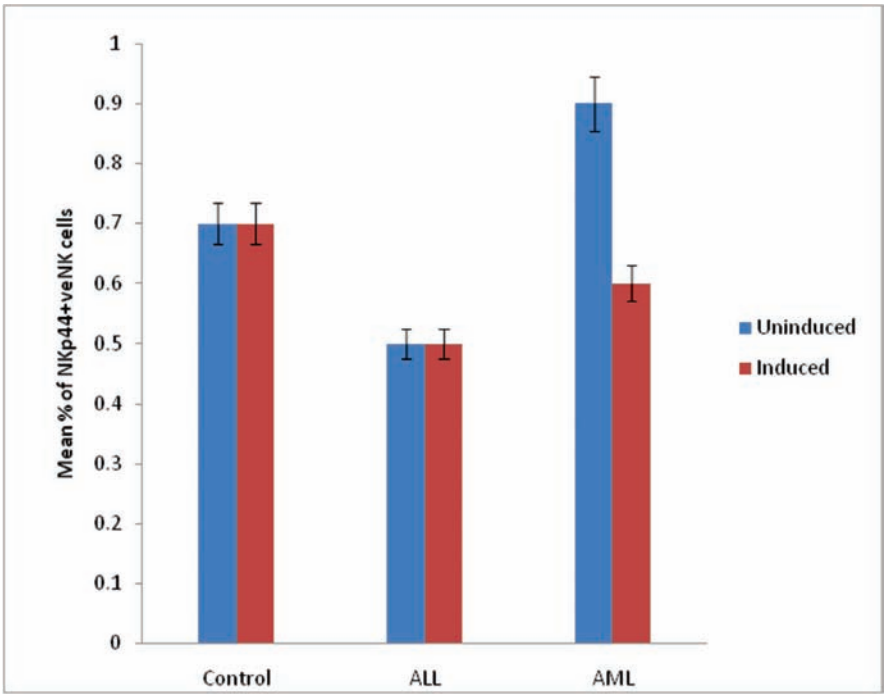


Fig. 3: NKp44 expression modulation on CD56+ve cell: AML NK cell highly sensitive to oxidative stress that lead to significant modulation of NCR NKp44 in comparison to ALL cells while ALL NK cells were insensitive to oxidative stress.


PATHOLOGY OF
INFECTIOUS
DISEASES

PATHOLOGY OF INFECTIOUS DISEASES

LEISHMANIASIS

1. Mechanism of Resistance *to* Miltefosine (MIL) in *Leishmania donovani*

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Ms. Vasundhra Bhandari, Mr. Deepak Kumar Deep, Ms. Aditya Verma

In collaboration with : Dr N S Negi, Dr. V Ramesh

Duration : 2012 -2016

Aims, Objectives & Background:

Widespread resistance against sodium antimony gluconate (SAG) has resulted in introduction of miltefosine (MIL) as the first line drug in parts of Bihar, however, long half-life treatment poses threat of development of resistance. Recent reports indicate a significant decline in its efficacy and high relapse rate. In this situation, understanding the mechanism of development of resistance towards miltefosine in the parasite has become the top priority to rescue the efficacy and longevity of this drug. In an earlier study we established that the genes modulated in experimental resistance do not show similar expression behavior in clinical isolates of *L. donovani* from miltefosine unresponsive cases.

Work done during the year:

To further explore the basic underlying mechanism responsible for development of MIL resistance / unresponsiveness in clinical isolates, we employed *Leishmania* whole genome oligonucleotide array approach to compare the gene expression pattern in isolates of *Leishmania donovani* obtained from VL patient at pre treatment and clinically relapsed stages.

To investigate the global mRNA expression profiles of *L. donovani* parasites from clinically relapsed group and from pre-treatment group, one color microarray based gene expression profiling was carried out using *L. infantum* whole genome 60mer oligonucleotide microarray slide [8X15K format]. Gene expression studies were performed between isolate of *L. donovani* from relapse group and from pre-treatment group (control sample). RNA was prepared from *L. donovani* isolate from the two groups using trizol method. The quality and integrity of RNA was assessed on RNA 6000 Nano Assay Chips on Bioanalyzer. The presence of three distinct ribosomal peaks (18S, 24S and 24S) confirmed quality of RNA extracted. Complementary RNA (cRNA) was generated from 1μg of total RNA using Quick-Amp Labeling kit (Agilent technologies) that directly incorporates Cy-3 labeled CTP into the cRNA. Hybridization was carried out at 65°C for 17 hours.

Hybridization was performed from three biological replicates of pre-treatment group isolate and from relapse group isolates to account for sample heterogeneity, and variations due to hybridization. The hybridized arrays were washed with gene expression hybridization buffers 1 and 2 (Agilent technologies) after addition of 0.005% Triton X-102. The slides were scanned immediately on a G2505C scanner (Agilent Technologies) to minimize the impact of environmental oxidants on signal intensities. The analysis was performed using GeneSpring GX 11.0 microarray data and pathway analysis tool. Identification of differentially expressed genes was performed by t-test (Unpaired) for the two groups, Confidence Level (p-value cut-off) < 0.05 and fold change analysis.

DNA microarray data were analyzed by custom R programs to illustrate the expression profile of *L. donovani* from relapse vs pre-treatment case by extrapolating on a chromosome map of *L. infantum*. As evident in chromosome map highly upregulated genes in relapse isolates were located on chromosome no 2, 9, 12, 21, 29 while the downregulated genes were located on chromosome no 4,11, 24 and 33 (Fig.1).

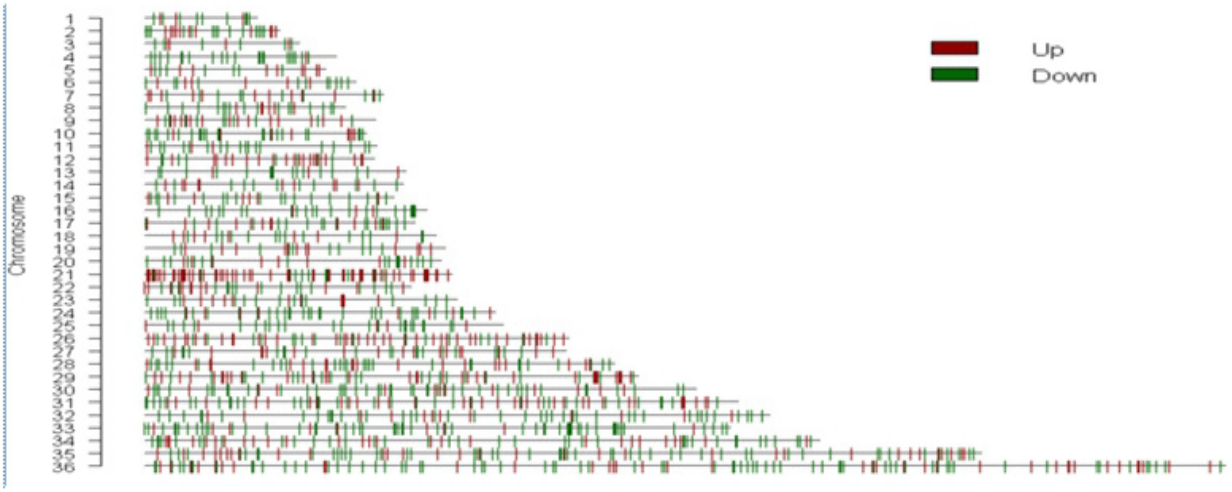


Fig. 1: Chromosome map showing differentially modulated genes in relapse group isolate

Differentially regulated genes (more than 2 fold modulated) were clustered using hierarchical clustering based on Pearson coefficient correlation algorithm. 2D cluster analysis was performed on gene exhibiting statistically significant variability between pre-treatment and relapse group isolate as determined by GeneSpring 11.0 software. Replicate clusters (3 each) were averaged prior to cluster analysis (Fig 2).

The present microarray study revealed important differences in gene expression pattern between relapse group isolate and pre-treatment group isolate. Approximately 1800 genes which comprised ~20% of total *Leishmania* genome were found to be differentially modulated (fold cut off value 1.5, $p<0.05$) in isolates from relapse group while 80% of the genes were unaltered. 7.4% genes were up regulated (n=668) while 12.4% were down regulated (n=1103). After BLAST2GO, AmiGO and KEGG pathway analysis we identified genes which represents various functional categories including metabolic pathways,

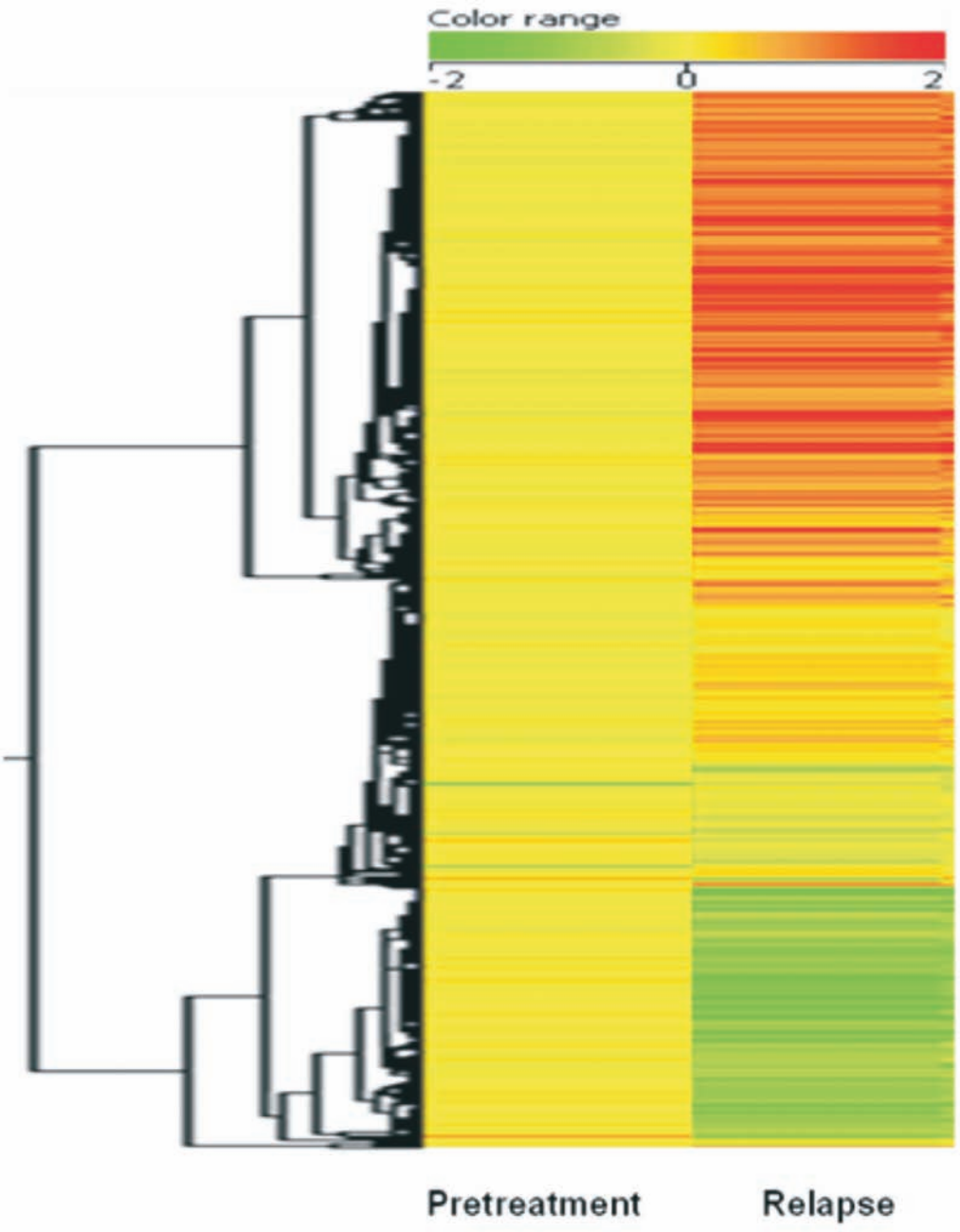


Fig. 2: The heat map showing comparison of the gene expression between pre-treatment and relapse group isolate of *L. donovani*.

transporters, genes involved in signal transduction pathway, nucleotide binding and cellular components. Unclassified proteins which include the hypothetical proteins (proteins with unknown function) comprised about 66% of total differentially modulated genes (Fig 3).

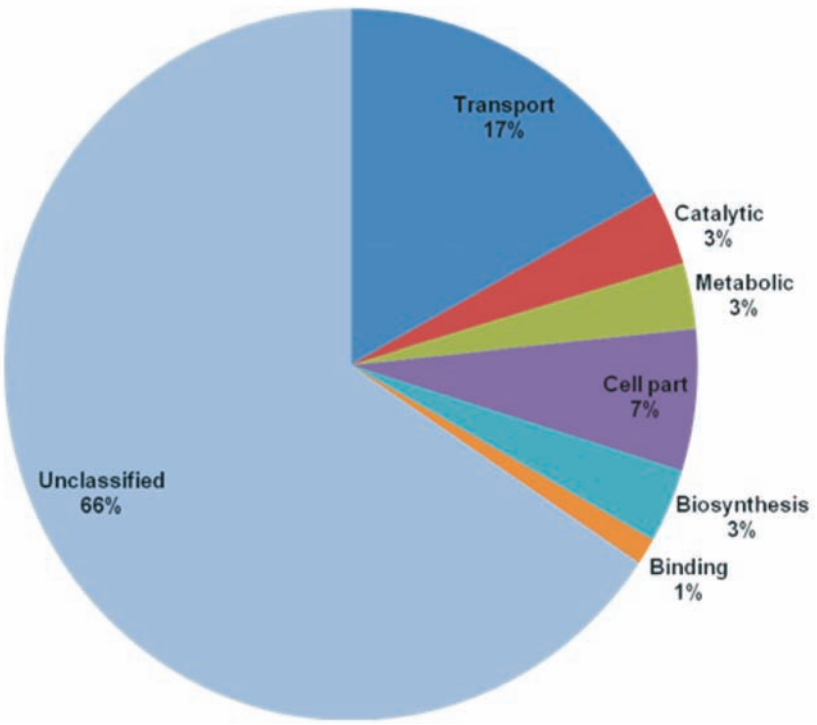


Fig. 3: Distribution of differentially expressed genes in relapse group isolate according to Gene Ontology (GO) function categories

Genes which were consistently modulated in relapse group isolate and selected for further analysis includes, Hypothetical protein conserved (LinJ.20.0380), Hypothetical protein (LinJ.20.0570), SMP2(LinJ.20.1340), Phosphoglucomutase (LinJ.21.0700), Hypothetical protein conserved (LinJ.34.3170), Protein kinase (LinJ.17.0440), MFS Transporter (LinJ.03.0390), Multidrug resistance like protein (LinJ.24.1510), ABCC5 (LinJ.31.1300), CAL-ATPase (LinJ.31.2080), VPTM (LinJ.33.2010), Amastin like surface protein (LinJ.34.1730), NUP155 (LinJ.36.7220), delta-12 fatty acid desaturase (LinJ.33.3420) and ABCF2 (LinJ.33.0340).

Characterization of Lipase like precursor protein over-expressed in lab generated miltefosine resistant Leishmania

Lipase precursor like protein was upregulated in lad generated miltefosine resistant *L. donovani* parasite. Episomal expression of Lipase precursor like protein in *L. donovani* was carried out and confirmed by western blot using monoclonal anti HA antibody as probe.

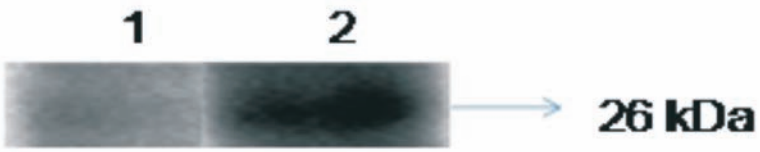


Fig. 4: Lane 1, mock transfectant *Ld*Neo (empty vector) showed no episomal expression of Lipase precursor like protein, Lane 2, *Ld*Lip++showing episomal expression of lipase precursor like protein.

Further, we assessed growth kinetics and susceptibility towards Miltefosine in transfected parasite.

Effect on growth

The growth of transfected parasites *Ld*Lip++ were monitored with wild type parasite *Ld*WT and mock transfectant LdNeo under Miltefosine pressure (3μM) at promastigote level for a period of 10 days. The transfectant *Ld*Lip++ showed better tolerance towards Miltefosine pressure while the growth patterns of *Ld*WT and LdNeo were comparable (Fig 5).

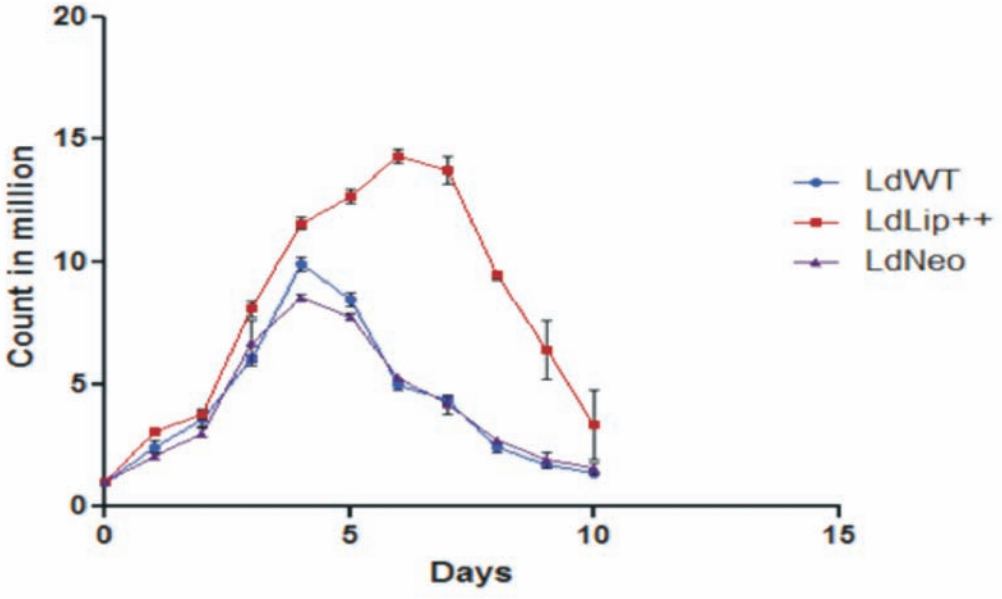


Fig. 5: Growth curve of **Ld**WT, *Ld*Neo and *Ld*Lip^{++}. Values are mean ± SD of two independent assays.

In vitro drug susceptibility

The transfected parasite *Ld*Lip^{++} were assessed for *in vitro* miltefosine susceptibility both at promastigote and at intracellular amastigote level. The *Ld*Lip++ parasite showed higher IC_{50} as compared to wild type(*Ld*WT) and mock transfectant (*Ld*Neo).

Table 1: Values are mean± SD of two independent assays.

	Miltefosine $IC_{50}\mu M \pm SD$	
Isolates	**Amastigote**	**Promastigote**
LdWT	**2.66±.68**	**1.4±0.64**
LdLipase++	**9.1±.57**	**3.8±0.90**
LdNeo	**3.9±1.4**	**1.5±0.4**

Future work: The selected genes from microarray results will be validated by real time PCR.

2. Studies on Mechanism of Resistance Towards Paromomycin in *Leishmania donovani* Parasite:

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Ms. Vasundhra Bhandari, Mr. Deepak Kumar Deep, Ms. Aditya Verma

In collaboration with : Dr N S Negi, Dr. V Ramesh

Duration : 2013-17

Aims, Objectives & Background:

Paromomycin (PMM) has been found to be very effective both in mono- as well as in combination therapy for the treatment of VL in India. Although no clinical resistance has yet been reported, appropriate measures should be taken to ensure its long term effectiveness. Therefore, there is an urgent need

to understand mechanism of resistance towards PMM in order to safeguard this drug. During the period under study, we induced PMM tolerance in three different field isolates to explore the mechanism of drug resistance. Susceptibility towards paromomycin in presence of inhibitors to ATP binding cassette (ABC) transporters was determined in three PMM resistant (PMM-R) *L. donovani* parasites.

Work done during the year:

Generation and characterization of PMM-R L. donovani parasites isolates

We induced PMM tolerance in 3 distinct parasite isolates by step wise increase in drug pressure up to 100μM (61.7μg/ml) at promastigote stage. PMM tolerance was stable in all the three isolates up to 10-12 passages without drug pressure. Parasites were further sub-cloned by serial dilution and paromomycin susceptibility was determined both at promastigote and intracellular amastigote stages. All the three PMM-R isolates showed more than 9 fold resistance at promastigote stage and approximately 6-7 fold resistance at intracellular amastigote stage as compared to their respective wild type isolates (Table 2).

Table 2: Sensitivity profile of PMM-R and their respective wild type isolates.

Parasite ID	IC50 Promastigote (μM±SD)	IC50 Amastigote (μM±SD)
573 WT	34.6 ±1.08	13.09±0.48
573 PMM-R	334.2 ±25.13	91.80±12.1
K133 WT	43.25 ± 0.77	12.29± 0.24
K133 PMM-R	480.14 ± 26.66	87.17± 5.78
568 WT	47.3 ± 14.1	14.2 ± 0.61
568PMM-R	548.8 ± 93.54	83.06±10.7

PMM susceptibility in presence of inhibitors to ABC transporters:

In our previous report, we have shown a marked increase in gene expression of ABC transporters in PMM-R isolate. To further validate the role of ABC transporters in paromomycin resistance, we determined the susceptibility of all three lab generated PMM resistant and corresponding wild type isolates towards paromomycin in presence of inhibitors to ABC transporters (Verapamil and Amlodipine). A significant increase of approx. 2 fold ($P < 0.05$) in susceptibility towards paromomycin was observed in PMM R parasites in presence of verapamil (Fig. 6). Similarly, we observed a significant increase (> 6 fold, $P = 0.01$) in PMM susceptibility in presence of amlodipine in the PMM-R isolates (Fig. 7).

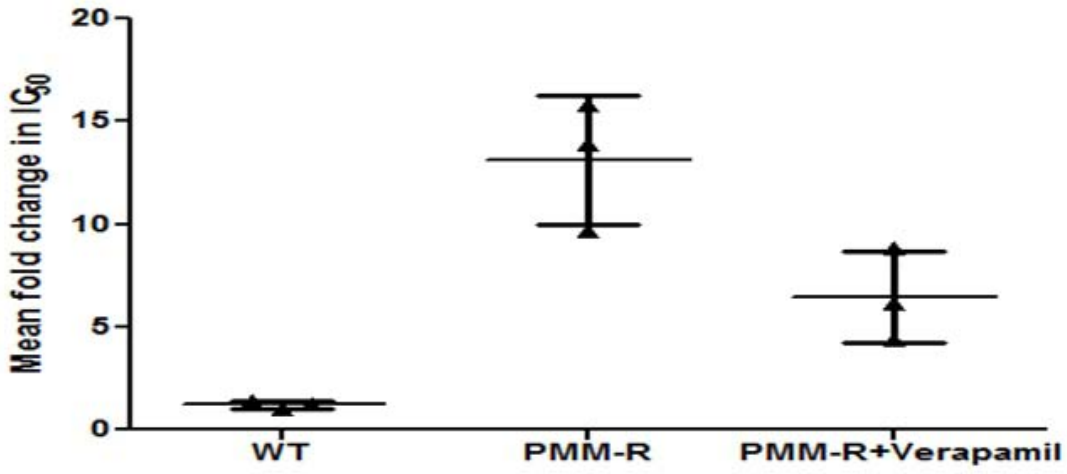


Fig. 6: Susceptibility of PMM-R isolates in presence of verapamil. *In vitro* susceptibility towards paromomycin in presence of verapamil (8μM) in three different PMM-R and WT isolates at promastigote stage. Each individual value represents mean fold change in IC50 ± SD of the results from two separate assays.

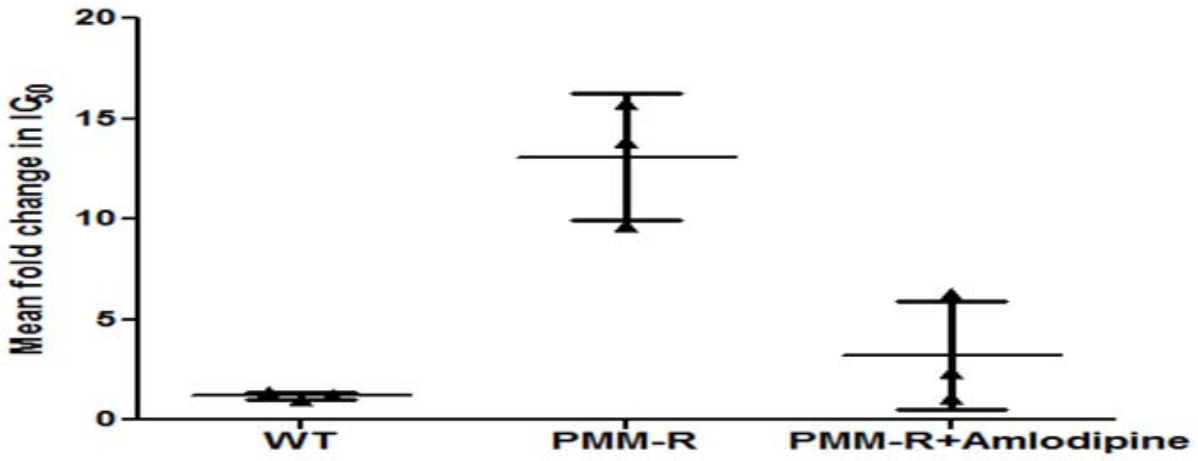


Fig 7: *In vitro* susceptibility towards paromomycin in presence of amlodipine (0.5μg/ml) in three different PMM-R and WT isolates at promastigote stage. Each individual value represents mean fold change in IC50 ± SD of the results from two separate assays.

Future Work

To determine the susceptibility of PMM-R isolates at intracellular amastigote level in presence of inhibitors. Comparative transcriptomic profiling of PMM resistant *L.donovani* isolate using genomic microarray technology to identify genes showing altered expression in PMM-R isolate followed by functional characterization of the selected gene (s).

3. Protective Immunogenicity of Centrin Knock-out Live Attenuated *Leishmania* parasite in the animal models and in human cells

Scientific staff : Dr. Poonam Salotra, Mr Kumar Avishek

In Collaboration with : Dr. Angamuthu Selvapandiyan, Dr. Hira Nakhasi, Dr. V. Ramesh, Dr. N. S. Negi

Duration : 2011-14

Aims, Objectives & Background:

Leishmaniasis is a parasitic disease that encompasses a range of clinical manifestations affecting people in tropical and subtropical regions of the world. However, there is still no vaccine available for use in humans. Currently only treatment option is chemotherapeutic which is costly, limited and associated with high relapse and resistance rates. Mutant parasites with known genetic defect have great potential to be used as live attenuated vaccine as they mimic natural infection without causing disease. Live attenuated *L. donovani* parasite deleted for a growth regulating gene 'centrin1' (*Ld*Cen1$^{-/-}$) is a potential vaccine candidate. It displayed attenuated growth only at the intracellular amastigote stage and has been found safe, protective and immunogenic in mice, hamsters and dogs. In the present study we evaluated immune response generated in human PBMCs by *Ld*Cen1$^{-/-}$ live attenuated vaccine candidate.

Work done during the year:

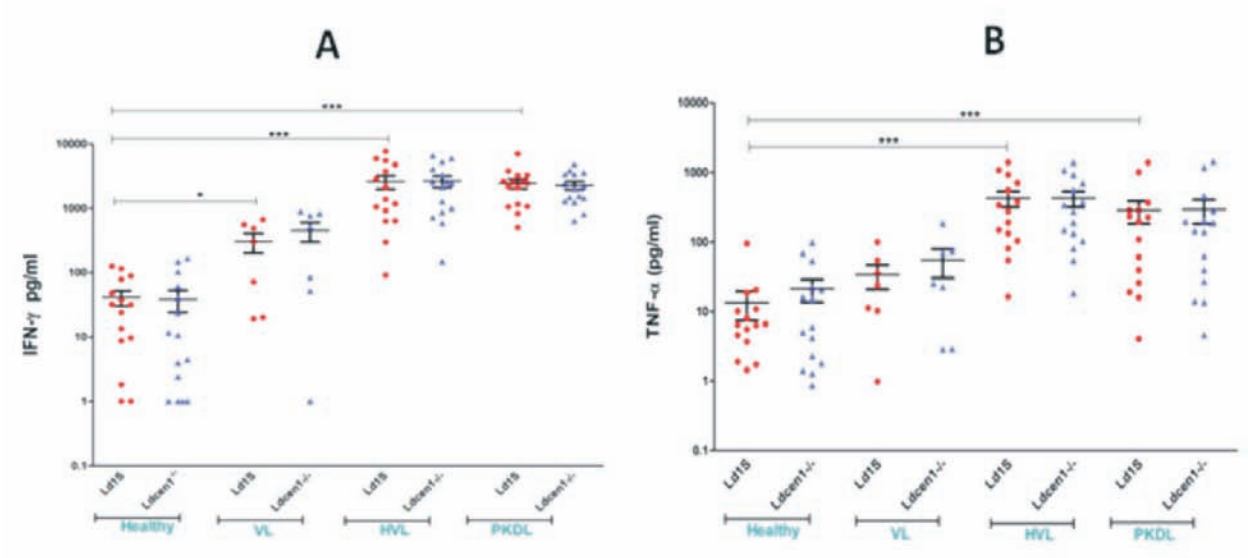
Evaluation of immune responses generated by LdCen$^{-/-}$ in human PBMCs

Th1 and Th2 type cytokine production were estimated in the human blood PBMCs in response to *Ld*Cen1$^{-/-}$and wild type (*Ld*1S) *L. donovani* parasite in

healthy, visceral leishmaniasis(VL), healed visceral leishmaniasis (HVL) and post kala-azar dermal leishmaniasis (PKDL) cases. In the previous year we reported cytokine responses in a small number of cases (n=6 for healthy, HVL and PKDL). Now we have extended this study to large sample number (n=15 for healthy, HVL and PKDL), in addition we have also included VL group (n=7). A total of 7 cytokines, representative of Th1/Th2 response (IL2, IL4, IL6, IL10, IL12, IFN-γ and TNF- α) were studied.

Th1 cytokines response

Th1-cells play a crucial role in human leishmaniasis as they provide protection and prevent disease progression. Th1 response activates the macrophages for killing of intracellular *Leishmania* parasites. Th1 response was evaluated by estimating levels of four cytokine (IL2, IL12, TNF-α and IFN-γ), by multiplex cytokine ELISA in supernatants of PBMCs exposed to either wild type (*Ld*1S) or *Ld*Cen1$^{-/-}$ parasites.



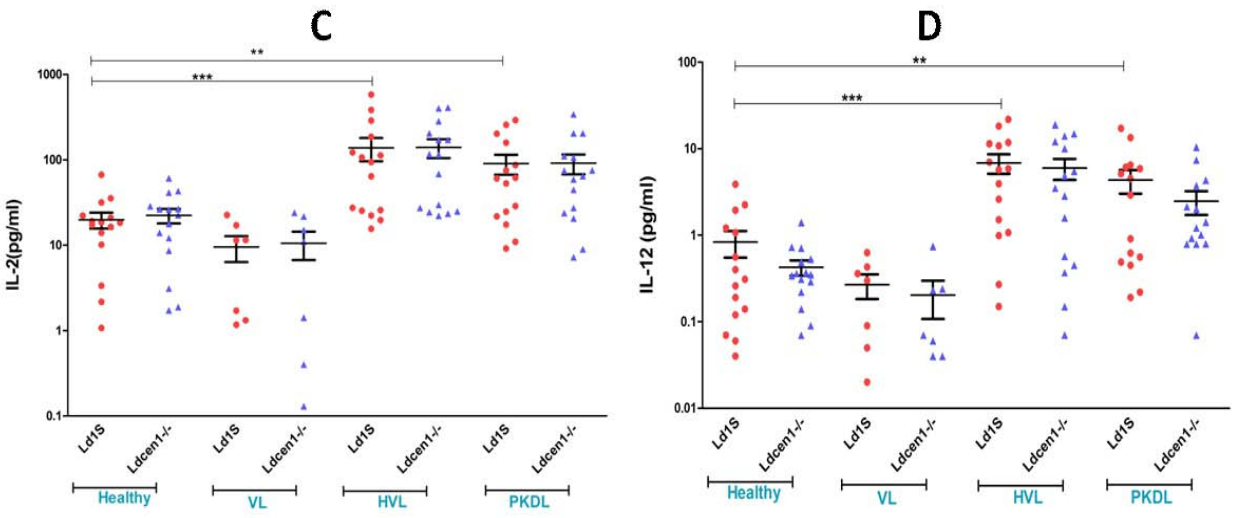


Fig. 8. Th1 cytokines level in supernatant of peripheral blood mononuclear cells (PBMCs) from Healthy (n=15), VL (n=7), PKDL (n=15) and HVL (n=15) cases in response to Wild type (*Ld*1S) and *Ld*Cen1$^{-/-}$ parasites. (A) TNF-α, (B) IFN-γ, (C) IL-2 and (D) IL-12. Value of control unstimulated cells were subtracted from the values of *Ld*1S and *Ld*Cen1$^{-/-}$ infected PBMCs. Data are given in Mean±SEM (pg/ml). Significance was determined by Mann-Whitney U test. P <0.05 is considered statistically significant.*=P<0.05, **=P<0.01, ***= P<0.001.

PBMCs from HVL and PKDL displayed significantly higher level of all the four cytokines compared to healthy group after infection with Ld1S and *Ld*Cen1$^{-/-}$ parasites. In VL group IFN-γ levels were significantly high while IL2, IL12 and TNF-α remained unaltered (Fig. 8). Th1 cytokine responses stimulated by *Ld*Cen1$^{-/-}$ were similar to that of the wild type.

Th2 cytokines response

Th2 immune response suppresses the action of Th1 function and helps in intracellular parasite growth and disease progression. Th2 response in this study was evaluated by estimating levels of three cytokines viz., IL4, IL6, and IL-10.

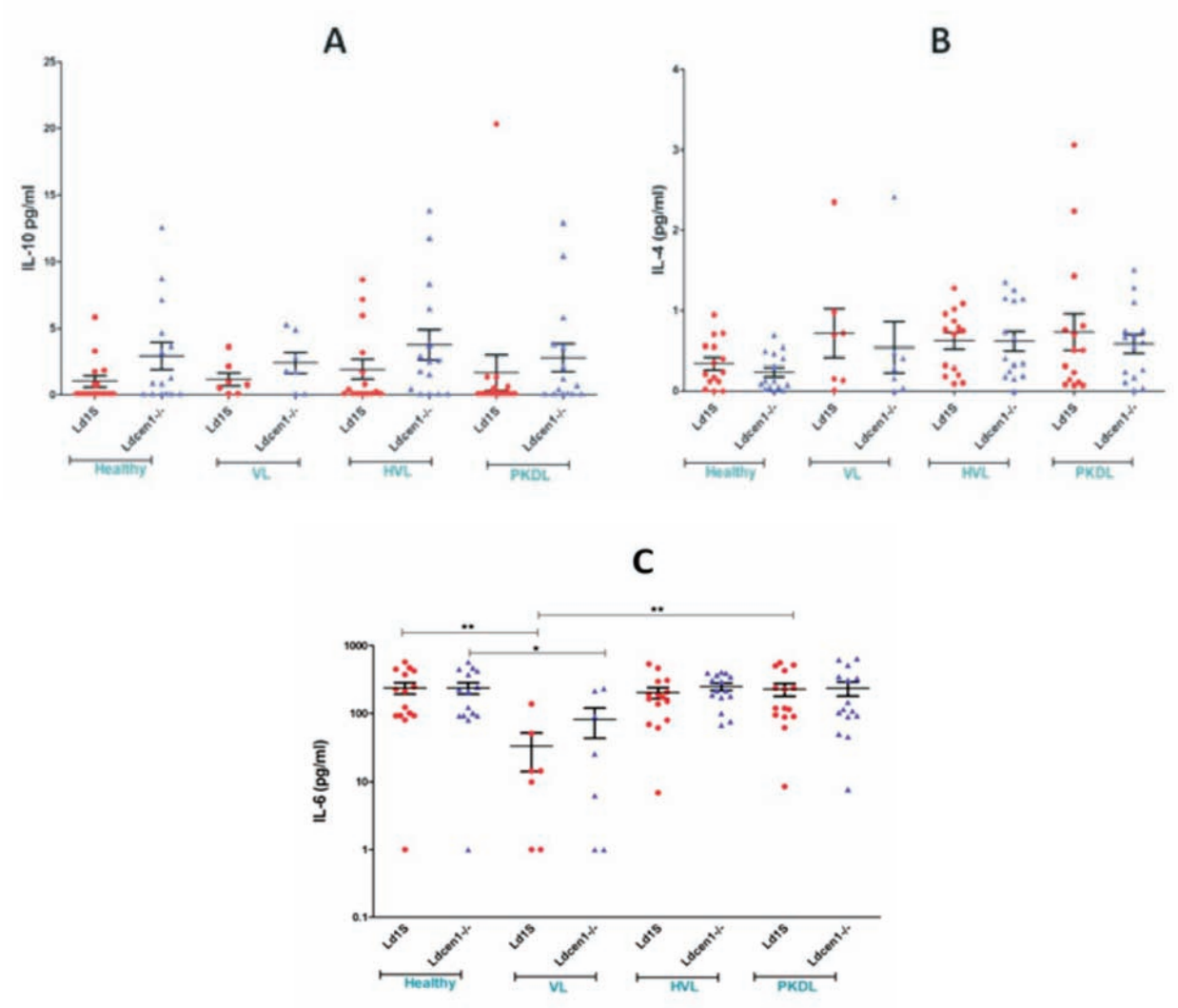


Fig. 9: Th2 cytokines level in supernatant of peripheral blood mononuclear cells (PBMCs) from Healthy (n=15), VL (n=7), PKDL (n=15) and HVL (n=15) patients in response to *Ld*1S and *Ld*Cen1$^{-/-}$ parasites. (A) IL-10, (B) IL-4 and (C) IL-6. Data are given in Mean±SEM (pg/ml). Significance was determined by Mann-Whitney U test. P <0.05 is considered statistically significant. *=P<0.05, **=P<0.01.

We observed non significant stimulation of IL-10 (Fig. 9A) and IL-4 (Fig. 9 B) after infection with wild type or *Ld*Cen1$^{-/-}$ parasite in any of the four groups compared to uninfected control cells. However, IL-6 was stimulated significantly after infection with both wild type and *Ld*Cen1$^{-/-}$ in healthy, HVL and PKDL groups. Levels of IL-6 in healthy, HVL and PKDL were also significantly high compared to the VL group.

Outcome

The analysis of immune responses elicited by *LdCen-/-* in human PBMCs from healthy individuals pre-exposed to *Leishmania* showed predominant Th1 response by the vaccine candidate, correlating to protection previously observed in the animals. Immune responses displayed by live attenuated *LdCen1*$^{-/-}$ parasite were similar to that of the wild type parasite, indicating its potential as a vaccine candidate.

4. Development of New Live Attenuated Vaccine Candidates for Kala-azar

Scientific staff : Dr. Poonam Salotra, Mr Kumar Avishek

In collaboration with : Dr. Angamuthu Selvapandiyan, Dr. Hira Nakhasi, Dr. V. Ramesh, Dr. N. S. Negi

Duration : 2011-15

Aims, Objectives & Background:

Study aims to generate new live attenuated *Leishmania* parasites as vaccine candidate by targeted deletion of genes that are highly expressed at the intracellular amastigote stage of the parasite. Amastigote-specific genes are likely to play central roles in survival of *Leishmania* parasite in the mammalian host. In the present study we report towards characterization of one such gene A1 which has been found to be highly expressed at the amastigote stage.

Work done during the year:

Bioinformatics analysis of A1:

Bioinformatics analysis was carried out to predict structure and other protein interacting regions present in A1. Bioinformatics analysis showed that A1 is

a disordered protein having 34% disorder region and 54% alpha helix, 14% beta strand, secondary structure (Fig 10). It has two protein binding regions for interaction with other proteins.

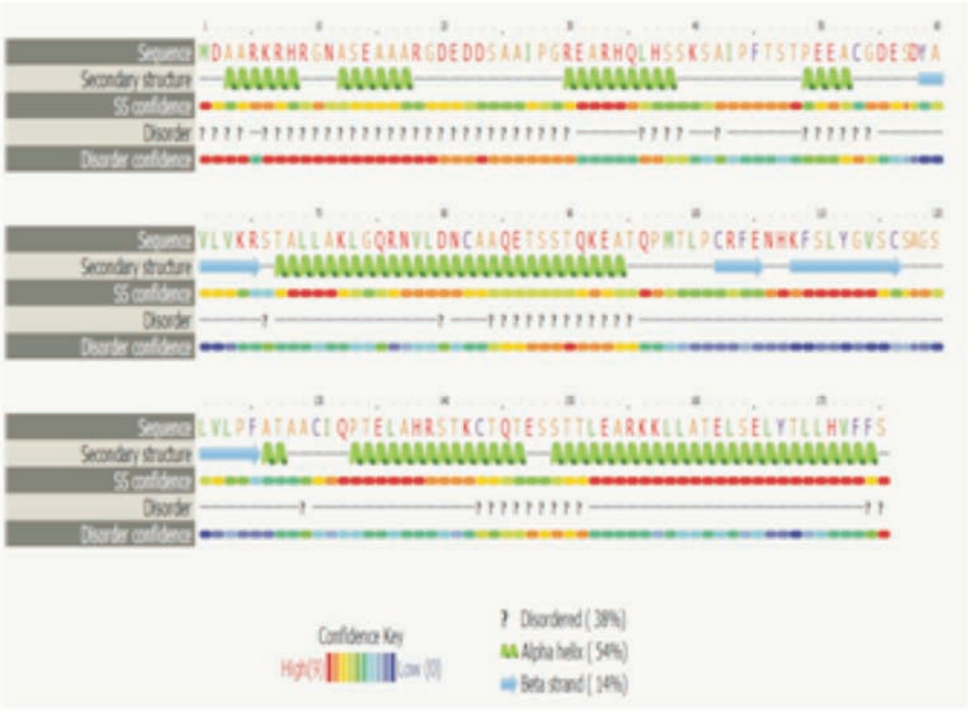


Fig. 10: Disorderness and secondary structure prediction of A1 protein by using Phyre online tool

Over expression of full length A1 gene in *L. donovani*

The *Leishmania* expression plasmid pKSNeo was used to express full-length A1 gene in *L. donovani*. A1 is 175 amino acid long protein with molecular weight ~ 21 kDA. As reported earlier, we have successfully cloned A1 gene in pKSNeo vector, clone was confirmed by restriction digestion and orientation of A1 in pKSNeo was confirmed by PCR. pKSNeo with A1 gene in right orientation was transfected in *Leishmania* parasite by electroporation. The transfected parasites were selected under G418 pressure and the over-expression was validated by western blotting of the total promastigote lysate probed with anti HA antibody (Fig 11).

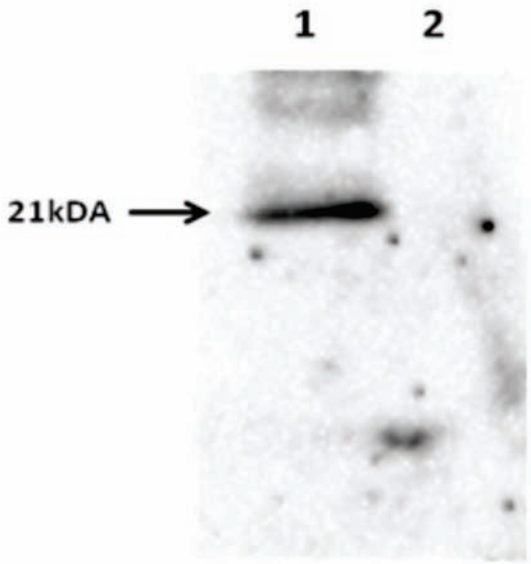


Fig. 11: Western blot to confirm the over-expression of A1 in *L. donovani* 100μg total promastigotes lysates were separated on a 12% SDS-PAGE gel and transferred to nitrocellulose membranes. Membrane was probed with anti HA antibody followed by rabbit IgG conjugated with HRP and developed using ECL. Lane 1: Lysate of parasite transfected with A1 gene, Lane 2: Lysate of parasite transfected with pKSNeo plasmid alone.

Effect of over-expression of A1 on growth of *Leishmania* parasite

The growth of parasite over-expressing A1 gene was monitored at the promastigote stage over a period of 10 days. Growth of A1 transfected parasite (*Ld*SA1) was comparable to the growth of controls transfected with the plasmid alone indicating that over-expression of A1 had no effect on the growth of the parasite (Fig 12).

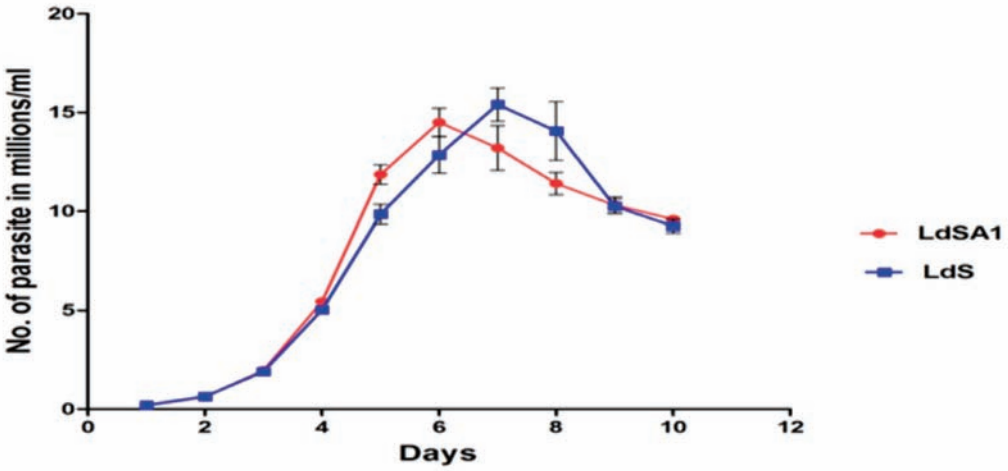


Fig. 12: Growth curve of *Leishmania* parasite over-expressing A1 gene (***Ld***SA1) and with pKSNeo plasmid alone. X-axis of the graph represents number in days while Y-axis represents number of parasite in millions.

Future Plan

Immuno-fluorescence analysis will be performed to determine the intracellular localization of A1. A1 gene knock-out study and effect of gene deletion on survival, growth and phenotype of parasite will be carried out.

5. Ubiquitin Related Modifier 1: A Post Translational Modification Machinery in *Leishmania donovani*

Scientific staff : Dr. Poonam Salotra, Ms. Vanila Sharma

In collaboration with : Dr. Angamuthu Selvapandiyan, Institute of Molecular Medicine, New Delhi

Duration : 2012-15

Aims, Objectives & Background:

Post translational modification is a diverse biological process that regulates the activity of target proteins. These modifications may occur in the form of

phosphorylation, acetylation, methylation or by covalent addition of entire protein molecule like ubiquitin. However in case of *Leishmania* this process is not well understood. Earlier, we reported a novel Ubiquitin like (Ubl) system in *Leishmania* comprising of an Ufm1-Uba5-Ufc1 ubiquitination like pathway. Here we show characterization of another Ubl: Ubiquitin related modifier-1 (Urm1) and its conjugation pathway in *L. donovani*. Last year we had reported the cloning, expression and purification of recombinant *Ld*Urm1 and *Ld*Uba4 proteins The study aims to characterize Ubiquitin-related modifier 1 (*Ld*Urm1) and its conjugation pathway in *L. donovani*.

Work done during the year:

In the year under report, immunoflourescence was done to identify the cellular localization of L*d*Urm1 and L*d*Uba4 proteins in promastigotes and amastigotes. We further performed assays to understand the mechanism of interaction between the two proteins in *Leishmania*. Co-immunoprecipitation (Co-IP) using anti-L*d*Urm1 antibody followed by MS analysis was conducted to identify the interacting targets of L*d*Urm1 in *Leishmania*.

Intracellular localization of LdUrm1 and LdUba4 in *Leishmania donovani*

Immunofluorescence studies showed that the *Ld*Urm1 is localized at the anterior end near flagellar reservoir believed to be early endosomes, both in promastigotes and axenic amastigotes (Fig 13 A and C). *Ld*Uba4 on the other hand was localized to cytoplasm in both promastigotes and axenic amastigote (Fig 13 B and D).

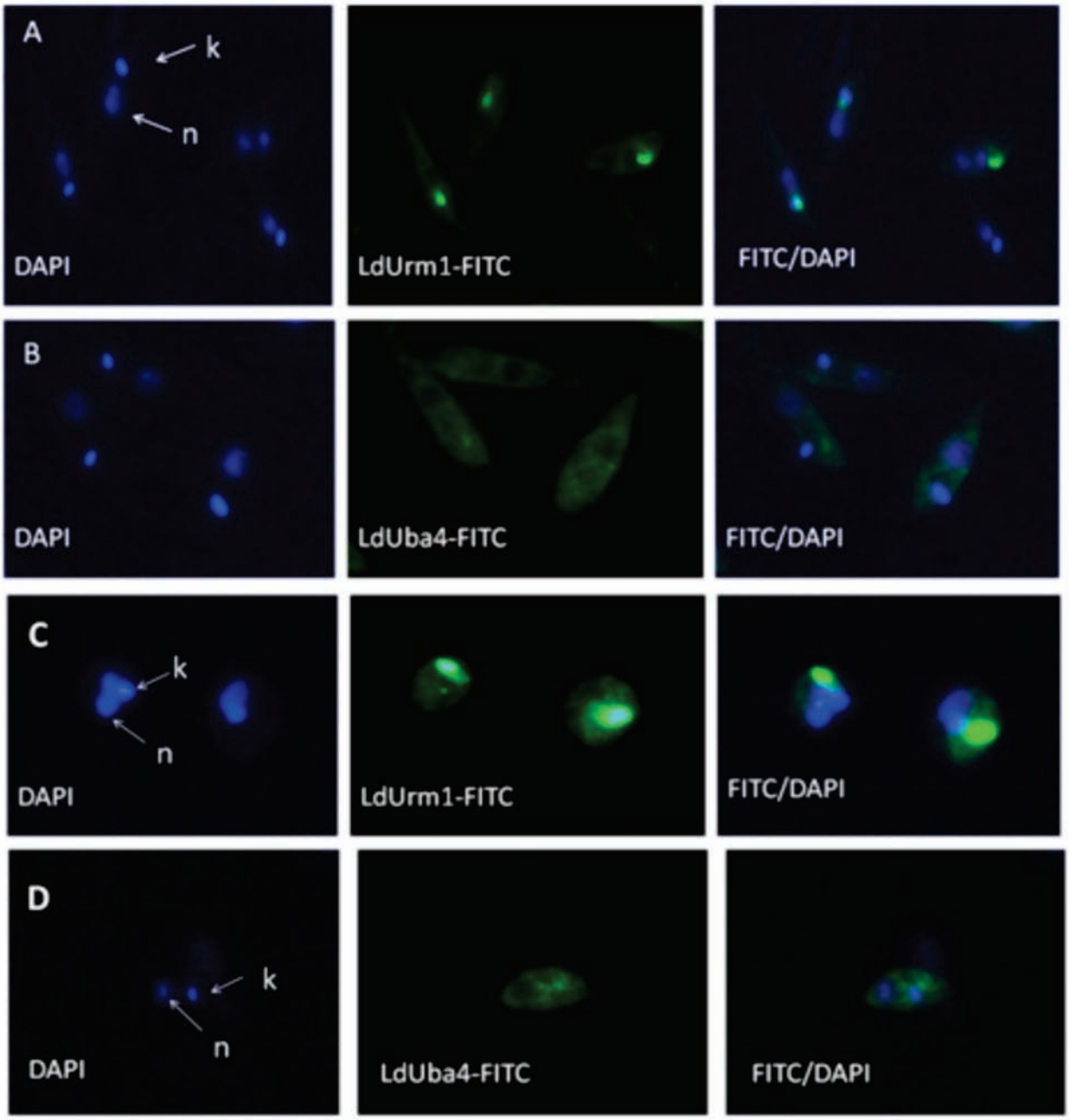


Fig. 13: Intracellular localization of *Ld*Urm1 and *Ld*Uba4 in *L. donovani*. *Ld*Urm1/ ***Ld***Uba4 was detected with anti-LdUrm1/ anti-*Ld*Uba4 respectively and visualized with FITC labeled anti-rabbit antibody. X100. k, kinetoplast; n, nucleus.

Demonstration of *Ld*Urm1-*Ld*Uba4 conjugation pathway *in vitro*.

To understand the interaction behaviour of LdUrm1 and LdUba4, the recombinant proteins were subjected to Co-IP followed by western blotting to reconstitute the

conjugation pathway in vitro. For this anti- LdUba4 antibody was immobilized onto Protein A agarose beads and incubated with (i) a mixture of LdUba4 and LdUrm1 pure proteins; (ii) LdUba4, LdUrm1 and ATP; (iii) *L. donovani* lysates. The resin was than eluted for isolation of the conjugates. The elutes were subjected to SDS-PAGE followed by immunoblotting with anti-LdUba4 antibody. A single band corresponding to the size of LdUba4 (~50kD) was seen on the blot (Fig 14A). Contrary to this, an additional band of higher molecular weight corresponding to the size of LdUrm1-LdUba4 conjugate (~87kD) was seen when ATP was added to the mix (Fig 14B). Interestingly, when protein A agarose beads were incubated with *L. donovani* lysate a band corresponding to the size of LdUba4 (~50kD) and a band corresponding to LdUrm1-LdUba4 conjugate (~60kD) was seen when probed with anti-LdUba4 antibody (Fig 14C). Taken together these results validate the fact that LdUrm1 and LdUba4 interact with each other in *Leishmania* to form a transient intermediate of high molecular weight, however the interaction is energy dependent.

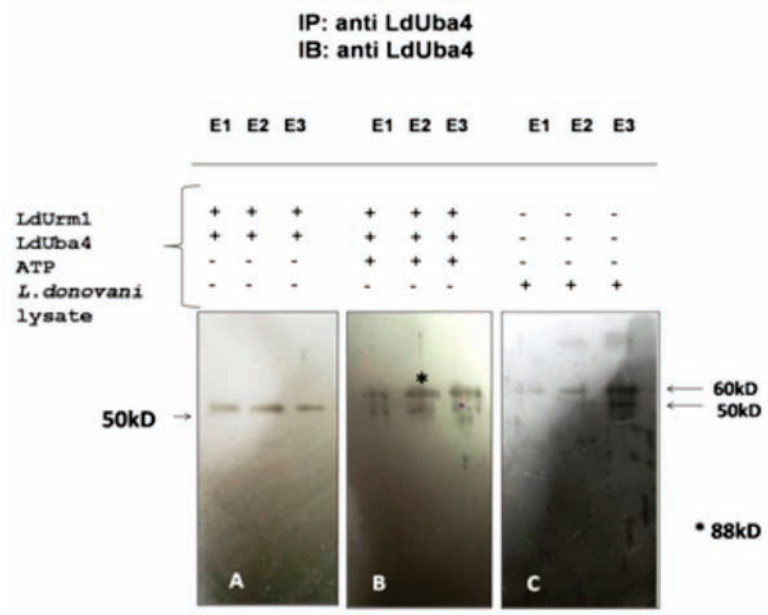


Fig. 14: Demonstration of interaction between the recombinant *Ld*Urm1 and *Ld*Uba4. Anti LdUba4 antibody was crosslinked with Protein A agarose beads and incubated with three different reaction mixtures **A.** *Ld*Urm1 and *Ld*Uba4 **B.** *Ld*Urm1 and *Ld*Uba4 in presence of ATP **C.** *L. donovani* lysate and three subsequent IP elution were taken. E1, E2, E3.

Immunoprecipitation and MS analysis: A functional proteomics approach to identify putative *Ld*Urm1 targets.

In order to investigate the putative targets of urmylation in *L. donovani* and its role as post translational modifier, cell extracts were prepared from *L. donovani* promastigote culture and *Ld*Urm1 associated proteins were retrieved by immuno-precipitation, separated by SDS/PAGE, and subjected to silver staining. The Co-IP elutes were subjected to western blot and probed with anti-*Ld*Urm1 antibody. A total of 6 bands appeared on immunoblot (Fig. 15, *right panel*). The bands were excised and analysed by mass spectrometry. The results revealed that *Ld*Urm1 is conjugated to *Leishmania* proteins that are majorly associated with early endosomal vesicular trafficking, cytoskeleton and cell division. Early endosome associated putative Rab-GTPase and Rab5 were found to be the putative targets of *Ld*Urm1 in MS analysis. From a structural and functional perspective, these findings collectively suggest a link between *Ld*Urm1 and early endosome associated hemoglobin internalization for *Leishmania*. A hypothetical protein target to *Ld*Urm1 with HECT domain characteristic to E3- like enzymes was also identified highlighting the possibility of involvement of an E3 enzyme into the conjugation pathway. However, the hypotheses need to be studied in depth and validated further.

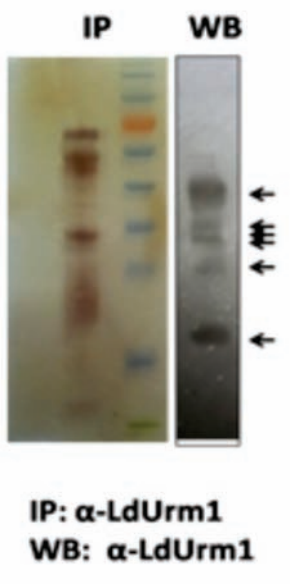


Fig. 15: Immunoprecipitation and MS analysis. SDS-PAGE depicting the Co-IP done using α-*Ld*Urm1 (*left panel*). Six distinct bands identified on immunoblot developed using α-*Ld*Urm1. The bands were excised and analysed by mass spectrometry to identify *Ld*Urm1 specific conjugates *(right panel).*

Future Plan:

Studies are underway to overexpress wild type (*Ld*Urm1^{WT}) and non-conjugatable form of *Ld*Urm1 (*Ld*Urm1$^{\Delta G)}$ to identify the significance of *Ld*Urm1 for the growth and survival of *Leishmania* both as promastigote and amastigote. This would help us to understand the role of Urm1- Ubl4 pathway in *Leishmania*.

6. Development of Loop-mediated Isothermal Amplification (LAMP) Assay for Diagnosis of *Leishmania* infection

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Dr. Sandeep Verma

In collaboration with : Dr. N.S. Negi and Dr. V. Ramesh, Safdarjung Hospital

Duration : 2012-14

Aims, Objectives & Background:

Parasite detection based diagnosis (stained smears, culture or histopathology) of VL and PKDL is invasive and has poor sensitivity, while immunological methods (Direct Agglutination Test, enzyme-linked immunosorbent assay etc.) are not conclusive for PKDL because of persistence of anti-leishmanial antibodies after VL, and are not reliable in immune-compromised patients. Previously, we established a genus specific loop mediated isothermal amplification (LAMP) assay based on 6 primers as a diagnostic tool for VL and PKDL. We have now applied LAMP assay on confirmed VL and PKDL samples at NIP, and on confirmed VL cases in endemic region (RMRI, Patna).

Work done during the year:

Sensitivity and specificity of LAMP assay in VL and PKDL diagnosis

VL (n=55) and PKDL (n=63) patients originating from Bihar (India) and reporting

to Safdarjung Hospital, New Delhi, were included in this study at the pre-treatment stage. The patients presented with characteristic symptoms of VL (fever, hepatosplenomegaly, anemia, and leucopenia etc.) or PKDL (on clinico-histopathologic observations) and only confirmed cases (Leishman–Donovan bodies and/or PCR positive) were included in this study. Venous blood (n=55) and BMA (n=15) samples were collected from VL patients while tissue biopsy (n=63) samples were collected from PKDL patients. Blood samples from healthy volunteers (endemic, n=22; non endemic, n=36), malaria patients (n=5), and tuberculosis patients (n=5), and tissue biopsy samples from leprosy patients (n=18), fungal disease (sporotrichosis or pityriasis lichenoides chronica) cases (n=6) and normal skin from PKDL patients (n=10) were collected as controls.

DNA isolated from blood of VL patients and tissue biopsy of PKDL patients and from healthy and other disease controls were subjected to LAMP assay. The LAMP assay was positive in 53 of 55 VL blood samples, giving sensitivity of 96.4% (95% Confidence interval, 87.7–99%). The LAMP assay was positive in 61 out of 63 PKDL tissue biopsy samples, giving sensitivity of 96.8% (95% Confidence interval, 89–99.1%). The LAMP assay was negative in all controls (n=102) comprised of blood from healthy individuals (endemic, n=22; non endemic, n=36), malaria (n=5), tuberculosis (n=5), and tissue biopsy from leprosy and fungal infections (n=10), giving specificity of 100% (95% Confidence interval, 96.4–100%), (Fig. 16 and Table 3).

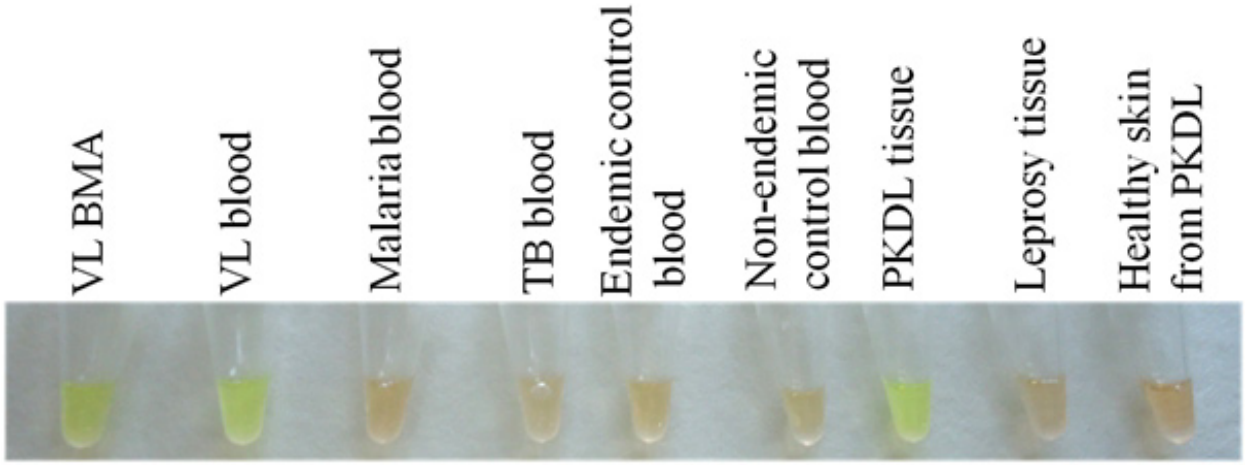


Fig. 16: LAMP assay with BMA and blood samples of VL, blood samples of malaria, tuberculosis (TB), endemic control, non-endemic control, and tissue DNA from PKDL, Leprosy and Healthy skin from PKDL cases. 2 μl of DNA isolated from clinical samples was used for amplification.

Table 3: Result of LAMP assay in VL and PKDL clinical samples and controls

Source of DNA	Total cases	Positive cases	% Positive
VL bone marrow	15	15	100
VL blood	55	53	96.4
Malaria blood	05	0	0
TB blood	05	0	0
Endemic controls	22	0	0
Non-endemic controls	36	0	0
PKDL lesions	63	61	96.8
Leprosy lesions	18	0	0
Fungal lesions	06	0	0
Normal tissue from PKDL	10	0	0

Sensitivity and specificity of LAMP assay at RMRI (Patna)

VL patients reporting to the RMRI (Patna) were included in this study at the pre-treatment stage. Venous blood from parasitological confirmed VL (LDB positive

splenic aspirates) cases (n=57) at pretreatment stage and from healthy endemic controls (n=40) was collected. The LAMP assay was performed with blood DNA samples and was found positive in all confirmed VL cases (n=57) while negative in all endemic healthy controls (n=40), results shown in Table 4, indicating a high sensitivity 100% (95% CI 93.7-100%) and specificity 100% (95% CI, 91.2–100%).

Table 4: Sensitivity and specificity of the LAMP assay in parasitological confirmed (LDB in splenic aspirates) VL cases (using blood) at RMRI (Patna)

Sample	Cases tested	Cases positive	Sensitivity/Specificity (95% CI)
VL	57	57	100% (93.7-100%)
Controls	40	0	100% (91.2-100%)

3rd party validation of LAMP assay by RMRI (Patna)

The personnel were trained to perform LAMP assay at RMRI (Patna). The LAMP assay was then performed with blood DNA from confirmed VL cases (n=50) and from healthy endemic controls (n=35) at RMRI. The assay was found highly sensitive (sensitivity=98%, 95% CI 89.5–99.7%) and specific (specificity=97.1%, 95% CI, 85.5–99.5) (Table 5).

Table 5: Third party validation at RMRI (Patna) on parasitological confirmed VL cases

Sample	Cases tested	Cases positive	Sensitivity/Specificity (95% CI)
VL	49	50	98% (89.5-99.7%)
Controls	35	1	97.1% (85.5-99.5%)

Outcome:

The assay was found highly sensitive and specific for diagnosis of VL and PKDL. Third party validation of the assay, carried out at RMRI, Patna, confirmed its utility as a potential diagnostic tool. The DNA sequence of the amplified target region was determined. Application for the patent of the newly developed LAMP assay was filed at Indian Patent Office.

CHLAMYDIASIS

1. Immunopathogenesis of Reactive Arthritis Induced by Chlamydia Trachomatis

Scientific Staff : Dr. Sangita Rastogi, Mr. Praveen Kumar (SRF-ICMR)

In collaboration with : Brig. (Dr.) DS Bhakuni, Army R & R hospital, New Delhi

Duration : 2010-14

Aims, Objectives & Background:

Chlamydia trachomatis-induced Reactive Arthritis (cReA) has been defined as a non-purulent arthritis that develops during or soon after extra-articular bacterial infections elsewhere in the body, but in which the microorganism cannot be recovered from the joint. It is well accepted today that cReA is caused by intra-articular persistent infection and, therefore, is of post-infectious origin.

Knowledge of the biology of chlamydiae is essential in understanding the pathogenesis, diagnosis and treatment of cReA. Chlamydiae are non-motile obligate intracellular bacteria, infecting a variety of human cell types, including epithelial, endothelial and smooth muscle cells, as well as macrophages and monocytes. At the sight of primary infection, *viz.:* urogenital tract, ocular conjunctiva or respiratory tract mucosa, the bacteria infect monocytes/ macrophages and are disseminated via the bloodstream to settle into the joint. Thereafter, the persistent chlamydiae cannot be detected using traditional culture techniques but can be located in synovial membrane and synovial fluid using various methods, including electron microscopy, immunofluorescence and PCR. Morphologically aberrant but viable, metabolically active persistent chlamydiae have been consistently documented in synovial tissue in post-chlamydial ReA. The search for *Chlamydia* or its components at the site of the primary infection or in the joint is the optimal approach to confirm chlamydial etiology of arthritis.

The most specific diagnosis of cReA is made by the detection of the pathogen in the joint itself using PCR and/ or other molecular amplification assays. However, during initial diagnostic testing, the use of immunofluorescence assay has the potential to serve as an easy detection method for *C. trachomatis*. The pathological processes due to chlamydial infection appear to be mediated by the immune reactivity. The host immune response stimulated by repeated episodes of infection increases the pathological lesions. Reinfection or persistence state causes pathological changes. Chlamydial heat shock protein 60 (chsp60) is a *Chlamydia* genus-specific protein, serving as a strong antigenic target for the immune system and it has been suggested that antibodies to chsp60 are markers of chronic inflammation. This study was initiated as an ICMR-SRF project and aimed to understand the mechanism of chlamydial immunopathogenesis in the synovium of cReA patients.

Work done during the year:

The present report summarizes the results obtained in 120 arthritic patients, *viz*.: 30 Reactive Arthritis (ReA), 40 Undifferentiated Spondyloarthropathy (uSpA), 50 Rheumatoid Arthritis (RA)/ Osteoarthritis (OA). This includes 71 patients enrolled during the previous years of the project while, during the reporting period, 49 male/ female age-matched arthritic patients, *viz.:* 10 ReA, 16 uSpA, 17 RA and 6 OA were further enrolled under the guidance of the collaborating rheumatologist from Department of Rheumatology & Clinical Immunology, Army hospital (R&R), New Delhi. European Spondyloarthropathy Study Group (ESSG) criteria was followed for the selection of ReA/ uSpA patients *(Amor et al 1995; Dougados et al 1991)* while RA patients were selected following the American College of Rheumatology (ACR) criteria *(Arnett et al 1988)*.

The elementary bodies of *C. trachomatis* were localized in the joint fluid of patients with ReA/ uSpA (26%) by direct immunofluorescence assay. On comparison of cytological findings with nPCR, there was moderate agreement between the tests, giving a Kappa co-efficient of 0.6. However, since majority of the patients with *C. trachomatis* infection in uSpA are asymptomatic for

urogenital infection, hence an attempt was made to develop a non-invasive diagnostic approach by detection of *C. trachomatis* in first voided urine in this group of arthritic patients. For this, urine sample was centrifuged and cells were fixed with acetone and stained by FITC-conjugated major outer membrane protein *C. trachomatis* antibodies for detection of Elementary Bodies (EBs) by direct immunoflurorescence assay using *MicroTrak C. trachomatis* Direct Specimen Test (*Trinity Biotech, USA*) while nucleic acid isolation was done by viral RNA (*Qiagen, USA*) kit. *C. trachomatis* MOMP was found in 17.2% (5/ 29) uSpA patients by immunofluorescence cytology while the cryptic plasmid gene of *C. trachomatis* (200 bp) was located in the nucleic acid of urine sediment and 13.3% (4/ 30) uSpA patients were found positive by PCR method.

Chlamydial heat shock protein 60 (chsp60) which is synthesized in increased amounts and is released into the extracellular milieu during persistence was also studied. Overall, 23.8% (10/ 42) ReA/ uSpA patients were positive for circulatory IgG antibodies against chsp60 protein in the serum ('p' value < 0.001 versus RA) (Fig. 1). Patients with uSpA showed evidence of higher antibodies to chsp60 (9/33; 27.2%) as compared to ReA (1/9; 11.1%) ('p' value < 0.6).

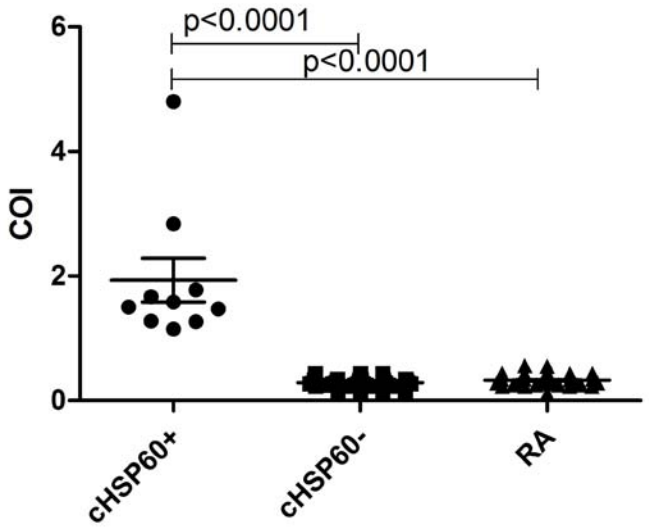


Fig. 1: Cut-off indices (COI) were compared between the ReA/ uSpA chsp60-positive, ReA/uSpA chsp60-negative and control chsp60-negative Rheumatoid Arthritis (RA) patients. Significant differences were observed between these groups.

Overall, the level of high sensitive C-reactive protein (hsCRP) in ReA/ uSpA patients was 0.0 - 84.5 mg/ ml (mean ± SD = 35.2 ± 28.6) while patients positive for chsp60 protein had 19.0 - 81.5 mg/ ml (mean ± SD = 48.1 ± 22.8). Although hsCRP was higher in chsp60 protein positive patients but this was found to be statistically insignificant ('p' value = 0.17) in comparison to the chsp60 protein negative ReA/ uSpA patients while it was observed that hsCRP value in chsp60 protein positive patients were significantly higher than both inflammatory and non-inflammatory controls respectively ('p' value = 0.01 versus RA, 'p' value = 0.0001 versus OA) (Fig. 2). The level of hsCRP in chsp60 protein negative ReA/ uSpA patients was significantly higher than non-inflammatory control OA patients (mean ± SD = 2.0 ± 1.22, 'p' value = 0.0001) while there was only mild increase with inflammatory RA controls (mean ± SD = 25.87 ± 21.4, 'p' value = 0.23).

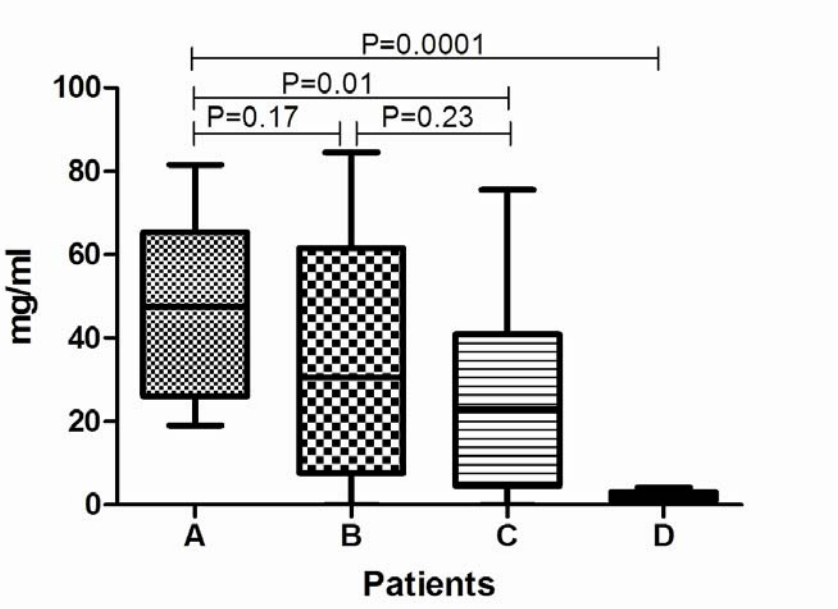


Fig. 2: Level of high sensitive C-reactive protein (mg/ ml) in different study groups (A - B) and control groups (C - D). 'p'value < 0.05 were considered to be significant.
A: chsp60-positive ReA/ uSpA; B: chsp60-negative ReA/ uSpA; C: chsp60-negative RA; D: chsp60-negative OA.
Abbreviations: ReA-Reactive Arthritis; uSpA- Undifferentiated Spondyloarthropathy; RA- Rheumatoid Arthritis; OA- Osteoarthritis

Future Plan:

HLA-B27 gene will be located in DNA of patients in order to find out genetic susceptibility to *Chlamydia*-induced reactive arthritis and its role in disease severity.

TUBERCULOSIS

1. Understanding the Underlying Mechanism of Macrophage Immune Modulation: Role of Resistin

Scientific Staff : Dr. Nasreen Z. Ehtesham, Ms. Aadinarayan Varma

In Collaboration with : Dr. Seyed E. Hasnain, IIT, Delhi

Duration : 2009-15

Aims, Objectives & Background:

Tuberculosis (TB), a progressive disease taking one human life every 15 s, globally, is caused by invasion of *Mycobacterium tuberculosis* (*M.tb*) into macrophage. There has been considerable interest about the host defence in *M. tuberculosis* infection. The innate immune system provides the first line of defense against invading pathogens. The innate immune system is a candidate for the production of elevated levels of cytokines in tuberculosis. The innate immune pathway is activated when certain antigens bind to specific receptors like toll-like receptors (TLRs).

Given the importance of resistin, which is mostly expressed in macrophages (cells involved in first line of defense) and contribute to the inflammatory response may be involved in the regulation of immune responses and play a potential role as a part of innate immune system

Novel attributes of Human Resistin

Our previous report shows that i) human resistin has a very stable secondary structure ii) human resistin forms higher order oligomers. This oligomerisation tendency, with a very stable secondary structure, raises some intriguing questions regarding its physiological relevance:

Work done in our laboratory show that this pro-inflammatory cytokine acts as a chaperone. Hence human resistin now can be classified as chaperokine. Chaperokine are those, which are chaperones in the cell and possess pro-inflammatory properties when they are in extracellular environments.

Work done during the year:

Expression changes of resistin during ER stress:

U937 were used for induction of ER stress either by Tunicamycin which inhibits N-linked glycosylation or Thapsigargin which causes ca^{2+}imbalance. Level of resistin was monitored using qPCR and by western. Resistin was shown to be overexpressed at transcript and protein levels in both dose and time-dependent manner during ER stress in U937 cells. (Fig. 1)

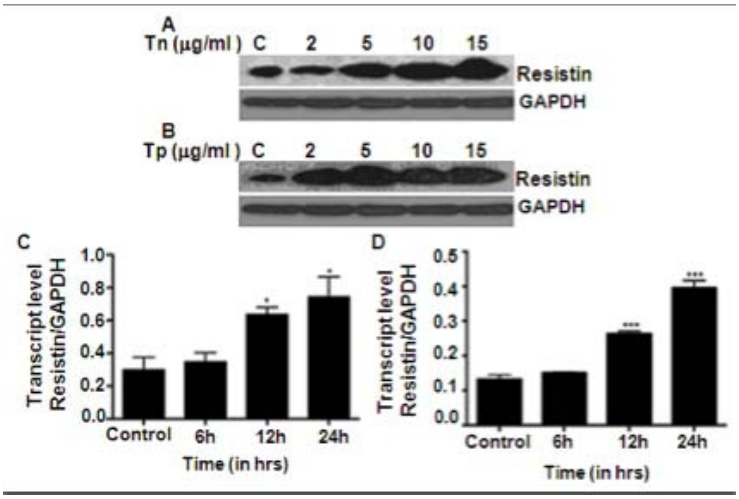


Fig. 1: Resistin is up-regulated during tunicamycin or thapsigargin treatment. U937 cells were treated with increasing concentrations (0, 2, 5, 10, and 15 µg/mL) of tn (A) or tp (B) for 24 h, and resistin expression levels were determined by Western blot. In another experiment, U937 cells were treated with 5 µg/mL of tn (C) or tp (D) for 0, 6, 12, and 24 h, and resistin transcript levels were quantified using qPCR. Note the increase in resistin mRNA levels over time during tn/tp-induced ER stress. Statistical analysis was performed using one-way ANOVA and post-Dunnett's multiple comparison test. ***P < 0.0001, *P < 0.01.

Resistin is retained in ER during ER stress

Owing to chaperone activity of resistin *in vitro* and expression level correlation with ER stress later we analyzed whether the secretion of resistin is effected during stress condition.

ER stress was induced in U937 cells using tunicamycin/ thapsigargin. Supernatant was collected to assay for secreted resistin using ELISA and cell were analysed by confocal microscopy to assay the intracellular resistin. (Fig. 2). Upon ER stress, tunicamycin/thapsigargin treatment leads to secretion impairment resulting in about 40-50% decline in resistin secretion The issue of differential secretion was examined by the localization of hRes during ER stress using confocal microscopy. Increase in expression of hRes after 24 hrs of treatment in terms of increased Cy3 intensity was evident, thus showing that under conditions of ER stress while resistin continues to be over-all induced, bulk of it remains within ER. These results demonstrate that tunicamycin/thapsigargin induced stress leads to reduced levels of secretory resistin but increased intracellular levels, localized to the ER.

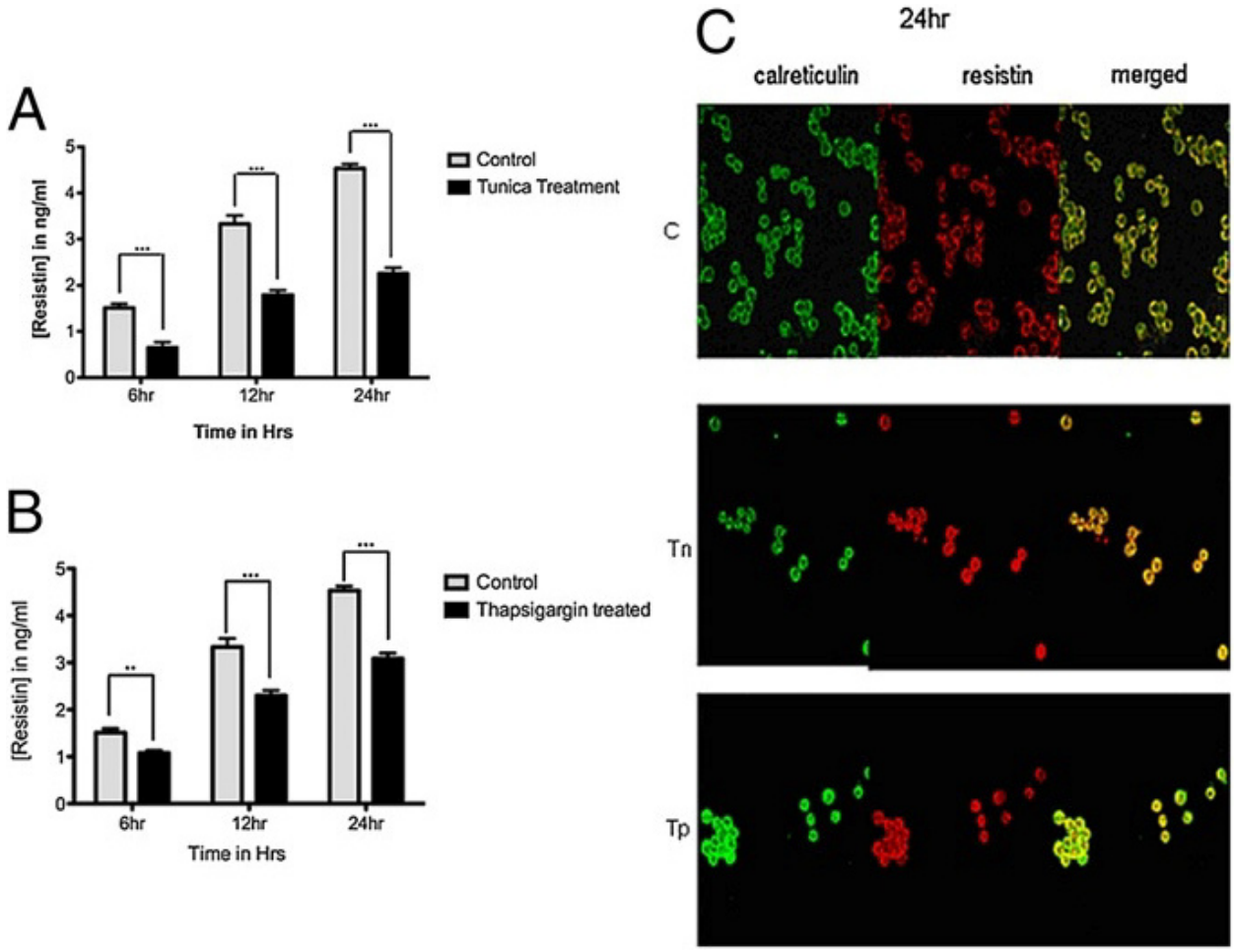


Fig. 2: Levels of secreted resistin were reduced under ER stress. U937 cells were treated with 5 μg/mL of tn (A) or of tp (B) for 6, 12, and 24 h. ELISA was performed with the supernatants of the treated cells. Note the reduced secretion of resistin in tn/tp-treated cells compared with untreated controls. Statistical analysis was performed by two-way ANOVA and post-Bonferroni test (***P < 0.0001, **P < 0.001 (C) Colocalization of resistin and calreticulin in ER. Untreated U937 cells and those treated with 5 μg/mL of tunicamycin (Tn) or thapsigargin (Tp) for 24 h were processed for confocal microscopy using respective fluorescence-labeled antibodies. ER marker (calreticulin) is shown in green color and resistin in red. Note the merging of the green and red signals in the merged column indicating colocalization of resistin in the ER.

Resistin rescued Hela cells during ER stress:

Further, in order to understand the effect of resistin we overexpressed resistin in HeLa which does not otherwise express resistin. Apoptosis was induced in transiently transfected cells and compared with untransfected cells. Percentage of cells undergoing apoptosis was scored using Annexin V staining These results demonstrate that resistin aids cells in restoring ER homeostasis by protecting them from apoptosis (Fig. 3).

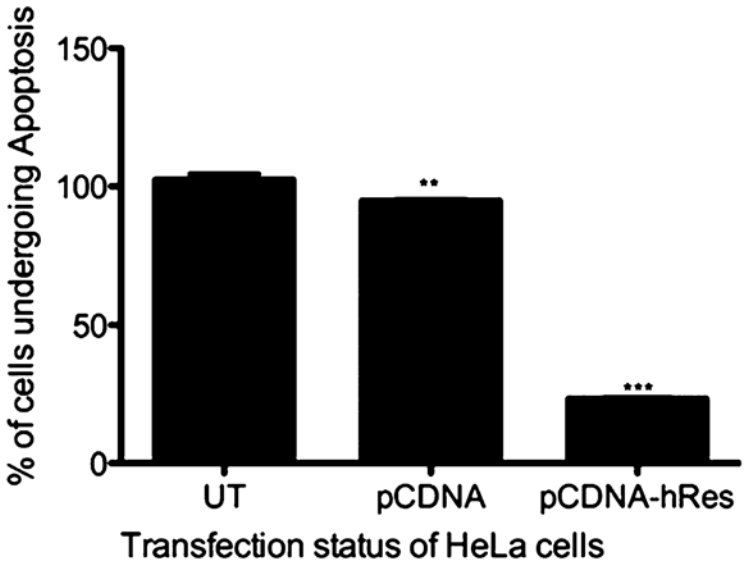


Fig. 3: Resistin expression causes an inhibition of apoptosis of HeLa cells. HeLa cells were transiently transfected with pCDNA plasmid vector, recombinant pCDNAhRes plasmid construct, or mock transfected (UT). Apoptosis was induced by treating cells with thapsigargin for 24 h, and the percentage of apoptosed cells was scored by Annexin V staining using FACS. The percentage of cells undergoing apoptosis in untransfected condition was normalized to 100 and results were plotted. Statistical analysis was performed by two-way ANOVA and post-Bonferroni test (***P < 0.0001, **P < 0.001).

Future Plan:

Owing to resistin overexpression during ER stress and its role in rescuing cells from ER stress induced apoptosis, role of resistin in restoring ER homeostasis will be analyzed using PCR arrays in order to identify the downstream targets.

In addition, changes in immune modulatory mechanisms that are mediated by resistin was also to be analyzed as the protein secretion is minimized during stress, where as in normal condition the protein is secreted out and mediates immune modulation as a pro-inflammatory cytokine.

2. Comparative Analyses of Non-pathogenic, Opportunistic and Totally Pathogenic Mycobacteria Reveal Genomic and Biochemical Variability and Highlight the Survival Attributes of *Mycobacterium tuberculosis*

Scientific Staff : Dr. Nasreen Z. Ehtesham, Javeed Ahmad

In collaboration with : Dr. Seyed E. Hasnain, IIT, Delhi

Duration : 2012-2015

Aims, Objectives & Background:

The evolution of *Mycobacterium* as a species is usually driven by processes including deletion, insertion, or a combination of these events, which aid in survival in different environmental conditions or geographic niches. This trend is also clearly evident from analyses of Mycobacterial genomes where a distinct pattern of decreasing genomic content is seen as one moves from non-pathogenic (NP) to opportunistic pathogens (OP) to true pathogens (TP). We therefore performed genome size analysis using 44 mycobacterium strains representing NP, OP and TP. One of the largest genomes in the *Mycobacterium* family is that of *Mycobacterium smegmatis*, a non-pathogenic mycobacterium, with approximately 6717 protein coding genes (genome size: 6.9Mb) whereas the other extreme is a True pathogenic mycobacterium, *Mycobacterium leprae*, with the smallest genome consisting of approximately 2770 protein coding genes (genome size: 3.3Mb).

Mycobacterium indicus pranii (*MIP*), has been earlier shown to have novel

immunomodulatory properties, and proven therapeutic value in the treatment of leprosy. This clinically 'benevolent' bacterium has been evolutionarily suggested to be at the point of transition to pathogenicity despite earlier data from DNA sequence analysis of select genes of *MIP* which showed 99% identity with corresponding genes of *M. intracellulare*. Comparative proteomic analyses of virulence factors of *M.tb* and their homologs in 12 different Mycobacterial species, including *MIP*, point toward gene cooption as an important mechanism in evolution of mycobacteria. Our results convincingly establish the very upstream evolutionary position of *MIP* and also highlight some important differences in the metabolic pathway of *Mycobacterium tuberculosis* H_{37}Rv which are of possible significance in virulence and pathogenesis.

Work done during the year:

METHODS:

Re-annotation of *MIP* proteome:

The prediction of protein function domains for the ORFs for *MIP* was carried out using InterPro and Pfam. The domain hits of individual proteins were compared to the Clusters of Orthologous Groups of proteins (COG) based annotation.

Functional relatedness of homologs:

Sequence identity above 60% between two proteins is required to have 90% functional similarity. We therefore tried to relate functional similarity based on Interpro/Pfam domain hits to the Coverage and Sequence Identity of BLASTp results between *MIP* and other *Mycobacterium* species.

The statistical significance of our approach was determined using data from DELTA-BLAST, which returns the domain hits of a protein from the Conserved Domain Database (CDD).

In order to obtain a sequence identity cut-off below which no functional similarity or homologs can be observed in *Mycobacterium* genus (13 species included in the analyses), we plotted number of 'tp' against sequence identity for *MIP* versus all twelve *Mycobacterium* species. We also performed an all against all BLASTp based homology searches between 13 mycobacterium species using sequence identity cut-off of 20% and e value < 1e-04.

Comparative Metabolic Pathway Analyses:

Analysis of metabolic enzymes was carried out based on the IUBMB EC numbers in the KEGG database (accessed in December 2012) for *MIP* (387 EC), *MIA* (394 EC), *MYCA1* (413 EC) and *MYCTU* (396 EC) genomes. Comparative metabolic pathway analysis between *MYCTU*, *MIA* and *MIP* was performed using iPath2.0

RESULTS:

Re-annotation of *MIP* Proteome:

InterPro/Pfam domain knowledge for *MIP* proteins was used to assign potential functions to ~83% of *MIP* proteome) *MIP* ORFs (Fig. 1). Out of the remaining 891, 164 proteins were annotated using the phylogenetic classification of proteins encoded in complete genomes known as COG but failed to match with any domain in Pfam and InterPro. Previously, 3870 (~70%) of *MIP* ORFs were assigned a putative function on the basis of COG (Fig. 1). Out of 1554 hypothetical proteins in *MIP* based on COG annotation, 656 have been assigned a putative function based on functional domain knowledge from InterPro/Pfam database.

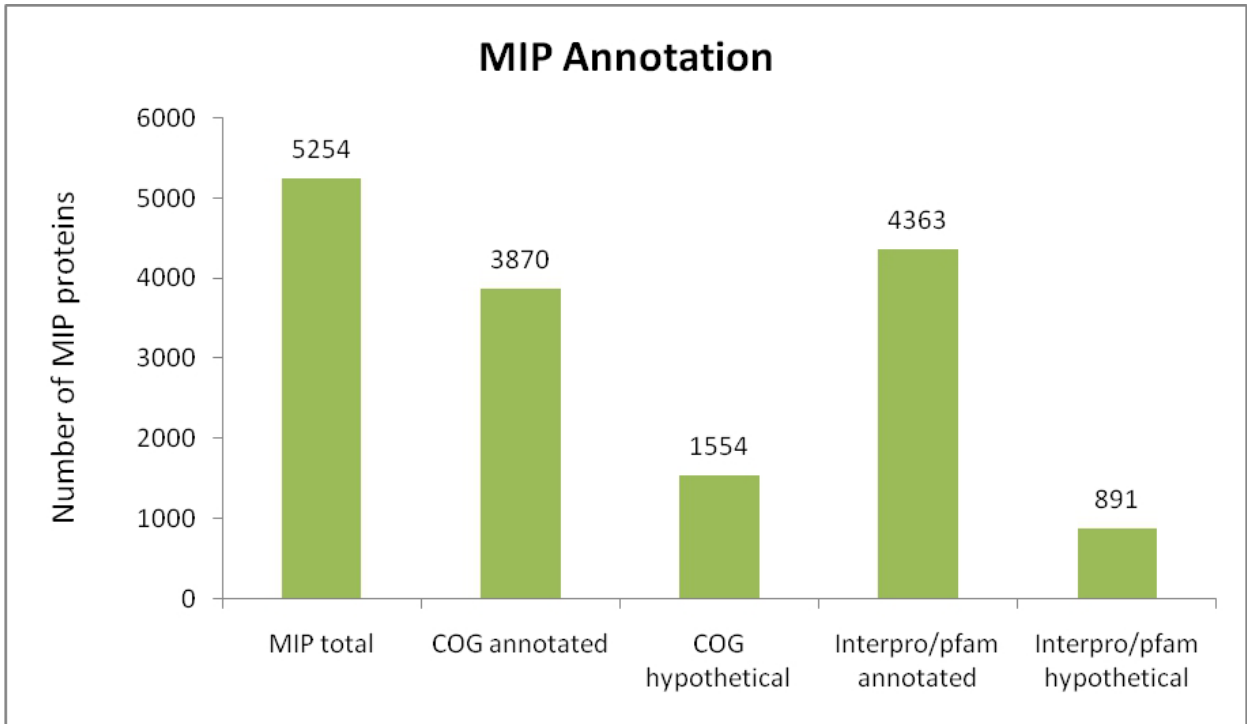


Fig. 1: Shows the comparative plot for annotation of MIP using COG and InterPro/Pfam.

Interestingly, 60 proteins were found to have conflicting COG and InterPro/Pfam based annotations. In such ambiguous cases, the protein sequences were further submitted in GENE3D to further confirm the annotation.. Thus, annotating a new proteome using Interpro not only provides better annotation coverage but also increases the confidence of annotation by providing in-depth knowledge about domains, motifs and structural annotation of the given protein sequence.

Comparative Genome Size Analysis

The complete genome sequences of the 44 Mycobacterial species used in our analyses were available in the public domain. The *Mycobacterium sp. MOTT36Y* (MMM, 5613626bp) represents the OP group of mycobacteria closest to *MIP* (5589007 bp), in terms of genome size. Amongst the OP group of mycobacteria, those closest to *Mycobacterium intracellulare* (5402402<->5501090 bp) are *Mycobacterium sp. MOTT36Y* (MMM, 5613626 bp), *Mycobacterium avium 104* (MYCA1, 5475491 bp) and *Mycobacterium abscessus ATCC 19977* (MYCAB, 5090491 bp). It is an interesting observation that based on the genome size

the MYCA1 genome - an OP, fits between *Mycobacterium intracellulare MOTT-64* (MIR, 5501090 bp) strain and *Mycobacterium intracellulare MOTT-02* (MIT, 5409696 bp) (Fig. 2)

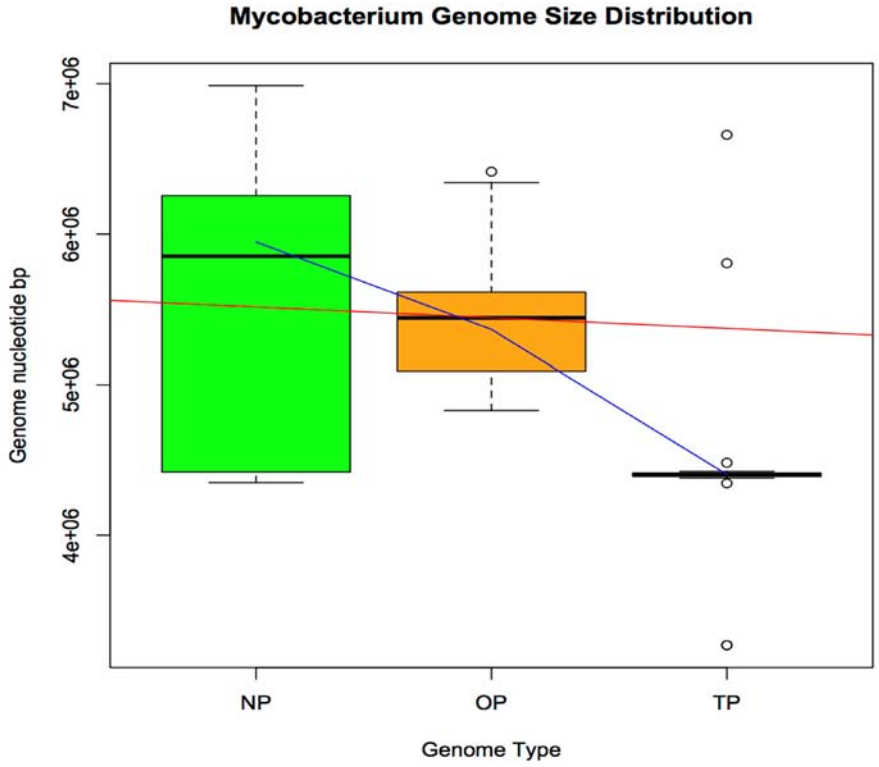


Fig. 2: The genome size of non-pathogenic (NP) mycobacteria is bigger than opportunistic (OP) and totally pathogenic Mycobacteria. The green box-plot represents the Non-Pathogenic (NP) genomes size, orange box represents Opportunistic Pathogen (OP), and True Pathogens (TP) is represented by pink box, which is very tight. The red line is the regression line and blue line is the lowess line. On an average the genome size of NP is bigger than OP and TP. The TP genomes are on an average smaller yet variable in size. The genome plasticity in TP possibly highlights their ability to evolve and survive in various environmental conditions.

For further comparative analyses 20% sequence identity cut-off with an e-value < 1e-4 was used to analyse BLASTp results. This cut off was used to determine the number of homologous protein coding genes shared between *MIP*, *MIA* and *MYCTU*. Our analysis of homologous protein coding genes shared between *MIP* (NP), *M. intracellulare ATCC13950* (*MIA*) (OP) and *Mycobacterium tuberculosis*

$H_{37}Rv$ (*MYCTU*) (TP) revealed 4995 (~95%) *MIP* proteins showing homology with *MIA*, whereas that between *MIP*, *MIA* and *MYCTU* was significantly less. 4153 (~79%) *MIP* proteins and 4093 (~79%) *MIA* proteins exhibited homology with *MYCTU* proteome, while 3301 (~82%) and 3295 (~82%) *MYCTU* proteins showed homology with *MIP* and *MIA* proteomes respectively (Fig. 3).

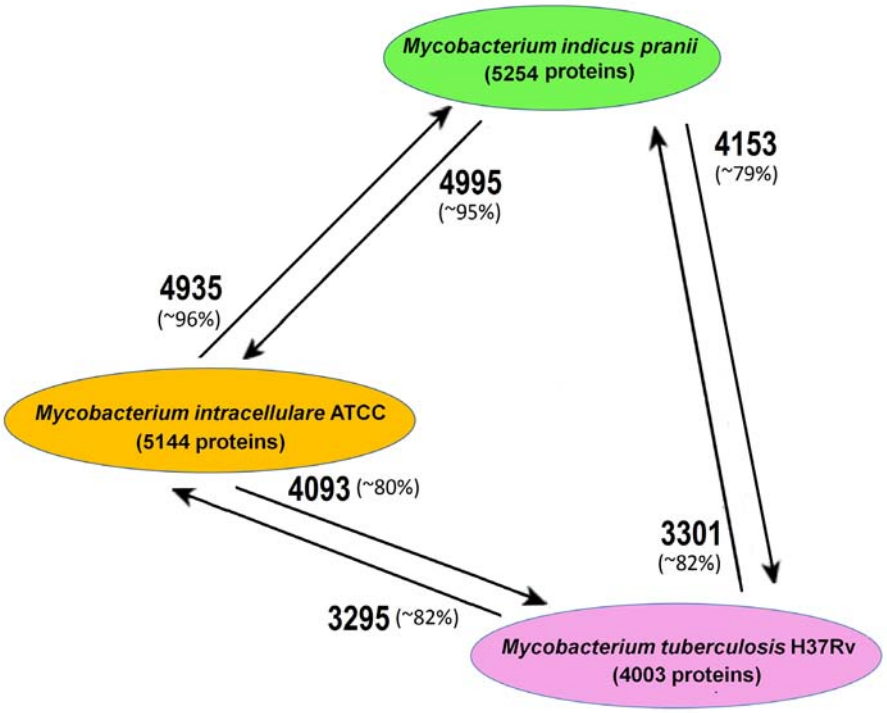
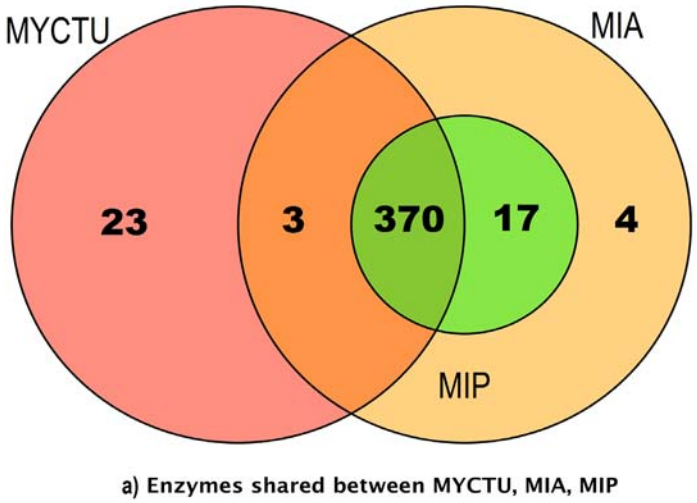


Fig. 3: Comparative genomics of select Mycobacterial genomes. *Mycobacterium indicus pranii* (shown in green colour - a non-pathogen), *Mycobacterium intracellulare ATCC 13950* (shown in orange colour - an opportunistic pathogen) and *Mycobacterium tuberculosis $H_{37}Rv$* (shown in pink colour - a pathogen) genomes were selected for comparative genomic analyses. We used BLAST, at the cut-off of 20% identity and e-value of 10e–04, to determine the number of homologous protein coding genes between them (shown as edge labels between the nodes).

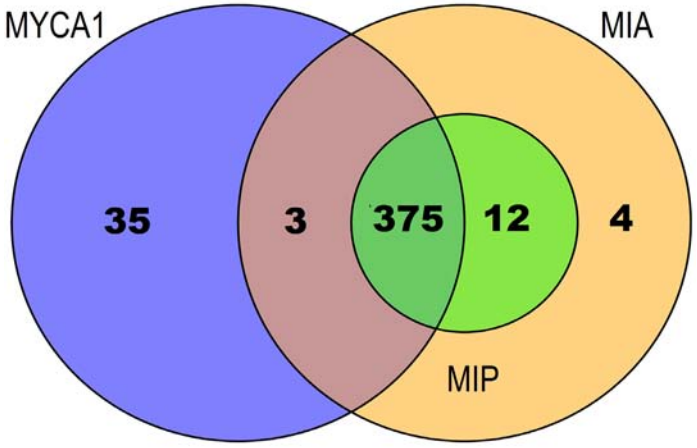
Comparative Metabolic Pathway Analyses:

There were 387 enzymes (E.C. numbers) common between *MIA* and *MIP* (part of MAC complex). When these two genomes were compared to *MYCTU* (part of *M.tb* complex) only 17 enzymes remain uniquely shared between the *MIA* and

MIP genomes (Fig. 4a). When compared to *MYCA1*, only 12 enzymes remain uniquely shared between *MIA* and *MIP* (Fig. 4b). Three enzymes - EC 1.8.7.1: sulfite reductase (ferredoxin), EC 2.7.1.6: galactokinase (phosphorylating) and EC 5.4.2.8: phospho mannose mutase were shared between both *MIA* and *MYCTU*, and *MIA* and *MYCA1*. As these enzymes were absent from *MIP* genome and shared between OP and TP, they may be linked to pathogenesis of the *Mycobacterium tuberculosis*.



a) Enzymes shared between MYCTU, MIA, MIP



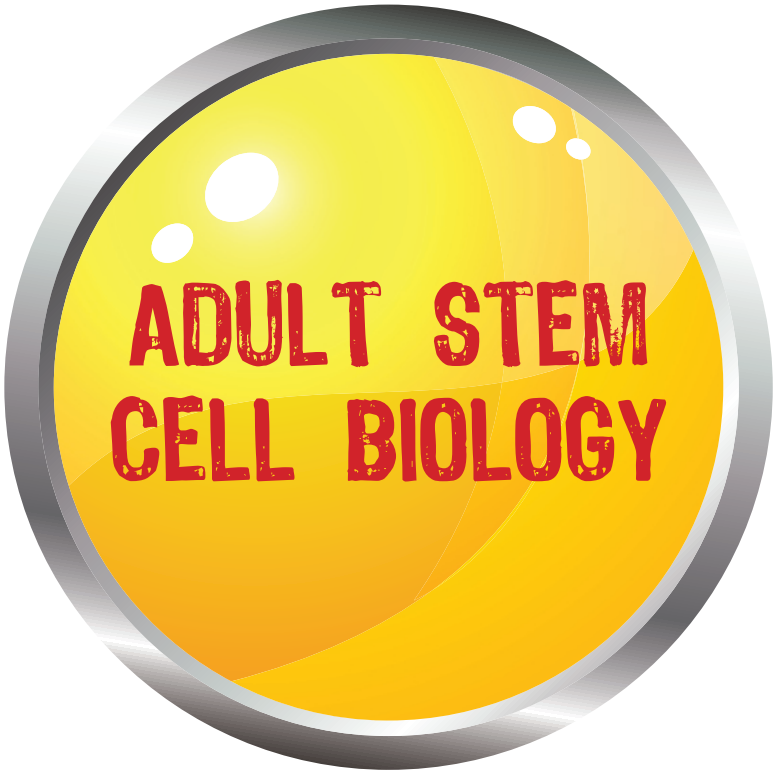
b) Enzymes shared between MYCA1, MIA, MIP

Fig. 4: The enzymatic similarity between MIP, MIA, MYCA1 and MYCTU highlights interesting enzymatic plasticity. The MIA (OP, orange) shares three enzymes (EC 1.8.7.1: sulfite reductase (ferredoxin), EC 2.7.1.6: galactokinase (phosphorylating) and EC 5.4.2.8: phosphomannose mutase) with MYCA1 (OP, blue) and MYCTU (TP, red), which are absent in MIP (NP, green). The MIA and MIP share 17 enzymes between them (part 1), which are absent in MYCTU. While they share 12 enzymes between them (part 2), which are absent in MYCA1.

Although the genome size of OPs (*MIA* and *MYCA1*) and TP (*MYCTU*) is reduced with respect to the MIP (a NP), our analysis indicates that the OP and

TP genomes have acquired few enzyme coding genes. It is tempting to suggest the likely association of these acquired enzymes with the virulence of these OPs and TPs. One of the three shared enzymes - EC 1.8.7.1 encodes a ferredoxin-dependent sulfite reductase (coded by *nirA* gene) is active during the dormant phase, and has been reported as a potential drug target for *Mycobacterium tuberculosis*.

Our systems biology analyses of pathogenic, non pathogenic Mycobacterial species provide strong evidence to suggest that despite having identical ribosomal RNA genes, except for notable differences in the 23S rRNA gene, with *M. intracellulare*, *MIP* (a NP with strong immunomodulatory properties) is a predecessor of *M. avium* complex and is placed at an evolutionarily transitory position with respect to fast grower and slow grower or a saprophyte and a seasoned pathogen. During the process of evolution, MIP evolved into MIA (an OP) by deleting few genes and acquired few enzyme coding genes (which may provide a common/evolutionary link between MYCA1 (OP) and MIA (OP)).


ADULT STEM
CELL BIOLOGY

ADULT STEM CELL BIOLOGY

1. A Novel Arithmetic Approach for Fool-proof Production of Growth Arrest in 3T3 Cells suitable for Human Epidermal Culture

Scientific Staff : Dr. L.K .Yerneni, Bijendra Kumar
Mr. Rishi Man Chugh (SRF, ICMR Project)

Duration : 2010-2014

Aims, Objectives & Background:

The present investigation is on the use of SWISS 3T3 cells to develop a culture system for growing Cultured Epithelial Autografts, by adopting a strategy similar to our earlier innovative process of growth arrest in NIH 3T3 feeder and to characterize the epidermal sheets produced thereof. The objectives are to find out correct exposure conditions for a fool-proof Mitomycin C induction of 3T3 feeder cell attenuation through arithmetic derivation of effective concentration based on cell number, to verify cell proliferative influence of such fool-proof growth arrested 3T3 cells on human epidermal keratinocytes and to characterize the human epidermal keratinocytes and epidermal sheets cultivated using such fool-proof growth arrested 3T3 cells.

Work done during the year:

A strategy of growth arrest in feeder cells as a cost effective substitute to gamma irradiation was adopted by way of using permutations of concentrations and doses of mitomycin C to provide a functional cellular substrate towards epidermal keratinocyte stem cell growth. It was shown that the effective permutations were initially identified through titration of a range of concentrations of mitomycin C with respect to a range of exposure feeder cell numbers. Subsequently a range

of volumes were conceived from permutations of concentrations and doses to generate varied feeder batches. This was followed by identification of an optimal feeder processing to produce maximal epidermal keratinocyte stem cell proliferation to speed-up cost-effective production of transplantable epithelia. An optimal batch of feeder cells exposed to an intermediate dose but a low concentration of mitomycin C and identified to produce maximal proliferation of epidermal keratinocyte stem cells and stratified epidermis was comparable if not superior to γ-*Irr* feeders.

Methods:

BrdU labelling:

Mitotic Index was estimated in slide flasks (Nunc) in which cultures were initiated with 70 viable 1st passage epidermal keratinocytes per flask containing the best performing feeders of B-r along with sub-optimal B-p and γ-Irr at a density of 15,000 per cm^2 and incubated with change of culture medium every alternate day for 10 days. Feeder cells were selectively removed with 0.02 % EDTA and the wells were incubated in non radio-active Bromodeoxy Uridine (BrdU) for 1 hour, fixed in Cornoy's fixative, treated with 4M HCl for antigen retrieval, neutralized with 0.1M sodium Borate, incubated in primary mouse monoclonal anti-BrdU antibody (Cat No.sc-32323, Santacruz) followed by visualization of fluorescence labeled nuclei after incubating in FITC labeled anti-mouse secondary goat polyclonal antibodies (Cat No. sc-2010, Santacruz Biotechnology Inc.). Every colony from triplicated slide flasks per feeder group was differentially counted for labeled and un-labeled nuclei. Briefly, each colony was photographed in both phase contrast and fluorescence modes on Nikon Diphot 300 microscope at 20X objective using Evolution QEi monochrome camera (Media Cybernetics); multiple images were taken if the colony was larger than the field and overlapping margins were demarcated by matching the images before counting of nuclei was performed using manual tag tool of Image Pro-Express express software version 6.0.

Statistics:

Separate dot plots were constructed to represent total number of cells and percent of BrdU positive cells per each colony in ascending order in every feeder group. Variance in distribution of BrdU positive cells among colonies of the three feeder groups was analyzed with Kruskal-Wallis H test and comparisons between two feeder groups were undertaken by Mann-Whitney non parametric U-test, while the overall BrdU positivity between two groups of feeders was tested by Chi square.

Significant Results:

The overall distribution of either cellularity or BrdU labeled keratinocytes across colonies was not significantly varied among the tested feeder groups (Fig. 1). Nevertheless, the percent of BrdU positive keratinocytes in B-r feeders at 30.1% (3243/10790) from 27 colonies was significantly ($P<0.01$) higher than B-p which revealed 20.5% (2000/9754) positivity from 20 colonies (Figure 2). This elevation appears to be contributed by the additional colony formation in B-r resulting in significant ($P<0.03$) distribution of labeled cells per colony in this group (Figures 2 and 3). On the contrary, the percent of labeled cell number in γ-Irr was found to be 28.9% (3270/11321) from 21 colonies and was not significantly different from that in B-r, but at the same time, γ-Irr was found to be significantly superior to B-p. The results indicated that it was the stimulation of colony initiation rather than increase in mitosis per colony that turned out to be the advantageous consequences of fine-tuning of mitomycin C treatment by including both concentration and dose per cell. It is apparent that feeders of B-r were superior to B-p in inducing a higher BrdU label, in spite of absence of significant variation in average cellularity per colony, indicating that an overall high cell turn over would eventually set in. The importance of dose titration of feeders with mitomycin C is further highlighted through improvement of the otherwise sub-optimal performance to match with that of γ-Irr feeders in achieving faster growth of keratinocytes.

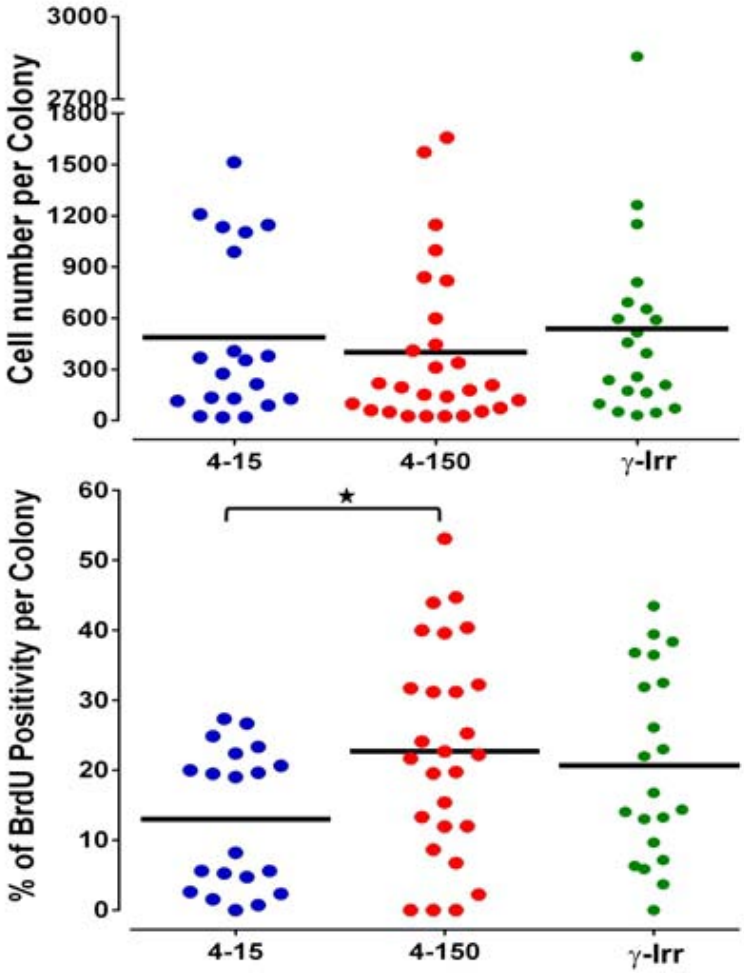


Fig. 1: Dot plots depicting distribution of cellularity (Top) BrdU positive cells (Bottom) across all keratinocyte colonies produced by 1st passage keratinocytes per feeder group. Feeder cells included B-r, B-p and the gamma irradiated feeders (γ-Irr). Significance ($P<0.03$) is indicated by asterisk.

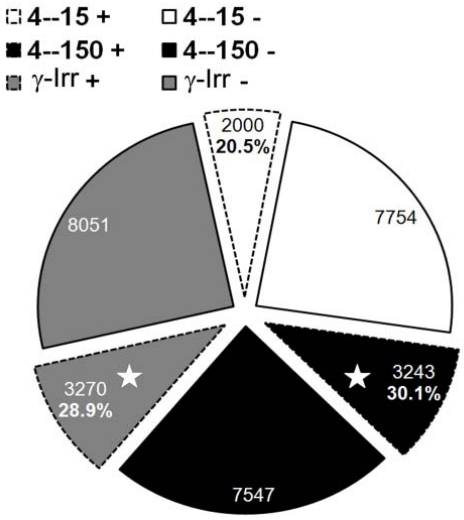


Fig. 2 (Top): Pie diagram showing overall BrdU positive & negative keratinocytes with corresponding percentages in cultures set up over feeder cells of B-r, B-p and γ-Irr. Significant ($p<0.01$) comparisons with B-p are indicated by white asterisk.

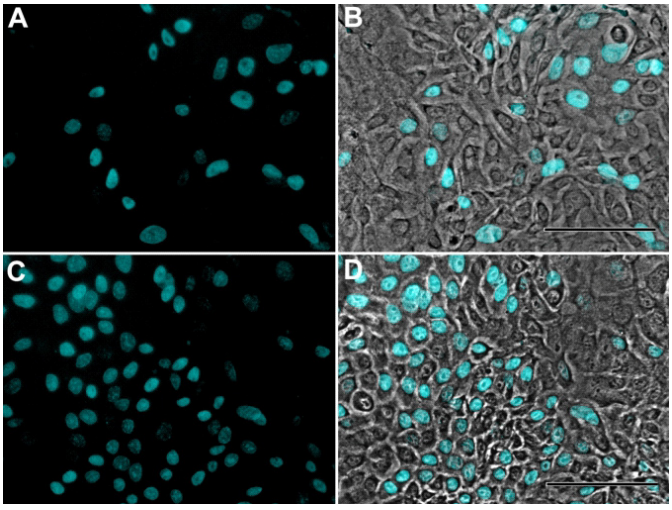


Fig. 3 (Bottom): Brdu labelling in human epidermal keratinocytes grown in presence of mitomycin C feeders of B-p (A & B) and B-r groups (C & D). The BrdU positive nuclei are blue-green (A & C) & merged with corresponding phase contrast images (B & D). Magnification bar = 10 μm.

The results indicated that it was the stimulation of colony initiation rather than increase in mitosis per colony that turned out to be the advantageous consequences of fine-tuning of mitomycin C treatment by including both concentration and dose per cell. It is apparent that feeders of B-r were superior

to B-p in inducing a higher BrdU label, in spite of absence of significant variation in average cellularity per colony, indicating that an overall high cell turn over would eventually set in. The importance of dose titration of feeders with mitomycin C is further highlighted through improvement of the otherwise sub-optimal performance to match with that of γ-Irr feeders in achieving faster growth of keratinocytes.

Future Plan:

It is proposed that in a clinical setting, every working feeder bank generated should once be evaluated for its optimal processing and the same could be adopted to produce CEA for application in burns. Further, work towards standardization of large-scale production of CEA from the skin biopsy needs to be initiated.

Patent filing:

A detailed description of new invention entitled, "A method for processing of feeder cells suitable for Adult Stem Cell Proliferation" was submitted to IPR division of (ICMR) for evaluation.

2. Technology to grow Non-xenogeneic CEA using Human Dermal Fibroblasts as Feeders

Scientific Staff : Dr. L.K .Yerneni, Bijendra Kumar
Ms. Hemlata Chouhan (JRF, CSIR Direct Fellowship)

Duration : 2013-16

Aims, Objectives & Background:

This project is initiated on similar lines of the completed research project aimed at establishing detailed manufacturing protocol for Cultured epithelial Autograft (CEA) using Swiss 3T3, but keeping in mind the xenogeneic nature of these culture

processes, an investigation is proposed to identify a cost-effective non-xenogeneic product suitable for resurfacing burn wounds. It is therefore, proposed to verify growth stimulatory influence of human dermal fibroblasts as feeders after applying our innovative approach to produce fool-proof and efficient growth arrest, and to characterize the epithelial autograft produced in this manner. The objectives are to find out accurate exposure conditions for a least toxic but effective Mitomycin C induction of human dermal fibroblast cell attenuation through a novel arithmetic derivation of effective concentrations from exposure cell density gradation experiments, to verify the optimal influence of such variedly growth arrested human dermal fibroblasts on proliferation & the basic characteristics of human epidermal keratinocytes, to characterize the human epidermal sheets cultured using such variedly growth arrested human dermal fibroblast cells to identify the best outcome, to grow epidermal sheets using human serum (commercially sourced) to simulate their production in clinical setting attaining total non-xenogeneic conditions, to demonstrate production of human epidermal keratinocyte cultures to be setup from human skin Biopsy employing the identified optimal feeders, and to test stability and Shelf life of final product & other QC parameters and to estimate mitomycin C residues in the final product.

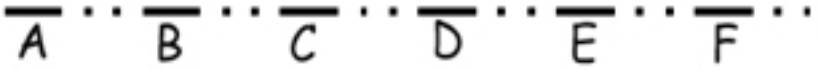
Work done during the year:

Methods:

Cell Density Gradient experiment:

1st passage primary neonatal foreskin dermal fibroblasts were obtained from (Genlantis, USA) which were expanded to establish a 2-tiered frozen bank of identical cells. Several rounds of preliminary experiments have led to determine 9th passage fibroblast plating for density gradient experiments in a way to achieve three different exposure cell numbers (ECN), viz., 9333, 36000, 51,556 cells/cm^2. The cultures were exposed to a 2 hour-pulse of mitomycin C (Sigma), at four concentrations of mitomycin C per ml at A, B, C & D which were X, X+5, 3X,

4X μg/ml, respectively, with appropriate solvent vehicle controls. The value of 'X' included as the minimal concentration of Mitomycin C effective to irreversibly arrest fibroblast cell proliferation, was deduced from preliminary experiments employing various concentrations of mitomycin C distributed on a longitudinal axis with lower concentration of A (equals to X-5) towards the left end and highest F (equals to 10X) towards the right.



At the end of exposure cells were detached replated into 24 well plates at a seeding density of 5000/cm^2. The cells were detached again and subjected to viable cell count by trypan blue exclusion at post reseeding days of 3, 6, 9 and 12.

Statistics:

The data were subjected to statistical analysis (Yerneni and Jayaraman, 2003). In brief, independent growth curve-plots were constructed for every concentration of mitomycin C with linear trend lines and r^2 values representing each exposure cell number (ECN). The significance of linearity between dose of mitomycin C, which was calculated by arithmetic conversion as ρg mitomycin C / cell and viable cell number for each of the cell counting days (3, 6, 9 and 12) was tested by correlation coefficient. The doses (Δ) were derived by an in house formula $\Delta = C\upsilon/\Sigma$ by substituting concentration (C), volume of treating solution (υ), and ECN (Σ).

Significant Results:

The results have clearly demonstrated that the effectiveness of any of the four tested concentration on cell survival (extinctions) was varied with ECN (Fig. 1).

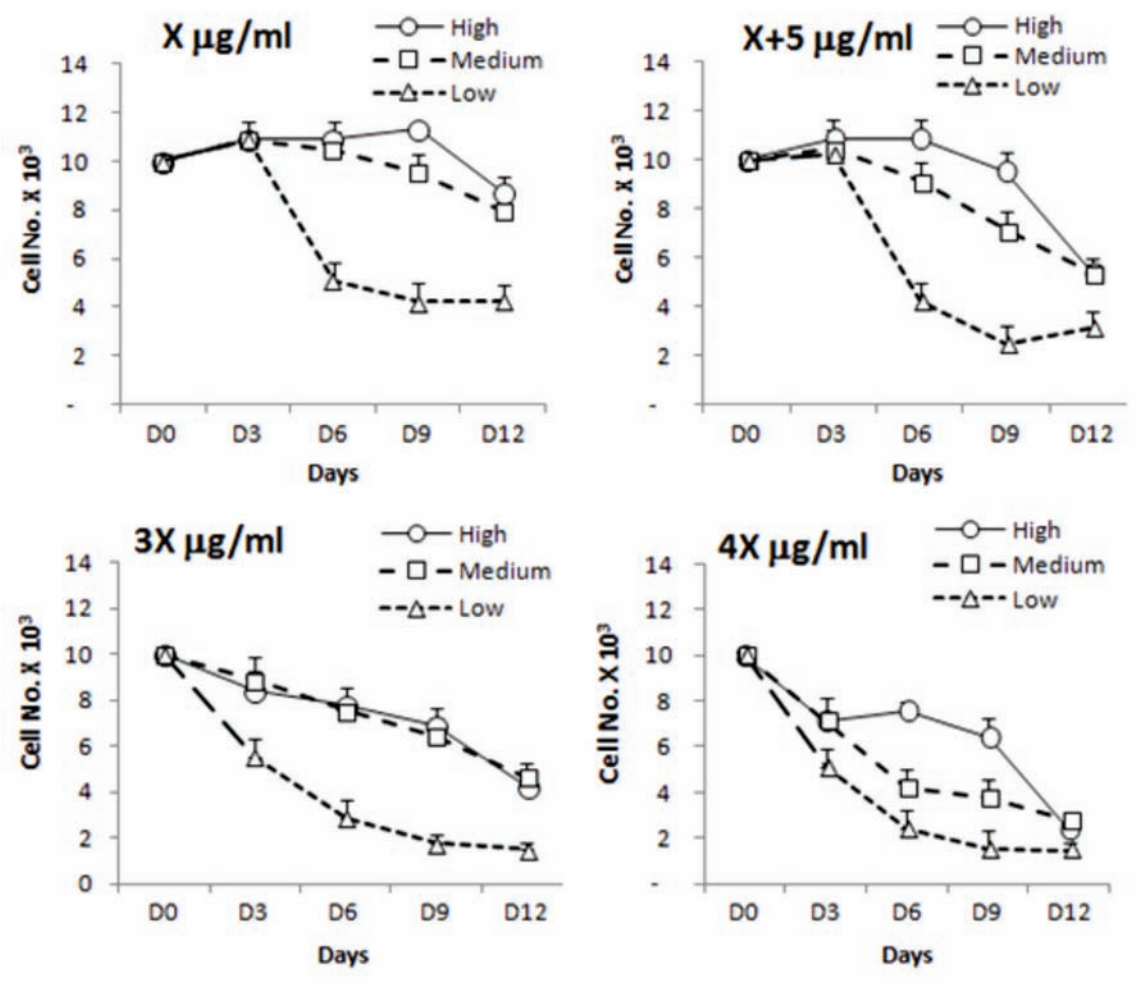


Fig. 1: Influence of various concentrations of mitomycin C on cell extinction/proliferation of Human dermal fibroblasts at 3 different exposure cell densities. The cells at Low (23.33 X 10^4 per cm^2), Medium (90 x 10^4 per cm^2) and High (128.89 x 10^4 per cm^2) cell densities were exposed to the given mitomycin C at concentrations and periodical viable cell counts were performed on post exposure days of 3,6,9 and 12. Cell numbers are presented as the mean with standard deviation from values of triplicate cultures.

But, the differential was maximal with an intermediate concentration of X+5. In order to see, if the differential is dependent on the calculated doses per cell, the later were arranged in ascending order correlated with significance of regression. It was found that the effectiveness is dose dependant rather than just concentration dependent, with lower doses being insignificant while the higher ones brought significant extinction of the treated cells (Table 1).

Table 1: Outcome of exposure of Human Dermal fibroblasts to a 2-hour pulse of various mitomycin C doses showing their relationship with the respective concentration, exposure cell density and correlation coefficient of cell number with time.

Regression analysis with concentrations in ascending order				Doses arranged in Ascending order	
Concen-tration C ug/ml	Exposure Cell No. ε (ECN)	Dose/ cell Δ pg/cell	Regression (R^2)	Dose/ cell Δ pg/cell	Regression (R^2)
X	High	78	0.105	78	0.105
	Medium	111	0.577	111	0.577
	Low	429	0.768	116	0.533
X+5	High	116	0.533	167	0.877
	Medium	167	0.877	233	0.939
	Low	643	0.799	310	0.83
3X	High	233	0.939	333	0.991
	Medium	333	0.991	429	0.768
	Low	1286	0.86	444	0.907
4X	High	310	0.83	643	0.799
	Medium	444	0.907	1286	0.86
	Low	1714	0.817	1714	0.817
$P<0.05$ if R^2 is > 0.658; Dose $(\Delta) = C\upsilon/\Sigma$; Low $\Sigma = 23.33 \times 10^4$ per cm^2, Medium $\Sigma = 90 \times 10^4$ per cm^2 High $\Sigma = 128.89 \times 10^4$ per cm^2					

Results are commensurate the proposed hypothesis that the feeder cell extinctions could be determined by dose modulation while maintaining lower possible concentrations of mitomycin C.

It will now be tested to find if various permutations of doses with a low concentration of mitomycin C would lead to regulation of feeder extinction rates through which an optimal net ratio of viable feeder cells versus epidermal keratinocytes could be achieved to optimize the latter's growth.


ENVIRONMENTAL
BIOLOGY

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

Scientific Staff : Dr. Arun Kumar Jain, Dr. Nida Akhtar, Mr. Shashi N. Kumar

In collaboration with : Dr Shashi Prateek, Consultant Gynaecologist
Dr K.C. Aggarwal, Consultant Pediatrician &
Dr Harish Chellani, Consultant Pediatrician
Safdarjang Hospital, New Delhi

Duration : 2012-15

Aims, Objectives & Background:

World Health Organization (WHO) reconfirms that most Indian cities are becoming death traps because of very high air pollution levels. India appears among the group of countries with highest particulate matter (PM) levels. Delhi is among the most polluted cities in the world today. Delhi has a population of approximately 18 million with a geographic area of 1,483 sq km and lies in the semi-arid zone over the sub-tropical belt with an altitude of 216 m above mean sea level. The gasoline/diesel exhaust emissions constitute the most important source of PAH pollution in urban India.

Rapid urbanization and mushrooming of industrialization has triggered the growth of transportation by all means including two wheeler sectors. Delhi alone accounts 1/8th of Total Vehicle Population in India. Consequently, these cities suffer from serious air quality problems including rise in PAHs. These

PAHs among the urban air toxics are of global concern due to their multiple effects on human population. PAHs are one of the potent and most widespread organic atmospheric pollutants formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense. Due to highest density of automobiles along with high density of population in Delhi, the risk associated with the human exposure to atmospheric PAHs is also the highest. PAHs are known to be mutagenic and include some of strongest known carcinogens (IARC, 1984). Attention is more importantly on PAHs bound to PM 2.5 and ultrafine fraction of the airborne particulates that are reportedly known for their higher health risk. Links between PAH exposure and elevated levels of DNA adducts, mutations, and reproductive defects have strengthened the notorious impact of PAHs. Women and children in developing countries are often exposed to high levels of air pollution including PAHs, which may negatively impact their health, due to household combustion of biomass fuel for cooking and heating. The present study proposed to investigate the health risks of polycyclic aromatic hydrocarbons (PAHs) and their association with low birth weight babies in a tropical megacity Delhi (India). To this end, 16 US EPA priority list PAHs were measured in the placental tissue, maternal and cord blood and urine samples collected from pregnant women admitted in Safdarjung Hospital, New Delhi.

Work done during the period:

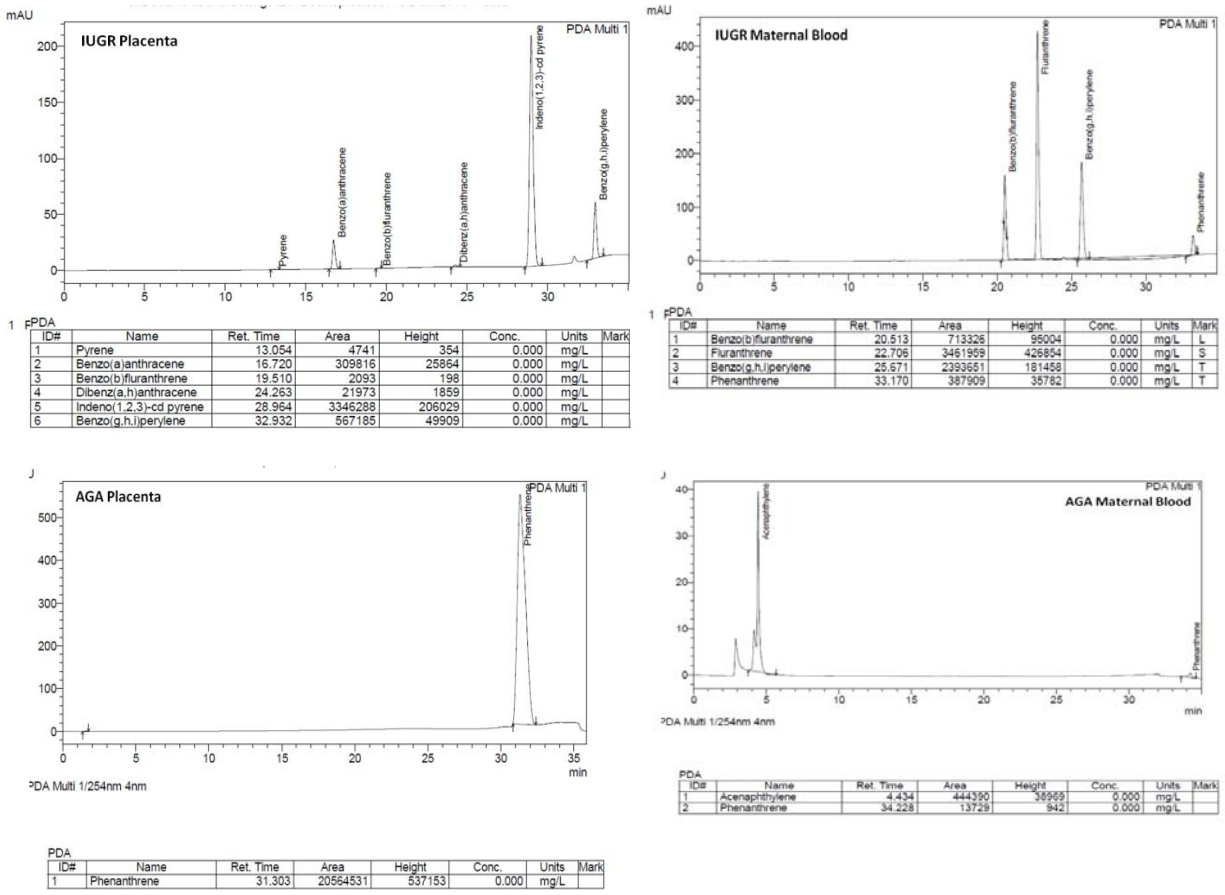
Women diagnosed as IUGR by ultrasonographic evidence during pre-natal check-up and/or giving birth to IUGR baby at the Department of Obstetrics and Gynaecology of Safdarjang Hospital, New Delhi, have been enrolled as cases were screened for presence of inclusion criteria and absence of exclusion criteria. While age and socio-economic status matched women with diagnosis of normal AGA baby during pre-natal check-up and or at the time of delivery have been enrolled as controls After the necessary ethical clearance and written informed consent from the participants samples of placenta, cord blood, maternal blood & urine sample were collected from the Department of Obstetrics and Gynaecology of Safdarjung Hospital, New Delhi. So far a total of 45 IUGR and 60 AGA (control) cases have been recruited for this study and samples of placenta, cord blood, maternal blood and urine are being collected and processed for PAHs exposure analysis. Details of residential history (Location and duration of residence), respiratory symptoms (Cough, Defaulting Breathing, Wheeze, Probable Asthma, etc.), home characteristics including heating and cooking sources and ventilation, and dietary habits of PAH-containing foods (i.e., fried, boiled, barbecued meat etc.) are recorded in the proforma questionnaire at the time of collection of sample along with informed consent. The questionnaire also addressed the psychosocial environment and typical daily activities, including usual routes and methods of travel outside the current area of residence during the past year.

Most common sub-clinical symptom was cough which was reported by more than 80% of cases. Similarly, weakness and fatigue were the other major problems faced by IUGR subjects. Although not statistically significant, the data showed lower average maternal weight and BMI in IUGR deliveries as compared to AGA deliveries. The mean weight of placenta collected from IUGR subjects was 470±112 g in comparison to 536±60 g in AGA subjects. The weight of baby ranged from 2.2-2.6 kg in IUGR delivered subjects and 3.2-3.8 kg in AGA subjects.

Selection of Polynuclear aromatic hydrocarbons for analysis by HPLC:

According to USEPA priority list the following PAHs are considered as a group in this profile: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo-[a,h]anthracene, and benzo[ghi]perylene, respectively. Initially attempts were made to optimize HPLC conditions for achieving best chromatographic separation of mixed PAHs. For this purpose several gradient elution methods using Acetonitrile/Water (in different proportions) and different flow rates of mobile phase from 0.8 to 1.5 ml/minute were evaluated with individual standards as well as a mixture of standards. The best separation of mixed 16 PAHs was achieved by using gradient elution conditions

After optimization of the analytical conditions the extracts obtained from placental tissue and blood as per the standardized protocol were analyzed for presence of the PAHs residues by HPLC using PDA Detector. The pollutants present in the samples were identified by comparing the retention time of the peaks observed in the sample chromatogram with the retention times recorded for the PAHs standard analysed with the same HPLC analytical conditions. Analysis of the HPLC chromatograms obtained from these extracts showed presence of one or more peaks. Some of these peaks could be recognised based on the RT of the standards. The PAHs recognized (Fig. 1) included Pyrene, Benzo(a)anthracene, Benzo(b)fluranthene, dibenz(a,h)anthracene, Indeno[1,2,3-cd] pyrene, Benzo(ghi) perylene, Flurenthene, Phenanthrene, Naphthlene, Benzo(a)pyrene, Acenaphthene, Chrysene, Acenaphthylene, etc. Figure 2 gives relative abundance of different PAHs in different samples.



ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Pyrene	13.054	4741	354	0.000	mg/L	
2	Benzo(a)anthracene	16.720	309816	25864	0.000	mg/L	
3	Benzo(b)fluranthrene	19.510	2093	198	0.000	mg/L	
4	Dibenz(a,h)anthracene	24.263	21973	1859	0.000	mg/L	
5	Indeno(1,2,3)-cd pyrene	28.964	3346288	206029	0.000	mg/L	
6	Benzo(g,h,i)perylene	32.932	567185	49909	0.000	mg/L	

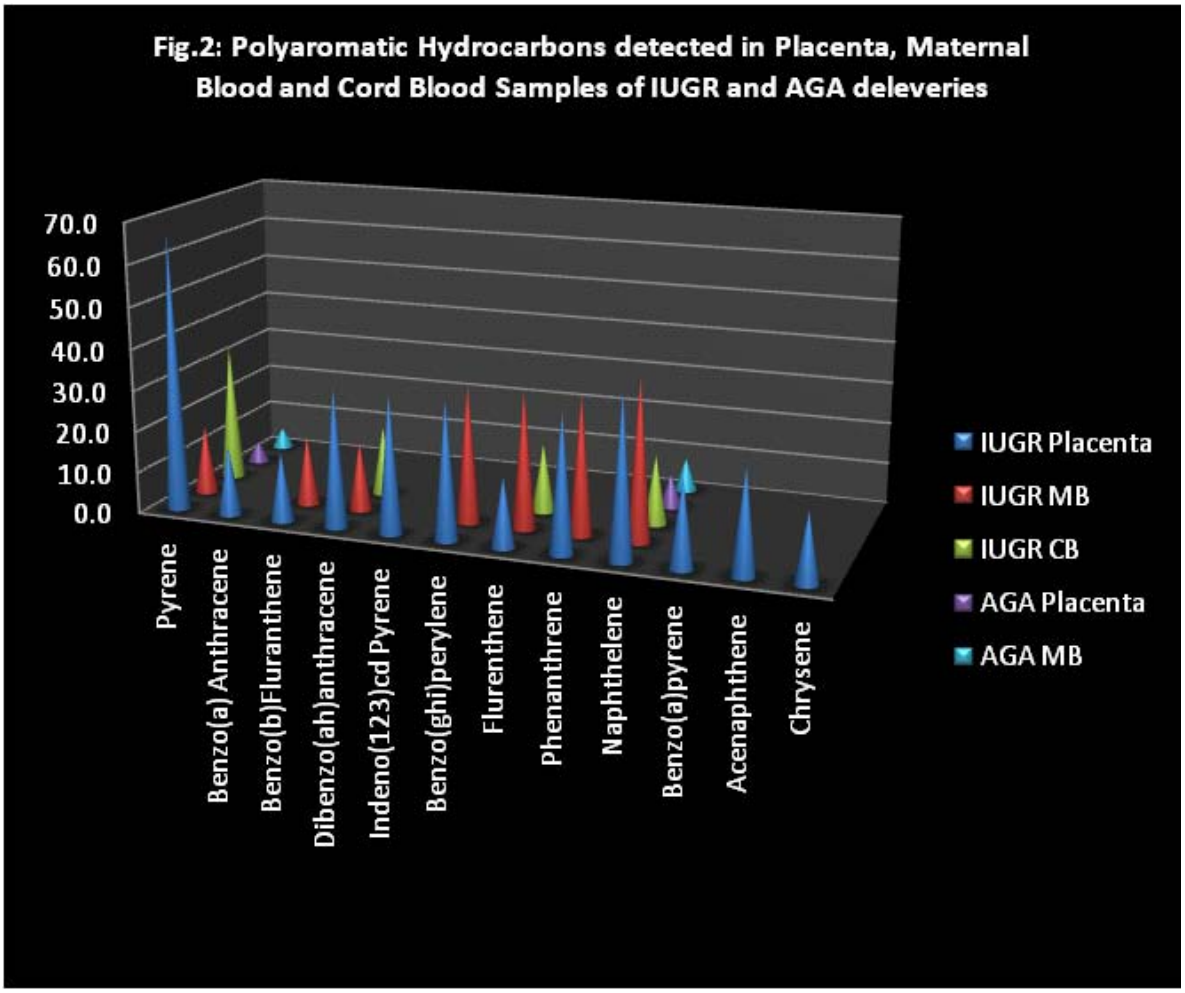
ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Benzo(b)fluranthrene	20.513	713326	95004	0.000	mg/L	L
2	Fluranthrene	22.706	3461959	426854	0.000	mg/L	S
3	Benzo(g,h,i)perylene	25.671	239355	181458	0.000	mg/L	T
4	Phenanthrene	33.170	387909	35782	0.000	mg/L	T

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Phenanthrene	31.303	20564531	537153	0.000	mg/L	

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Acenaphthylene	4.434	444390	38969	0.000	mg/L	
2	Phenanthrene	34.228	13729	942	0.000	mg/L	

Fig.1: Chromatograms of Placenta and Maternal Blood extracts from IUGR and AGA subjects

On the basis of the preliminary results, Naphthalene is highly detected in 39% IUGR and 8% AGA cases. Pregnant women may be exposed through indoor air pollution, moth balls and tobacco smoke. Second highest PAH, we have detected is pyrene in 32% of IUGR cases while 5% control also showed the presence of pyrene may be exposed through dietary sources (grilled and smoked food) and second hand cigarette smoke. More than 25% women were exposed to acenaphthene and phenanthrene and 21% through acenaphthylene and benzo (a) pyrene. There is no information available from studies on humans to tell



what effects can result from being exposed to individual PAHs at certain levels. The ongoing study will provide the information about human PAH exposure in Delhi and may further help in better understanding of the mechanism behind the adverse outcome of pregnancy vis-à-vis PAH exposure in Delhi. The study is being continued.


ACADEMIC
ACTIVITIES

WORKSHOPS/SEMINARS ORGANIZED AT NATIONAL INSTITUTE OF PATHOLOGY

1. Organized 13th Smt. Pushpa Sriramachari Foundation Day Oration delivered by Dr. Bhan entitled "Rotavirus Infection in India: Disease Burden, and Development of Affordable & Efficacious Vaccine"on 7th May 2014.

2. Lecture on "Proteomics and critical aspects of 2-D gel electrophoresis" delivered by Dr. Vipan Manhas, (Bio-Rad Laboratories) during the training course on 29th July 2013.

3. Organized DNB appraisal on 19th Sept. 2013.

4. Workshop on Electron Microscopy for Diagnostics and Biomedical Research from 23rd-27th September 2013.

5. Brain storming session to discuss tools and challenges in "Understanding of Biological Complexities through integrated Biology" by M/s Agilent Technologies on 26th September 2013.

6. Physical demonstration on newly installed Real Time PCR on 10th October 2013.

7. Organized workshop on Electron Microscopy for Diagnostics and Biomedical Research from September 23rd to 27th 2013.

8. Organized Scientific Advisory Committee meeting at NIP on 26th Nov. 2013.

AWARDS/PATENTS

AWARDS

1. Dr Poonam Salotra was appointed Member of RTAG (Regional Technical Advisory Group), WHO, 2013.

2. Dr Poonam Salotra was appointed member of the WHO Advisory Panel on Parasitic Diseases (Leishmaniasis) in August 2013.

PATENTS

1. Patent granted for "Development of DOT–BLOT assay for diagnosis of sequalae to *Chlamydia trachomatis* infection using cHSP60.

2. Development of primary cervical epithelial cell line from cervical lavage: New Patent has been filed as per ATCC deposition certification (3563/Del /2012).

3. Indian patent filed by ICMR (application no. 349/DEL/2014 dated 6 Feb, 2014) for "Loop mediated isothermal amplification (LAMP) assay for a reliable and rapid diagnosis of *Leishmania* infection".

EXTRAMURAL PROJECTS (2013-14)

NEW PROJECTS

1. **Investigations on Paromomycin resistance in Leishmania donovani using molecular and biochemical tools.**
   Dr. Poonam Salotra, ICMR, 2014-2017.

2. **Identification and characterization of artemisinin resistance associated genes in Leishmania**
   Dr. Poonam Salotra, ICMR, 2014-2017.

ONGOING PROJECTS

1. **Study on micro-RNA signatures associated with Breast Cancer Stem like Cells (CSCs) and their role in drug response.**
   Dr. Sunita Saxena, Dr. BSA Raju- ICMR (2013-15)

2. **Second phase of biomedical informatics centers of ICMR.**
   Dr. Sujala Kapur, Dr. AK Jain- ICMR (2013-17)

3. **Understanding the role of androgen receptor signalling in breast cancer.**
   Dr. Sunita Saxena, Dr. Jatin Mehta- ICMR (2013-15)

4. **Molecular mechanism of drug resistance in Acute Myeloid Leukemia (AML): Role of ATP-Binding Cassette (ABC) transporters.**
   Dr. Sujala Kapur, Dr. Pradeep Chauhan- ICMR (2013-15)

5. **Epigenetic studies in esophageal cancer in high risk region of north-east India.**
   Dr. Sunita Saxena, Dr. Sujala Kapur- Twining Program of DBT (2011-14)

6. **Development of new live attenuated vaccine candidates for kala-azar**
Dr. Poonam Salotra, DBT 2011-2015.

7. **Proteomic analysis of Leishmania donovani membrane components involved in host-parasite interaction.**
Post doctoral Fellowship project by ICMR, 2014-2016.

8. **Detection of *Chlamydia trachomatis* in synovial samples from patients with undifferentiated spondyloarthropathy/ reactive arthritis.**
Dr. Sangita Rastogi- ICMR-SRF 2010-14

9. **A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture**
Dr. LK Yerneni,-ICMR (2010-2014)

COMPLETED PROJECTS

1. **Genome wide analysis of genetic alterations in patients with esophageal cancer from north east India using single nucleotide polymorphism array.**
Dr. Sujala Kapur, Dr. Sunita Saxena- ICMR (2011-14)

2. **Immunogenetic profile of nasopharyngeal cancer.**
Dr. Sujala Kapur, Dr. Sunita Saxena- DBT (2010-13)

3. **Protective immunogenicity of Centrin KO live attenuated Leishmania parasite in the animal models and in the human cells.**
Dr. Poonam Salotra, DBT, 2011-2014.

4. **New tools for monitoring drug resistance and treatment response in Visceral Leishmaniasis in the Indian subcontinent.**
Dr. Poonam Salotra, European Commission, 2009-2013.

5. **Study on the effect of siRNA mediated androgen receptor gene silencing on androgen signaling pathway in breast carcinoma.**
Dr. Anurupa Chakraborty- Post-Doctoral Fellowship, ICMR (2010-12)

6. **Study on effects of anti-cancer drugs on breast cancer stem cells.**
Mrs. Shanti Lata- ICMR-SRF (2011-13)

7. **Virtual centre of excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis*.**
Dr. Nasreen Ehtesham DBT (2008-13)

8. **Understanding the functional role of PHLPP1 in IFNγ-mediated innate immune responses of macrophages.**
Dr. Nasreen Ehtesham DBT (2011-14)

PUBLICATIONS

1. Sharma Anita, Das Bhudev Chander, Sehgal Ashok, Mehrotra Ravi, Kar Premashish, Sardana Sarita, Hunan Rup, Mahanta Jagdish, Purkayastha Joydeep, **Saxena Sunita,** Kapur Sujala, Chatterjee Indranil, Sharma Joginder Kumar. *GSTM1* and *GSTT1* polymorphism and susceptibility to esophageal cancer in high- and low-risk regions of India ***Tumor Biology***: Volume 34, Issue 5, Page 3249-3257, 2013.

2. Kothari Charu, Gaind Rajni, Singh Laishram C, Sinha Anju, Kumari Vidya, Arya Sugandha, Chellani Harish, Saxena Sunita and Deb Monorama. Community acquisition of beta-lactamase producing Enterobacteriaceae in neonatal gut. ***BMC Microbiology*****,** 13: 136, 2013.

3. Pandrangi SL, Chikati R, Chauhan PS, Kumar CS, Banarji A, Saxena S."Effects of ellipticine on ALDH1A1 expressing Breast Cancer Stem Cells - an In vitro and In silico Study", ***Tumour Biol*****,** 2014 Jan;35(1):723-37. doi: 10.1007/s13277-013-1099-y. Epub, Aug 28, 2013.

4. Lakhanpal M, Yadav DS, Regina Devi T, Singh LC, Singh KJ, Latha PS, Chauhan P, Verma Y, Zomavia E, Sharma J, Kataki AC, Saxena S, Kapur S. Association of Interleukin-1B(-511) polymorphism with tobacco associated cancers in Northeast India: A study in Oral and Gastric Cancers. ***Cancer Genetics*** 2014 Jan-Feb; 207(1-2):1 11.doi:10.1016. cancergen..01.002. Epub2014 Jan16, 2014.

5. Pandrangi Latha Santhi, Appalaraju Sarangadhara Bagadhi, Sinha Kumar Navin, Kumar Manoj, Dada Rima, Lakhanpal Meena, Soni Abha, Malvia Shreshtha, Simon Sheeba, Chintamani Chintamani, Mohil Singh Ravindar, Bhatnagar Dinesh, Saxena Sunita. Establishment and characterization of two primary breast cancer cell lines from young Indian breast cancer patients: mutation analysis. ***Cancer Cell International***., 14:14.DOI: 10.1186/1475-2867-14-14, 2014.

6. Krayter L, Bumb RA, Azmi K, Wuttke J, Malik MD, Schnur LF, **Salotra** P, Schönian G. Multilocus microsatellite typing reveals a genetic relationship but, also, genetic differences between Indian strains of *Leishmania tropica* causing cutaneous leishmaniasis and those causing visceral leishmaniasis. ***Parasit Vectors***, 25;7(1): 123, 2014.

7. Kulshrestha A, Sharma V, Singh R, **Salotra** P. Comparative transcript expression analysis of miltefosine-sensitive and miltefosine-resistant *Leishmania donovani*. ***Parasitol Res***. 113(3): 1171-1184, 2014.

8. Katara, GK, Raj, A, Kumar, R, Avishek, K, Kaushal, H, Ansari, NA, Bumb, RA, **Salotra P**. Analysis of localized immune responses reveals presence of Th17 and Treg cells in cutaneous leishmaniasis due to *Leishmania tropica*. ***BMC Immunol***. 14(1): 52, 2013.

9. Bhandari, V., Kumar, D., Verma, S., Srividya, G., Negi, NS., Singh, R. and **Salotra P**. Increased parasite surface antigen-2 expression in clinical isolates of *Leishmania donovani* augments antimony resistance. ***Biochem Biophys Res Commun***. 440(4): 646-651, 2013.

10. Ramesh, V., Avishek, K., Sharma, V. and **Salotra P.** Combination Therapy with Amphotericin-B and Miltefosine for Post-kala-azar Dermal Leishmaniasis: A Preliminary Report. ***Acta Derm Venereol***. 94(2): 242-243, 2014.

11. Prajapati, VK., Sharma, S., Rai, M., Ostyn, B., **Salotra, P.,** Vanaerschot, M., Dujardin, JC. and Sundar S. In vitro Susceptibility of *Leishmania donovani* to Miltefosine in Indian Visceral Leishmaniasis. ***Am J Trop Med Hyg***, (4):750-754, 2013.

12. Clinco-epidemiologic study of cutaneous leishmaniasis in Bikaner, Rajasthan, India. Aara N, Khandelwal K, Bumb RA, Mehta RD, Ghiya BC, Jakhar R, Dodd C, **Salotra** P, Satoskar AR. ***Am J Trop Med Hyg***, 89(1): 111-115, 2013.

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36. Chugh S, Sarkar R, Garg VK, **Singh A**, Keisham C. Multifocal fixed drug eruption with COX-2 inhibitor-celecoxib. **Indian J Dermatol**; 58(2): 142-44, **2013**.

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38. Mahajan L, Pandit H, Madan T, **Gautam P**, Yadav AK, Warke H, Sundaram CS, Sirdeshmukh R, Sarma PU, Kishore U, Surolia A. Human surfactant protein D alters oxidative stress and HMGA1 expression to induce p53 apoptotic pathway in eosinophil leukemic cell line. ***PLoS One***, 31; 8(12):e85046, 2013.

39. **Gautam P**, Nair SC, Ramamoorthy K, Swamy CV, Nagaraj R. Analysis of human blood plasma proteome from ten healthy volunteers from Indian population. ***PLoS One***, 20; 8(8): e72584, 2013.

40. Polisetty RV, **Gautam P**, Gupta MK, Sharma R, Uppin MS, Challa S, Ankathi P, Purohit AK, Renu D, Harsha HC, Pandey A, Sirdeshmukh R. Heterogeneous Nuclear Ribonucleoproteins and Their Interactors Are a Major Class of Deregulated Proteins in Anaplastic Astrocytoma: A Grade III Malignant Glioma. ***J Proteome Res***, 5; 12(7): 3128-38, 2013.

ACCEPTED

1. Ihsan R, Chauhan PS, Mishra AK, **Singh LC**, Sharma JD, Zomawia E, Verma Y, Kapur S, **Saxena S**. Investigation on Copy Number Polymorphism of GSTM1 and GSTT1 in Susceptibility to Lung Cancer in a High-Risk Population from North East India ***Indian J Med Res.***

2. Mishi Kaushal Wasson, Pradeep Singh Chauhan, L.C. Singh, Dheeraj Katara, Jagannath Dev Sharma, Eric Zomawia, Amal Kataki, Sujala Kapur, **Sunita Saxena**. Association of DNA Repair and Cell Cycle Gene Variations with Breast Cancer Risk in Northeast Indian Population: A Multiple Interaction Analysis***, Tumor Biology***.

3. Dhirendra Singh Yadav, Indranil Chattopadhyay, Anand Verma,Thoudam Regina Devi,L.C Singh, Jagannath Dev Sharma, Amal Ch Kataki, Sunita Saxena, Sujala Kapur. A Pilot study evaluating genetic alterations that drive tobacco and betel quid associated Oral Cancer in North -East India**,** ***Tumor Biology***.

4. Syed Asad Rahman, Yadvir Singh, Sakshi Kohli, Javeed Ahmad, **Nasreen Z. Ehtesham**, Anil K. Tyagi, and Seyed E. Hasnain. Comparative Analyses of non-pathogenic, opportunistic and totally pathogenic ***Mycobacteria*** reveals genomic and biochemical svariability and highlights the survival attributes of M*ycobacterium tuberculosis* ***mBio*** *(In Press) 2014*

5. Chordoid Meningioma- A diagnostic dilemma ***Journal of Cancer Research and Therapeutics***.

6. Kumar P, Bhakuni DS, **Rastogi S.** Clinical significance of circulatory chlamydial heat shock protein-60 antibodies in spondyloarthropathy patients. ***BMC Infectious Diseases***, 2013.

7. **Rastogi S,** Kumar P, Bhakuni DS (2014). Is the role of *Chlamydia trachomatis* underestimated in reactive arthritis patients in India. ***International Journal of Infectious Diseases***, 2014.

Chapter in Book

1. **Salotra, P**, Singh, R Seifert, Karin. Visceral leishmaniasis – Current treatments and needs. *In* Trypanosomatid Diseases: Molecular Routes to Drug Discovery, First edition. Edited by T. Jäger, O. Koch, and L. Flohé. Published by Wiley-VCH Verlag GmbH & Co. UK (2013).

SCIENTIFIC ACTIVITIES

DR. SUNITA SAXENA

1. Invited to attend Scientific Advisory Committee meeting of Institute of Cytology & Preventive Oncology held on 2nd April 2013 at ICPO, Noida.

2. Invited to attend Nasi Scopus Young Scientists Award 2012 meeting held on 4th April 2013 at Stanford Biodesign Centre, AIIMS, New Delhi.

3. Participated in the viva-voce examination for Ph.D. of Ms. Thoudam Regina Devi held on 11th April, 2013 at BITS, Plani.

4. Invited to attend Selection Committee meeting for award of ICMR Post-Doctoral Fellowship (PDF) 7th Batch held on 17th April 2013 at ICMR, New Delhi.

5. Invited to attend meeting regarding reviewing the research collaboration agreement received from FDA, USA in connection with Dr. Poonam Salotra's joint US patent on "Live attenuated *Leishmania* vaccines' held on 23rd April 2013 at ICMR, New Delhi.

6. Invited to attend IVth meeting of the Project Review Steering Group to review the progress of the project on "Development of PC based fully automatic Batch analyser for Clinical Chemistry" held on 30th April 2013 at BMRC-205, Vellore Institute of Technology, Vellore, Tamilnadu.

7. Invited to attend meeting of Expert Group held on 13th May 2013 at ICMR, New Delhi.

8. Invited to attend meeting on translational research projects of NJIL & OMD, Agra held on 15th May 2013 at ICMR, New Delhi.

9. Invited to attend meeting of Expert Advisory Committee on development of protocols for treatment and patient care in hospital and trauma centres/ blocks of the six new AIIMS held on 20th May 2013 at Nirman Bhavan, New Delhi.

10. Invited to attend meeting of NOTTO Centre, Safdarjang Hospital, New Delhi held on 24th May 2013.

11. Invited to attend international conference on "Empowering Women in Developing Countries through Information & Communication Technologies" held during 1st -3rd June 2013 at JUIT Waknaghat, District Solan (HP).

12. Participated in Annual US-India Biopharma & Healthcare Summit held at Massachusetts, USA held during 21st June – 23rd June 2013.

13. Invited to attend 1st meeting of Advisory Committee for Bhopal Memorial Hospital & Research Centre, Bhopal held on 29th – 30th June 2013.

14. Invited to attend Collaborative Research Agreement meeting held on 1st July 2013 at ICMR, New Delhi.

15. Invited to attend Governing Body meeting of the ICMR held on 4th July 2013 at Nirman Bhawan, New Delhi

16. Invited to attend fourth meeting of the Expert Advisory committee on development of Protocols on Subject Committee held on 9th July 2013 at Nirman Bhawan, New Delhi.

17. Chaired the Ethical Committee Meeting, Safdarjang Hospital, New Delhi held on 11th July 2013.

18. Invited to attend meeting of NOTTO Centre at Safdarjang Hospital, New Delhi held on 15th July 2013.

19. Invited to attend 5th meeting of the DSMB on Curcumin Clinical Trial in CaCz Cancer held on 29th July 2013 in Department of Biotechnology, New Delhi.

20. Invited to attend meeting of the Expert Advisory committee on development of protocols for treatment and patient care in hospitals and trauma Centres/ blocks of the six new AIIMS held on 30th July 2013.

21. Invited to attend and deliver talk on Integrated analysis of cancer genome : Approach to understand cancer biology and identification of biomarker's in 2nd Dr. V. Ramalingaswamy Oration held on 8th August, 2013

22. Invited to attend Expert Group Meeting on 'Early diagnostic test for Oral Cancer Screening' meeting held on 8th August 2013 at ICMR, New Delhi.

23. Invited to attend meeting regarding discuss requirement of space availability in proposed NBCC building held on 19th August 2013 at ICMR, New Delhi.

24. Invited to attend 2nd meeting of the Scientific Advisory Committee of the National Centre for Disease Informatics and Research, Bangalore held on 24th August, 2013.

25. Invited to examinership for practical examination of the National Board of Examination held on 5th & 6th September 2013 at Govt. Medical College, Chandigarh.

26. Invited to attend "Planning workshop in Cancer Screening Strategies" organized by the ICPO, Noida at India International Center, New Delhi on 13th September 2013.

27. Invited to attend ICMR Awards/Prizes distribution function held on 24th September 2013 at New Delhi.

28. Invited to attend "Understanding Biological Complexities though Integrated Biology" organized by Agilent Technologies on 26th September 2013 in New Delhi.

29. Chaired the Ethical Committee meeting of Safdarjang Hospital New Delhi held on 26th September 2013.

30. Invited to attend Collaborative Research Agreement meeting held on 27th September 2013 at ICMR, New Delhi.

31. Invited to attend Research Advisory committee meeting of Rajiv Gandhi Cancer Institute and Research Centre, New Delhi held on 3rd October 2013.

32. Invited to attend Award of ICMR Post Doctoral Fellowship meeting held on 7th October 2013 at ICMR, New Delhi.

33. Invited to attend and deliver talk on "Role of Tissue Microarray in Pathology in UP PATHCON" 2013, held in New Delhi.

34. Invited to attend National Consultation Workshop on "Ethical Aspect of Biomedical Research involving Human Participants – The Present Context" held from 28th – 29th October 2013 in New Delhi.

35. Appointed as appraiser of DNB candidates of the Rajiv Gandhi Cancer Institute & Research Centre, New Delhi.

36. Chaired Ethical Committee meeting of Safdarjang Hospital, New Delhi held on 11th November 2013.

37. Invited to attend Task force Study project entitled "Early Detection of Oral Cancer through C test Brush Test" held at ICMR, New Delhi on 11th November 2013.

38. Invited to attend Scientific Advisory Committee meeting held on 12th -13th November, 2013 at Regional Medical Research Centre, Dibrugargh, Assam.

39. Invited to participat and chair the session on "Lung Cancer Classification – Changing Paradigms" and deliver talk on "Application of Tissue Microarray in Pathology" in 1st Indian Congress 2013 (Onco Pathology) Pre-Conference Workshop held at Kempinski Ambience Hotel, New Delhi during 21st – 24th November 2013.

40. Invited to attend the meeting of the Task Force on 'Early Detection of Oral Cancer' held in New Delhi on 28th November 2013.

41. Appointed as appraiser for Northern Railway Central Hospital for appraisal of DNB trainees by the National Board of Examination, New Delhi.

42. Invited to attend Scientific Advisory committee meeting of National Institute for Research in Reproductive Health, Mumbai held on 18th – 19th December 2013.

43. Invited to attend viva-voce Examination of Ms. Mishi Kaushal held at Birla Institute of Technology & Science, Pilani on 20th December 2013.

44. Invited to participate as Chairperson in "World Conference on Infectious Diseases" held at Chennai Trade Convention Centre, Chennai, Tamilnadu during 18th -22nd December 2013.

45. Invited to attend and review the progress of Tissue Bank in Biomaterial Centre, NOTTO, Safdarjang Hospital held on 3rd January 2013 at Safdarjang Hospital, New Delhi.

46. Invited to participate as guest faculty in National CME on Molecular Pathology of Cancer held on 10th January 2014 at Dr. B. Borooah Cancer Institute, Guwahati, Assam.

47. Invited to attend joint Scientific Advisory committee (SAC) meeting for NIRT, Chennai and NJILOMD, Agra held on 10th and 11th January 2014 at National JALMA Institute for Leprosy & other Mycobacterial Disease, Agra.

48. Invited to attend the launching indigenous affordable devices for detection of diabetes held on 13th January 2014 in New Delhi.

49. Invited to chaired Institute Ethics Committee meeting held on 27th January 2014 at Safdarjang Hospital, New Delhi.

50. Invited to attend Doctoral Committee (DC) of Department of Medical Elementology & toxicology, Jamia Hamdard University, New Delhi held on 29th January 2014.

51. Dr. Sunita Saxena invited to attend and chair 2nd Session of "Medanta Hepatopathology CME" organized by Department of Digestive and Hepatobiliary Sciences, Medanta on 3rd February 2014 in Gurgaon.

52. Invited to attend World Cancer Day held on 4th February, 2014 at Institute of Cytology & Preventive Oncology, Noida.

53. Invited to attend Post Summit Strategy Session in New Delhi on 6th February 2014 organized by USA-India Chamber of Commerce, New Delhi.

54. Invited to attend Meeting of the ICMR condemnation Board held in ICMR on 10th February 2014.

55. Invited to attend meeting of the 'Expert Committee' for the Establishment of the molecular laboratory at RLTRI, Raipur held at ICMR, New Delhi on 11th February 2014.

56. Invited to attend Ph.D. viva-voce examination of Ms. Rakshan Ihsan held on 24th February 2014 at Birla Institute of Technology & Science, Pilani.

57. Attended the meeting of Translational Research of ICMR held on 6th March, 2014 at ICMR, New Delhi.

58. Invited to attended Curtain Raiser of the film "Scientifically Yours" on Indian Women Scientists held on 6th March 2014 at Vigyan Prasar, Department of Science and Technology jointly with NISCAIR, CSIR, New Delhi.

59. Attended IAPM (Delhi Chapter) meeting held on 9th March 2014 organized by Department of Pathology, Army Hospital (R&R), New Delhi.

60. Invited to attend "To Mark the Economic, Political and Social achievements of Women" on the occasion of International Women's Day (IWD) organized by Amity University, Noida on 10th March 2014.

61. Invited to attend meeting of Translational Research to discuss phase II technogies held in ICMR, New Delhi on 10th March 2014.

62. Invited to attend as Co-chairperson of Screening committee the screening of the pilot projects of Cancer Biology at National Institute of Immunology, New Delhi held on 11th – 13th March 2014.

63. Invited to attend ICMR-AF Workshop on Chronic Non-communicable Disseases, New Delhi organized under Object Research Priorities for Indo-Finland Partnerships in Chronic diseases held at ICMR, New Delhi during 11th – 13th March 2014

64. Invited to attend Review Committee meeting for review of ICMR Post-Doctoral Fellowships held on 12th March, 2014 at ICMR New Delhi.

65. Invited to attend Selection Committee meeting for the selection of candidates for the award of PDF of ICMR held on 13th March 2014 at ICMR, New Delhi.

DR POONAM SALOTRA

1. Member of 5th RTAG (Regional Technical Advisory Group), WHO meeting on "Elimination of Kala-azar" held at Paro, Bhutan, September 2013.

2. Member of Indian delegation for promoting Indo-Africa collaboration to Pasteur Institute, Tunis, Tunisia, May 2013.

3. Invited speaker at 5th World Leishmania Congress (WL5) held at at Porto de Galinhas, Brazil, May 2013.

4. Appointed member of selection committee for award of INSPIRE faculty fellowship interviews held in May 2013, at DIPSAR, New Delhi.

5. Invited participant in the DBT-BCIL Mentorship Workshop for Women Scientist on "Writing Effective Scientific Proposals" at INSA, New Delhi, July 2013.

6. Participated in the 10th foundation day lecture of National Brain Research Institute on "The Brain today and Tomorrow looking in and looking out." held at India International Centre, New Delhi on 16th Dec, 2013,

7. Attended a talk on "Accelerating progress in children nutrition- Is there a way forward" by Dr M K Bhan, Former Secretary, DBT, organized at INSA, New Delhi in March 2014.

8. Organized a meeting at NIP to discuss the developments and outlook on live attenuated *Leishmania* Vaccine candidate (Ld Cen$^{-/-}$) with participants Dr Nakhasi, CBER, FDA, USA, Dr Selvapaniyan, IMM, Delhi, Dr R. K. Singh, Gennova, Pune, Dr. S Sinha, DBT and Dr Anamika Gambhir, DBT on 10th July 2013.

9. Attended a series of meetings with ICMR IPR cell to discuss the Research Collaboration Agreement for producing GMP-grade live attenuated *Leishmania* vaccine candidate (Ld Cen$^{-/-}$) in 2013-14.

10. Invited participant as a committee member for discussion of a new potential vaccine against leishmaniasis, organized by DBT at National Institute of Immunology, New Delhi in February 2014.

11. Served as Co-ordinator for Ph.D. program at National Institute of Pathology.

12. Continued as Co-ordinator of weekly Journal Club/ Seminars at National Institute of Pathology.

13. Reviewer for several projects submitted for funding to ICMR, CSIR, DBT and DST.

14. Served as Associate Editor for the journal BMC Infect Dis.

15. Appointed reviewer of manuscripts submitted to various journals including PLoS NTD, Antimicrob Agents & Chemotherapy, J of Antimicrobial Chemotherapy, BMC Immunology, Molecular Biochemical Parasitology etc.

DR. NASREEN EHTESHAM

1. Attended an International meeting "Bioworld 2013" organized by IIT Delhi from December 9th -11th 2013

2. Delivered an invited lecture in the international conference on "Cellular and Molecular Mechanism of Disease process" held in Kashmir from 13th -16th April 2014.

3. Member of Screening committee for post of Scientist "C" in the area of Genetics/ Mol Biology

4. Faculty and examiner for Pre PhD course at NIP for BITS, Pilani and Symbiosis university, Pune.

5. Evaluated a thesis submitted at BITS, Pilani and also was an external examiner for PhD thesis at BITS, Pilani

6. Reviewed manuscript for Cytokines, J. BioSciences, IJMR and Current Sciences

7. Reviewed projects submitted to DBT and ICMR.

8. Delivered an invited lecture at Dr. Reddy's Institute of Life Sciences, Hyderabad 12th April 2013.

DR. SANGITA RASTOGI

1. Attended an innovative update session on '*Molecular Probes New Development: Labeling and detection strategies in fluorescence microscopy*" organized by m/s Life Technologies at Hyatt Regency, New Delhi on 17th May 2013.

2. Participated in '*Workshop on Clinical Proteomics*' at Institute of Bioinformatics (IOB), Bengaluru from 29th July – 1st August 2013.

3. Convened Institutional Animal Ethics Committee meeting (IAEC) at National Institute of Pathology, New Delhi on 7th October 2013.

4. Attended and presented poster on *'Clinical significance of circulatory chlamydial heat shock protein-60 antibodies in spondyloarthropathy patients'* at 2nd International Science Symposium on HIV & Infectious Diseases held at Chennai from 30th January 2013 – 1st February 2014.

5. Attended lecture titled, *'Health situation in India- Future perspective'* delivered by Padma Shri Ranjit Roy Chaudhury on the occasion of National Science Day at Jamia Hamdard University, New Delhi on 28th February 2014.

6. Awarded Travel Grant by DST for participating in ICID, Cape Town, SA (2014).

7. Faculty for Pre-Ph.D. courses of Symbiosis University programme:

   - *Cell Biology*
   - *Introduction to Research Methodology*

DR LAXMAN KR YERNENI

1. Examiner of a Ph.D. thesis on epidermal stem cell characterization at Stem Cell Facility, AIIMS, New Delhi.

2. Participated in a Brain Storming Meeting on "Clinical Research Using Stem Cells' on 31st October 2013, conducted by Stem Cell Unit of BMS, ICMR.

3. Delivered a talk entitled "Cultured Epithelium and Potential Application in Burns" during one day Symposium on Organ Donation and National Organ & Tissue Transplantation Organization (NOTTO) Initiatives on the occasion of Fourth Indian Organ Donation Day held on 28th March, 2013 at Safdarjung Hospital, New Deli

DR. RUCHI SINGH

1. Invited reviewer for manuscript submitted to Journal of Clinical Microbiology, Antimicrobial Agents and Chemotherapy, J of Antimicrobial Chemotherapy, Journal of Vector Borne Diseases, ISRN parasitology.

2. Member, Editorial board of Journal ISRN Parasitology.

3. Reviewer for short term studentship projects of ICMR (2013-14).

DR. FOUZIA

1. Posters presented :21 – 24th November, 2013- Indian Cancer Congress (ICC)2013,New Delhi. "Potential role of ER, PR and Her-2/neu as progonostic markers in surface epithelial ovarian tumors".

2. Posters presented 15-16th February, 2014- "Lymphoma biology to therapy".13th Annual International Conference RGCON 2014, New Delhi. "A rare case of histologic transformation: Hodgkin's to Non Hodgkin's lymphoma".

3. Attended conference - One day CME in Cytopathology, Quarterly meet Delhi Chapter IAPM on 23rd March 2013.

4. Attended - Quarterly meet Delhi Chapter IAPM on 13th April 2013.

5. Attended - "One day CME in Diagnostic Pathology" at Jamia Hamdard Hospital, New Delhi on 7th September 2013.

6. Attended 2nd Annual conference of Delhi Chapter of Indian Academy of Cytologists 2013 on 28th September 2013.

7. One day CME in Pathology at B.L Kapoor Hospital, 19th November 2013 New Delhi

8. Indian Cancer Congress (ICC) 2013, New Delhi on 21st – 24th November 2013

9. Workshop on Her-2 neu testing at VMMC & Safdarjung Hospital on 17th Jan 2014

10. 13th Annual International Conference RGCON, New Delhi on 15th-16th February 2014

11. 29th Annual Conference, IAPM, Delhi chapter, R&R Hospital, New Delhi on 9th March 2014

STUDENTS ACTIVITIES

1. Mr Himanshu Kaushal participated and presented work in Immunocon 2013 conference held from 15th – 17th Nov, 2013 on the topic "*In vitro* evaluation of protective role of $CD8^+$ T cells in individuals immune to *Leishmania donovani*".

2. Mr Kumar Avishek participated and presented work in World congress of infectious diseases (WCID) Conference held from 18nd to 22th Dec, 2013 in Chennai on the topic "*Ex vivo* evaluation of cellular immune responses elicited by genetically modified live attenuated *Leishmania* parasite vaccine candidates in human PBMCs".

3. Mr Kumar Avishek participated in Immunocon Conference held from 15th to 17th Nov, 2013 in New Delhi and presented poster entitled as "*Ex vivo* evaluation of immunogenicity of Centrin1 gene deleted live attenuated Leishmania vaccine candidate".

4. Mr. Praveen Kumar Participated in "*Immunology Day Celebration*" organized by Indian Immunology Society (IIS) at AIIMS, New Delhi on 27th April 2013.

5. Mr. Praveen Kumar Selected and participated in workshop on "*Scientific Writing*" at Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow (UP) organized by Indian Rheumatology Association (IRA) from 14th - 15th September 2013.

6. Mr. Praveen Kumar Delivered oral presentation titled *"Is major outer membrane protein of Chlamydia trachomatis a major antigenic protein in genitourinary-induced reactive arthritis?"* at IMMUNOCON-2013 at Delhi University conference centre organized by Indian Immunology Society (IIS) & UCMS from 15th - 17th November 2013.

7. Ms Namita Singh participated in symposium on *"Impact of Endocrine Disruptors on Reproductive Health"*, held on 20th July 2013 at AIIMS, New Delhi.

8. Ms Namita Singh participated in workshop on *"Application of Statistical Softwares in Medical Research"* during 5th - 7th August, 2013 at Institute of Cytology and Preventive Oncology (ICMR), Noida.

9. Ms Namita Singh poster presentation entitled *"Role of tumor necrosis factor alpha and cyclooxygenases in women undergoing Chlamydia trachomatis infected spontaneous abortion"*, Singh Namita, Das Banashree, Rastogi Sangita in IMMUNOCON-2013, 15th - 17th November 2013 held at New Delhi.

10 Ms. Priya Prasad Poster presentation entitled *"Role of oxidative stress in Chlamydia trachomatis infected first trimester spontaneous aborters"*, Prasad Priya, Singh Namita, Das Banashree, Rastogi Sangita in IMMUNOCON-2013, 15th- 17th November 2013 held at New Delhi.

11. Mr. Md. Khubaib attended International conference BioWorld 2013: computational biology in Disease and Disorder held on 9th-11th December 2013 at Kusuma School of Biological Sciences, IIT Delhi.

12. Mr. Saurabh Pandey attended International conference BioWorld 2013: computational biology in Disease and Disorder held on 9th-11th December 2013 at Kusuma School of Biological Sciences, IIT Delhi.

13. Mr. Javeed Ahmad attended, Immunology Day Celebration" held on 27th April 2013 at All India Institute of Medical Sciences, New Delhi.

14. Mr. Javeed Ahmad participated in the, National Cancer Congress 2014- Blood and Marrow Transplantation, A journey Towards Excellence" on Februray 2nd 2014 at Hotel Hilton, Delhi.

15. Mr. Javeed Ahmad attended International conference BioWorld 2013: computational biology in Disease and Disorder held on 9th -11th December 2013 at Kusuma School of Biological Sciences, IIT, Delhi.


SCIENTIFIC
ADVISORY
COMMITTEE

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1. **Dr. Subhroto Sinha** ***Chairperson***
   Director,
   National Brain Research Centre (NBRC),
   Near NSG Campus,
   Nainwal Mode, Manesar,
   Gurgaon, - 122 050,
   HARYANA.

2. **Dr. Shubhda Chiplankar**
   Director,
   Advance Center for Treatment,
   Research & Education in Cancer,
   Kharghar,
   Navi Mumbai – 410 210.

3. **Dr. Dhananjaya Saranath**
   601-B, Kalpak Gulistan,
   9A Perry Cross Road,
   Bandra West,
   Mumbai - 400 050.

4. **Dr. N.K. Mehra**
   Professor & Head,
   Department of Transplant Immunology & Immunogenetics,
   All India Institute of Medical Sciences,
   Ansari Nagar,
   New Delhi – 110 029.

5. **Dr. Chitra Sarkar**
   Professor,
   Department of Pathology,
   All India Institute of Medical Sciences,
   Ansari Nagar,
   New Delhi – 110 029.

6. **Dr. Kiran Katoch**
C/o Dr. Rohini Katoch Sepat, IPS,
Suprintendent of Police,
Near 1st Cross Degree College,
Ooragaum,
Kolar Gold Fields,
Karnataka – 563 117.

7. **Dr. S.K. Raza**
Director,
Institute of Pesticides Formulation Technology,
Sector – 20, Udyog Nagar,
Gurgaon – 122 018.

8. **Dr. Pooja Sakhuja**
Professor & Head,
Department of Pathology,
G.B. Pant Hospital,
Jawahar Lal Nehru Marg,
New Delhi – 110 002

9. **Dr. Sudha Bhatacharya**
Professor & Dean,
Department of Environmental Sciences,
Jawahar Lal Nehru University,
New Delhi.

10. **Dr. J.K. Batra**
Scientist VIII & Dy. Director,
National Institute of Immunology,
Aruna Asaf Ali Marg,
New Delhi – 110 067.

11. **Prof. Jaya S. Tyagi**
Professor,
Department of Biotechnology,
All India Institute of Medical Sciences,
NEW DELHI – 110 029

12. **Dr. Ravi Sirdeshmukh**
Distinguished Scientist & Asso. Director,
Institute of Bioinformatics,
Unit 1, Discoverer, 7th Floor,
International Tech Park Ltd.,
Whitefield Road,
Bangalore – 560 066,

H.No. 17-3/A,
Dharmapuri Colony,
Uppal,
Hyderabad – 500 039.

13. **Dr. Ashwini Kumar**
Industrial Toxicology Research Centre,
Post Box No. 80,
Mahatma Gandhi Marg,
Lucknow – 226 001.

14. **Dr. Ashok Mukhopadhyay**
Scientist VI,
National Institute of Immunology,
Aruna Asaf Ali Marg,
New Delhi-110067

15. **Dr. Ravi Mehrotra**
Director,
Institute of Cytology & Preventive Oncology,
Research-cum-Clinical Complex,
1-7, Sector-39, Near Degree College,
Opposite City Centre,
NOIDA-201 301 (U.P.).

16. **Dr. Vijay Kumar**
Scientist 'G' & Head,
Division of B.M.S.,
Indian Council of Medical Research,
Ansari Nagar,
New Delhi – 110 029.


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CPCSEA Nominees

Dr. D N Rao, Professor, Dept. of Biochemistry, AIIMS, New Delhi - *Main CPCSEA Nominee*

Dr. Smriti Rekha Dutta - *Link CPCSEA Nominee*

Scientist Member

Dr. Harmeet Singh Rehan, Prof., & Head, Dept. of Pharmacology, LHMC, New Delhi - *Chairman*

Social Scientist Member

Dr. B B Batra, Chief Medical Officer, NFSG, CGHS, New Delhi

Veterinay Consultant

Dr. P K Yadav, Sr. Veterinary Officer, Experimental Animal Facility, AIIMS, New Delhi

NIP Members

Dr. Poonam Salotra, Scientist 'F' - *Chairman*
Dr. Sangita Rastogi, Scientist 'E' - *Member Secretary*
Dr. Usha Agrawal, Scientist 'E'
Dr. L K Yerneni, Scientist 'D'


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Immunologist,
AIIMS, New Delhi

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AIIMS, New Delhi

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Dr. B. S. A. Raju
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Mrs. R. Saratha
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Mr. A.K. Srivastava
Dr. Poonam Salotra
Dr. Sangita Rastogi
Dr. S. Kapur
Dr. A.K. Jain
Dr. L K Yerneni
Mrs. R. Saratha

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MOLECULAR BIOLOGY LABORATORY

In the year under report, total of 150 clinical samples were received from VL patients (blood, bone marrow), PKDL patients (blood, tissue biopsy and slit aspirates) and CL patients (tissue biopsy). Samples were processed for detection and species identification of *L. donovani/ L. tropica* infections by PCR, PCR-RFLP, LAMP, Q-PCR and rk39 strip test.

Staff: Dr Poonam Salotra, Dr Ruchi Singh, Mr. Sandeep Verma, Ms Vasundhra Bhandari, Ms Vanila Sharma, Mr Himanshu Kaushal, Mr Kumar Avishek, Mr Deepak Kumar Deep, MS Aditya, Mr Uday, Ms Kamlesh Sharma, Mr. Anish Saxena, Mr RC Chhetri.

INFLAMMATION BIOLOGY AND CELL SIGNALING LABORATORY (IBCL)

A rapid test capable of reliably detecting the presence of *Mycobacterium tuberculosis* is vital for early detection and treatment of the disease. Highly specific signature sequences have been identified and a PCR-based method has been developed for detection of *Mycobacterium tuberculosis* in patients. Development of kit will be aimed at minimizing the time period between sample collection and its accurate analysis. Samples are being collected for screening.

Staff: Dr. Nasreen Z. Ehtesham , Dr. Naresh Arora, Dr. Manish Bhuwan, Mr. Aadinarayan Varma, Mr. Md. Khubaib, Mr. Saurabh Pandey, Mr. Javeed Ahmad, Ms. Simran Kaur Arora

FLOW CYTOMETRY LABORATORY

A total of 925 samples of blood samples from leukaemic patients were acquired and analysed for surface antigen expression, intracellular cytokine responses and apoptosis.

Scientific Staff: Dr. S. Verma, Mr. P.D. Sharma

CONFOCAL LASER SCANNING MICROSCOPY LAB:

Total of 54 images were acquired on confocal laser scanning microscope.

Scientific Staff: Dr S. Saxena, Dr. S. Verma, Mr. P. D. Sharma

MICROBIOLOGY LABORATORY

In the year under report, clinical samples (synovial fluid, blood and urine) were received from total of 49 arthritic patients (10 with reactive arthritis, 16 with undifferentiated spondyloarthropathy, 17 with rheumatoid arthritis and 6 with osteoarthritis). Samples were processed for doing diagnostic and immunological research on *Chlamydia trachomatis*-induced reactive arthritis.

Staff: Dr. Sangita Rastogi, Mr. Praveen Kumar, Ms Namita Singh, Ms Priya Prasad, Mr. Kamal Dev.

ANIMAL HOUSE FACILITY (AHF)

The Animal House at National Institute of Pathology (NIP) is a centralized facility registered (Registration Number: 102/ 1999 CPCSEA) with the *Committee for the Purpose of Control and Supervision of Experiments on Animals* (CPCSEA), MoEF, GOI and provides technical service for various small animal research projects in accordance to the CPCSEA guidelines. Investigators are required to fill up Form 'B' for *Institutional Animal Ethics Committee* (IAEC) approval and also requested to maintain and submit necessary forms before and after completion of experiments. The AHF is equipped with modular Individually Ventilated Cages (IVC) units for housing mice, rats, guinea pigs and hamsters. During 2013-14, two new research projects submitted by the Institute's scientists were given ethical clearance for animal experimentation by the IAEC.

Staff: Dr. Sangita Rastogi, Mr. Kuldeep Kumar Sharma, Mr. Bala Dutt

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