



ANNUAL REPORT

2014-15

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

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RESEARCH ACTIVITIES



TUMOR BIOLOGY

TUMOR BIOLOGY

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, India

Aims, Objectives and Background:

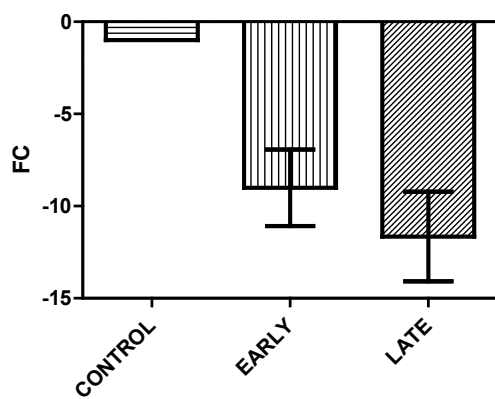
The present study is aimed to investigate genetic and epigenetic factors associated with early onset breast cancer in Indian women with objective to elucidate molecular pathogenesis by highlighting the changes in gene expression and methylation profile.

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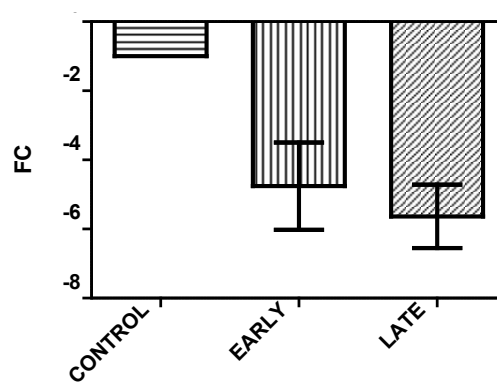
Work done during the year:

Study of gene expression profiling of 20 early (<40 years) and 20 late onset breast tumors (>55 years) and 5 distant normal tissues as controls, showed 1400 genes differentially expressed in early onset breast cancers compared to 2600 genes that are differentially expressed in late onset breast cancers. Validation of microarray data was done by confirming differential gene expression pattern for genes such as CCNJ, MATN3, and SOX18 in clinical samples.

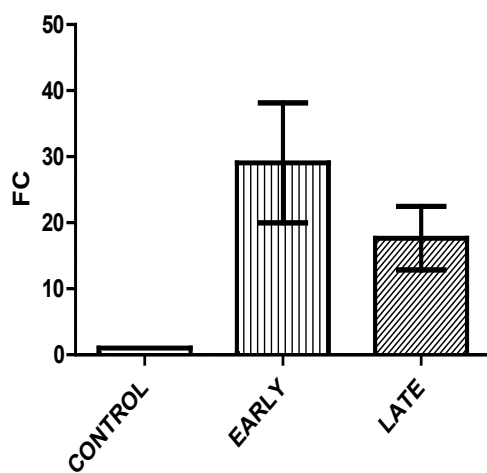
During the year under report we have selected MMP pathway genes for validation as they were found differentially expressed in early tumors. The gene expression data had shown upregulation of *MMP1*, *MMP13*, *MMP14*, *MMP3*, *MMP11* genes in early breast cancer compared to late breast cancer. Expression of MMP molecules, *MMP1*, *MMP13*, *MMP14*, *MMP3*, *MMP11*, and *ADAMTS1*, *ADAMTS5* was studied in 40 breast tumors, and 20 control tissue by real time PCR fold change was calculated and plotted. Fig.1 (a-g).



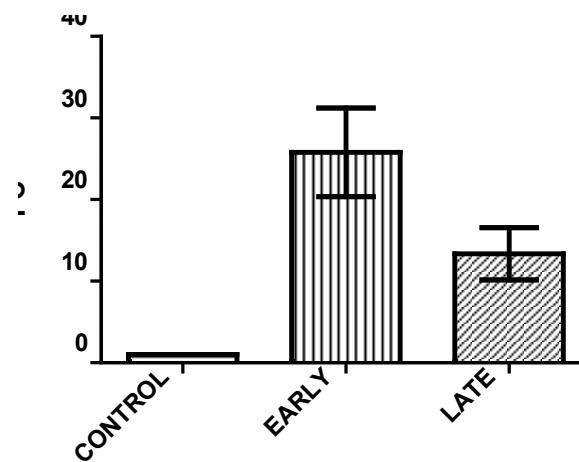
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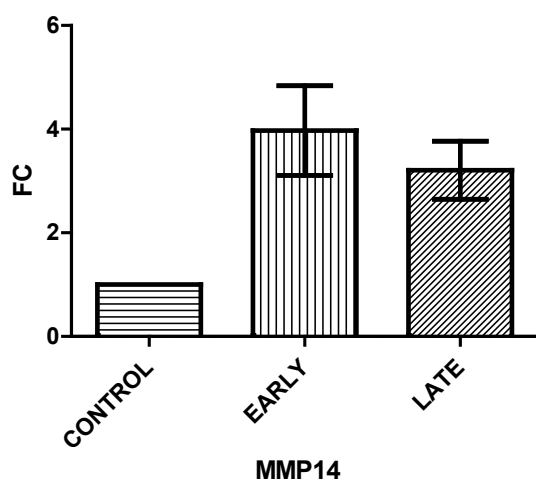
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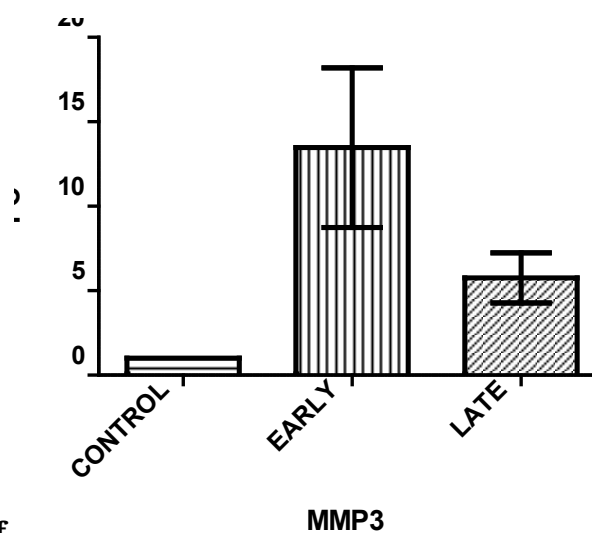
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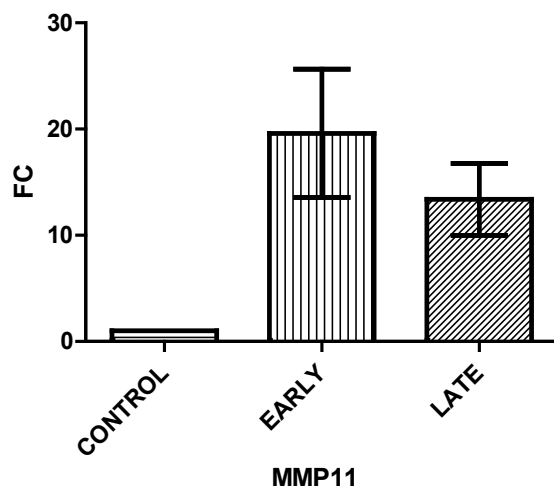
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e.



f.



g.

Fig. 1(a-g). Showing expression of MMPs family genes, in early and late onset tumors with control.

MMPs showed a very high average RQ fold change in early and late tumor compared to control tissue. A significant difference in expression of MMP13 was found between early tumors and late tumors ($p = 0.02$). Although expression of MMP1, *MMP11*, *MMP14*, *MMP3* genes were also found high in early tumors in comparison to late, the difference was not found statistically significant. Although expression of *ADAMTS1* and *ADAMTS5* was found significantly low in both early and late breast cancer compared to control, but the expression was also found significantly different in early and late tumors also, *ADAMTS1* $p=0.02$ and *ADAMTS5* $p=0.04$.

2. 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, Ms. Renu Yadav

Duration : 2013-15

Aims, Objectives and Background:

Cancer stem cells are known to be responsible for drug resistance and recurrence of tumors. This study is designed to estimate proportion of cancer stem cells (CD44+)

CD24-) in various breast cancer cell lines with correlation to their phenotype (ER+/- and triple negative) and study their unique miRNA and gene expression and methylation profiles compared to bulk tumor cells. This would help in identification of relevant pathways associated with stem cell characteristics and drug response.

Work done during the year:

During the year under report we have screened T47D, MDA-MB-453, MDA-MB-468, and ZR-75-1 cell lines to estimate the proportion of CD44⁺/CD24⁻ breast cancer stem cells (Figure 1).

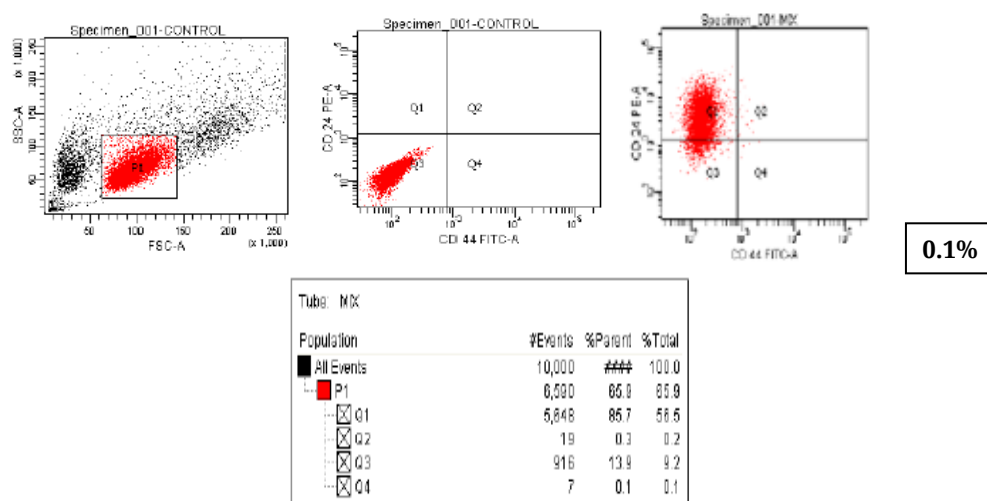


Fig 1a. T47D (CD44⁺/CD24⁻) profile

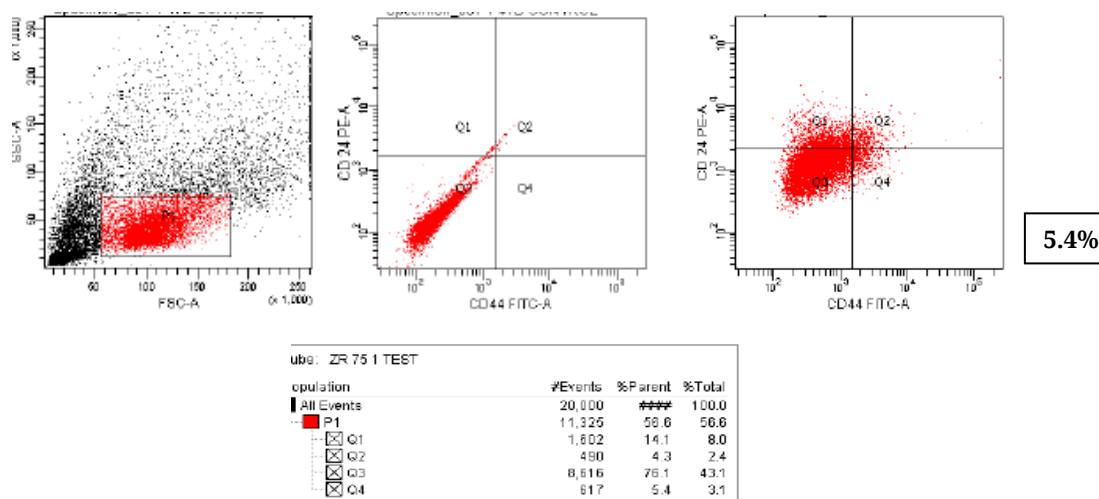


Fig 1b. ZR-75-1(CD44⁺/CD24⁻) profile

Fig.1. Representative figure showing seggreagationo of CD44⁺/CD24⁻ population

Further cancer stem cells were enriched in these cell lines using mammosphere assay, which were further utilized for profiling of microRNA expression in these breast CSC. Among differentially expressed microRNAs in breast CSC, 223 miRNAs were found to be 2 fold differentially expressed in compared to bulk cells. Hierarchical clustering has identified, distinct clusters associated with the cancer stem cells (Figure 2).

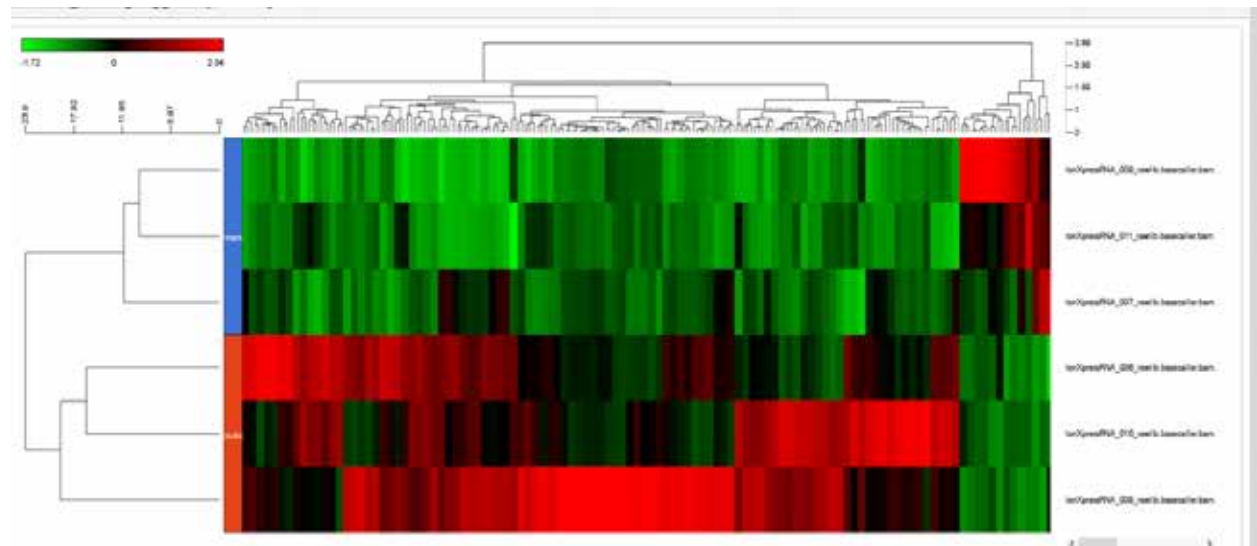


Fig. 2. showing the hierarchical clustering of miRNA in cancer stem cells and bulk cells

The preliminary analysis of pathways of these miRNAs was done by using bioinformatics tools for identification of the target genes through which these miRNAs might be functioning. We identified targets genes for approximately 88 microRNAs which were significantly differentially expressed in cancer stem cells, which revealed likely involvement of several key cancer associated genes that are targets for the miRNAs identified in the present study (Fig 3). Also these targets form part of pathways that are associated with cancer, however, such targets identified by using bioinformatics tools need validation by experiments.

Work done during the year:

Whole exome sequencing of 14 breast tumors, 7 early (≤ 40 years) and 7 are late onset (≥ 55 years) and 3 controls was done during the year under report and analyzed the data to identify genetic variations that are unique to early onset and late onset tumors. Upon analysis we found 2886 single nucleotide variations and 239 indels that are unique to early onset cases. In late onset tumors we found 5232 single nucleotide variations and 521 indels that are unique to late onset tumors. In addition we found 1991 single nucleotide variations and 137 indels that are common in both early and late onset tumors. Chromosome wise distribution of the variants showed highest number of variants in chromosome 1 followed by chromosome 2 and 6 (figure 1).

Preliminary pathways analysis showed involvement of cAMP, axon guidance, ECM receptor signaling TNF signaling pathways in early onset breast cancers (figure 2a) while in late onset tumors regulation of endocytosis, regulation of keratins, Rap1 signaling pathways (figure 2b) are disrupted.

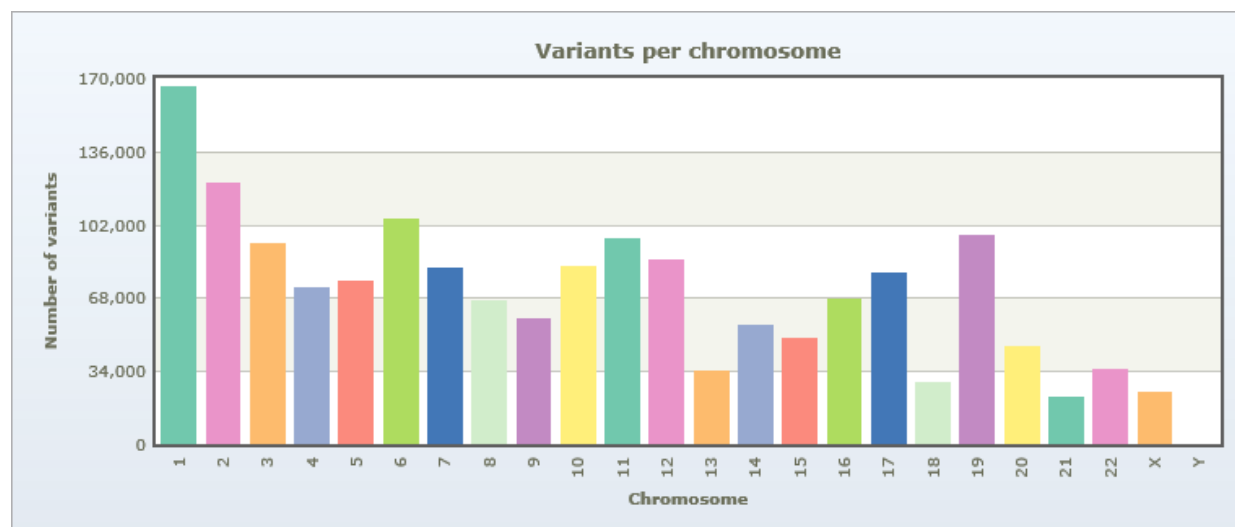


Fig. 1. Distrubution of variants among chromosomes in breast cancer patients.

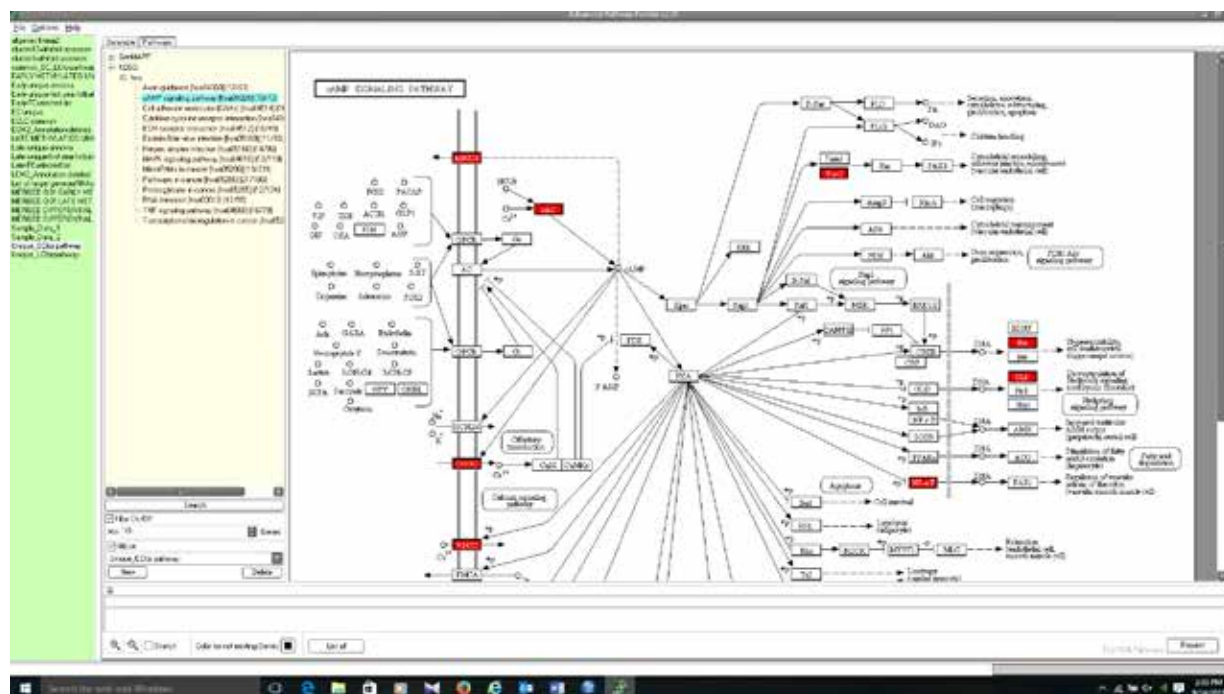


Fig.2a. Representative figure showing disruption of cAMP pathway in early onset tumors

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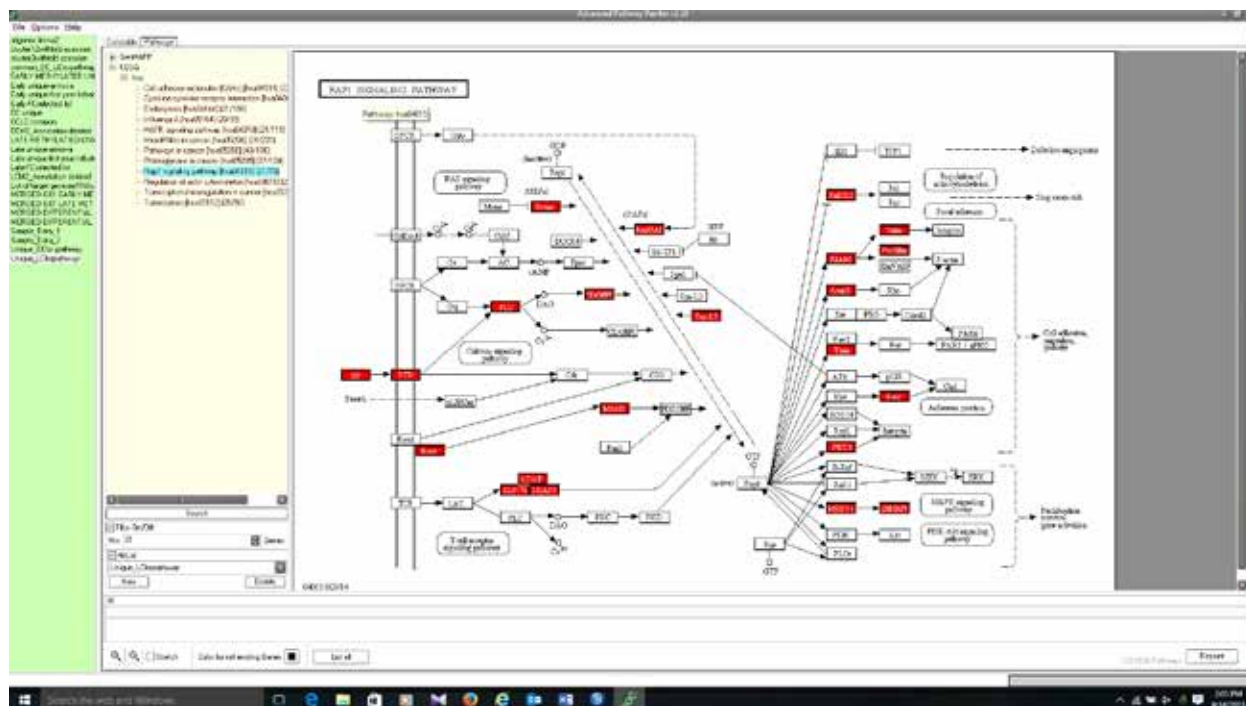


Fig.2b. Representative figure showing disruption of Rap1 pathway in early late onset tumors

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

Scientific staff : Dr Jatin Mehta, Dr. Sunita Saxena

Duration : 2013-15

Aims, Objectives and Background:

Breast cancer is a heterogeneous disease that encompasses a range of phenotypically distinct tumor 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 as well. The rationale for using Androgen receptors targets in breast cancer is underpinned by the fact that Androgen receptor is not only over expressed in breast cancer but contributes to breast cancer progression by stimulating WNT and HER2 pathway. Aim of the present study has been to understand AR signalling in breast cancer so as to provide the rationale for targeting of different types of breast cancer cells for the purpose of therapeutic intervention.

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Work done during the year:

In order to observe the effects of DHT on cell apoptosis, we selected three well known drugs namely paclitaxel, cyclophosphamide and 5-fluorouracil used in chemotherapy of breast cancer. To study the effect of AR on apoptosis, IC50 values of the chemotherapeutic drugs paclitaxel, 5-fluorouracil and cyclophosphamide were determined to be 17.62 μ M, 0.506 μ M and 3.45 μ M respectively. The effect of AR on the process of apoptosis was studied by adding IC50 concentration of the paclitaxel, 5-fluorouracil and cyclophosphamide drugs in presence of DHT, bicalutamide (AR antagonist) or both. It was observed that there was a significant increase in the cell survival on DHT treatment as compared to the vehicle treated cells, even in presence of IC50 concentration of the 3 therapeutic drugs, suggesting that AR reverses the chemotherapeutic drug induced apoptosis in MDA-MB-453 cells. Moreover, the

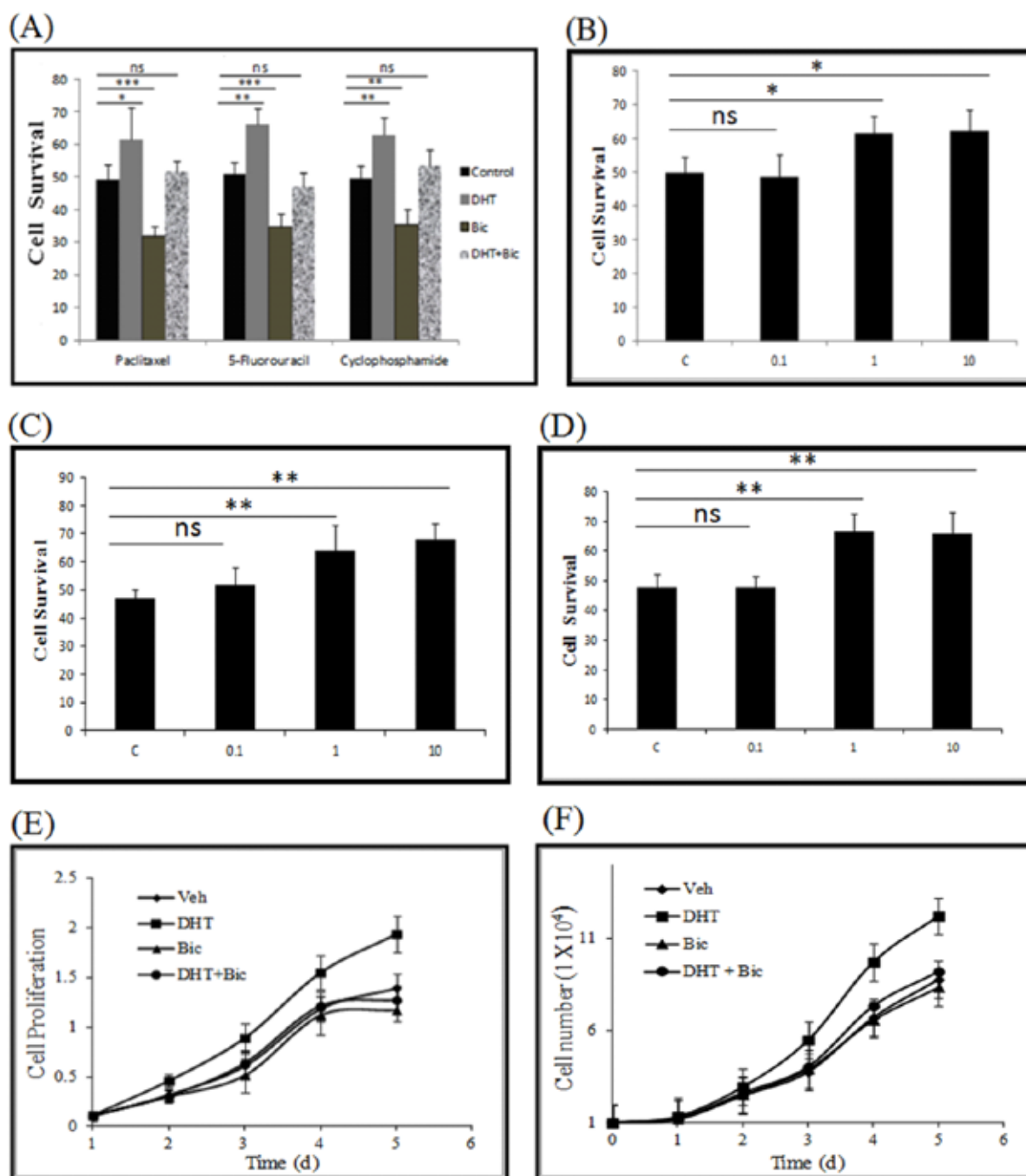


Fig. 1. DHT stimulation decreases apoptosis and induces cell proliferation in MDA-MB-453 cells. (A) Effect of DHT stimulation on cell survival caused by paclitaxel, 5-fluorouracil and cyclophosphamide in presence of 10nM/L of DHT (+) or vehicle control (-) for 24 hrs. (B-D) Increased cell survival cause by concentration dependent increase in DHT (nM/L) in presence the drugs (E) Role of AR in regulating MDA-MB-453 cell proliferation on DHT stimulation. (F) Live cell counting was done with trypan blue done to estimate cell the survival. DHT treated cells showed 1.55 fold (P=0.003) difference over vehicle treated cells.

cell survival was significantly decreased in the presence of bicalutamide alone as compared to the vehicle treated cells, with paclitaxel having a highly significant decrease in cell survival caused by increase in chemotherapeutic drug induced apoptosis followed by 5-fluorouracil and cyclophosphamide (Figure 1).

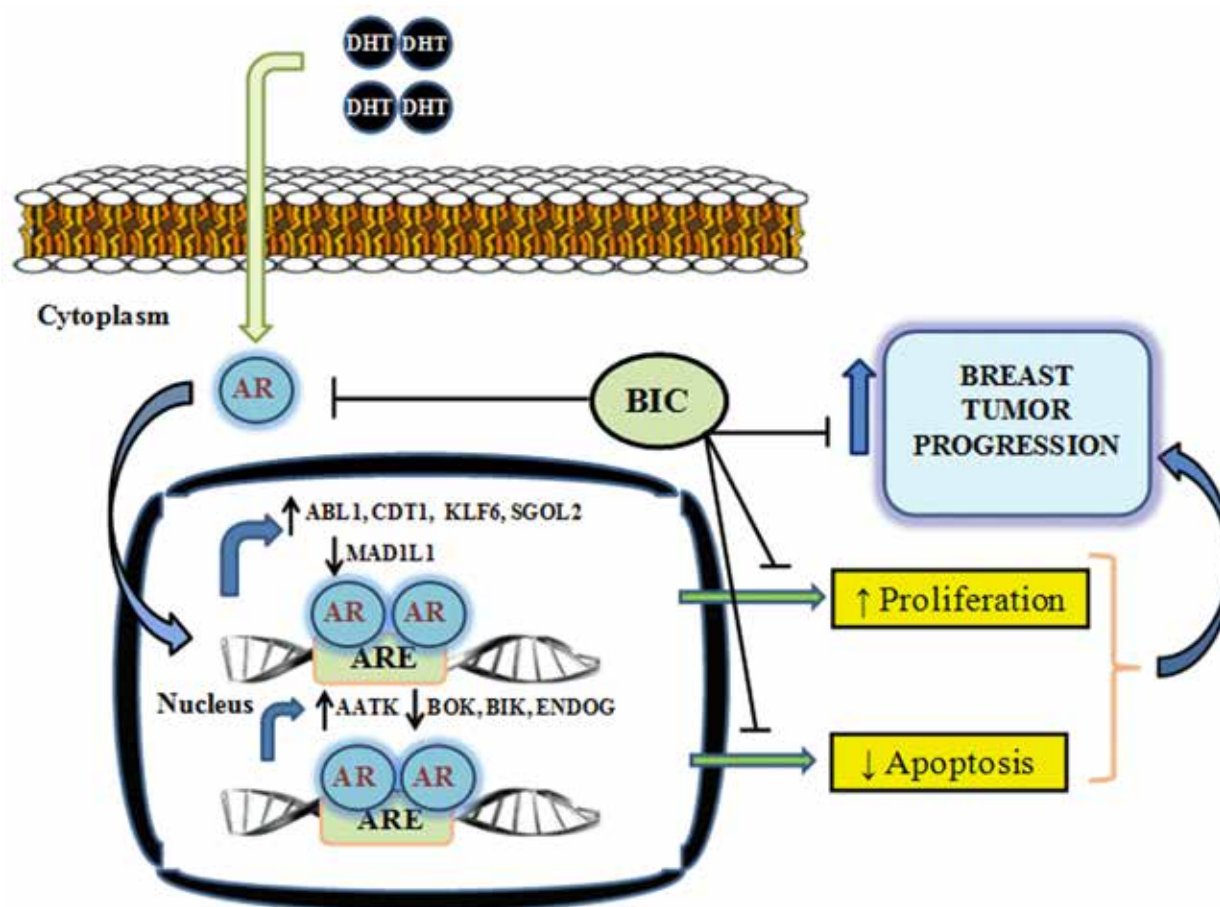


Fig. 2. Proposed model showing AR regulation of the breast tumour progression. Androgen receptor (AR) on DHT stimulation translocates into the nucleus and bind to its cognate androgen response elements (AREs). Inside the nucleus, AR up regulates the expression of ABL1, CDT1, KLF6 and SGOL2, while it represses MAD1L1 expression to induce cell proliferation. At the same it induces AATK expression and down regulates the expression of BOK, BIK and ENDOG to decrease apoptosis and promotes breast cancer progression. Bicalutamide (BIC) reverses the effect of AR on cell cycle and apoptosis by binding and preventing its activation. Due to its ability to negate the effects of AR, bicalutamide can be used to block breast cancer progression.

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

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

Duration : 2013-15

Aims, Objectives and Background:

AKR1B10 has been recently regarded not only as a potential diagnostic and/or prognostic marker in carcinomas but also as a therapeutic target for the prevention and treatment of several types of cancer. AKR1B10 has been implicated in the regulation of retinoid metabolism. Its role in the regulation of retinoic acid homeostasis may contribute to carcinogenesis. Additionally, cigarette smoke condensate exposure amplified AKR1B10 expression in both normal human epidermal and squamous cell carcinoma cell lines. In our previous study, gain of AKR1B10 was seen in breast cancer patients with betel quid chewing history. These observations implicate AKR1B10 as a tobacco exposure and response gene. Nevertheless, 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. To study the role of AKR1B10 in tobacco associated breast carcinogenesis, AKR1B10 expression will be analyzed in breast cancers and panel of breast cancer cell lines. Effect of tobacco exposure will be examined by exposing the cells to NNK (4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone)/nicotine. To check the effect of AKR1B10 suppression cells will be exposed to its competitive inhibitors sulindac and Oleanolic acid.

Work done during the year:

AKR1B10 expression in breast tumors

Seventy formalin fixed paraffin-embedded samples were analyzed for AKR1B10 expression through immunohistochemistry. AKR1B10 positivity was found in 50% of the samples with both cytoplasmic and nuclear localization. Fourteen to fifteen

percent samples had strong and weak positivity respectively whereas moderate positivity was seen in 20% of samples. Expression of AKR1B10 was not associated with ER, PR and Her2neu expression however it showed significant correlation with patients with history of tobacco use.

AKR1B10 expression in breast cancer cell lines

MCF-7, MDA-MB 231, MDA-MB 435 and HBL100 cell lines were cultured in DMEM media with 10%FBS and 5% CO₂. T47D and ZR75-1 cell lines were cultured in RPMI media with 10%FBS and 5% CO₂. The RNA was extracted from respective cell lines and converted to cDNA (High Capacity cDNA archival kit, Applied Biosystem) using a total of 1µg of total RNA. This cDNA was further used for analyzing for AKR1B10 expression using B-Actin expression as control by RT-PCR (Figure 1).

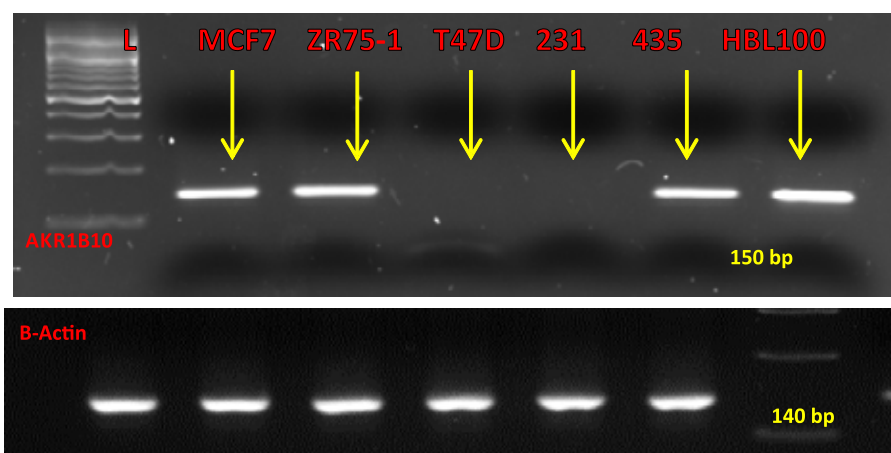
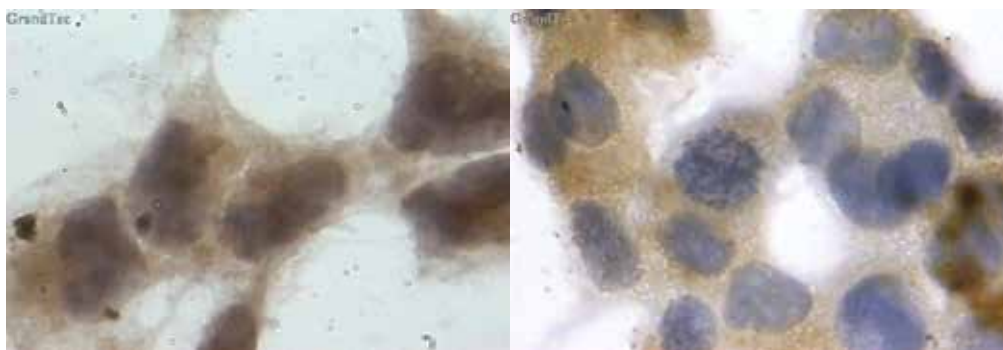


Fig. 1: RT-PCR for AKR1B10 expression in breast cancer cell lines.

Expression of AKR1B10 gene was found high in HBL100, MDA-MB 435, MCF-7 and ZR75-1 cell lines and absent in T47D and MDA-MB 231 cell lines. The expression of AKR1B10 was further confirmed by Immunocytochemistry in MCF-7, ZR 75-1 and MDA MB 231. (Figure 2).



A.

B.

Fig. 2: AKR1B10 expression in A. MCF-7, B. ZR 75-1100X magnification

Effect of NNK (4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone) and STE (Smokeless tobacco extract) on breast cancer cells: Cytotoxicity Assay

Breast cancer MCF-7, ZR 75-1 and MDA MB 231 cells (2000/well) were plated in a 96-well plated and incubated with medium containing 1, 5, 10, 20, 50 and 100 μM NNK and 5, 10, 20, 50 and 100 $\mu\text{g/ml}$ STE or 0.02% of DMSO which served as a vehicle control in a final volume of 100 ml for 24–72 hrs at 37°C. The dose and time kinetics of NNK and STE treatment in breast cancer cells (MCF-7, ZR 75-1 and MDA MB 231) were determined by MTT assay (Figure 3). Exposure to NNK increased cell proliferation in the breast cancer cell lines in a dose dependent manner with an optimal dose of 10 μM of NNK in MCF-7 and MDA MB 231 and 20 μM in ZR 75-1. Exposure to STE increased cell proliferation in ZR 75-1 in a dose dependent manner with an optimal dose 20 $\mu\text{g/ml}$ STE (figure 4). These optimal doses will be used in all the subsequent experiments.

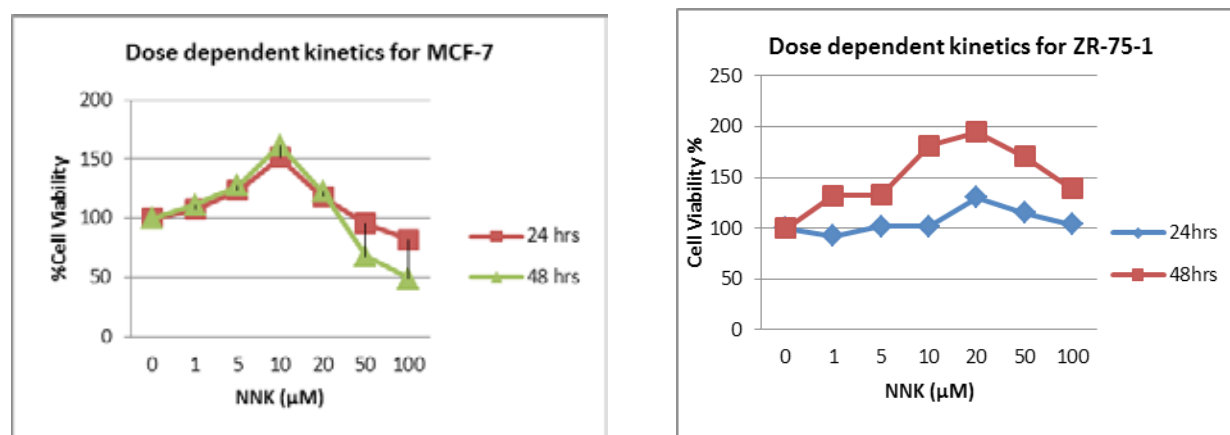


Fig. 3: Dose and Time dependent kinetics of NNK treatment on MCF-7 and ZR 75-1 cells using MTT assay.

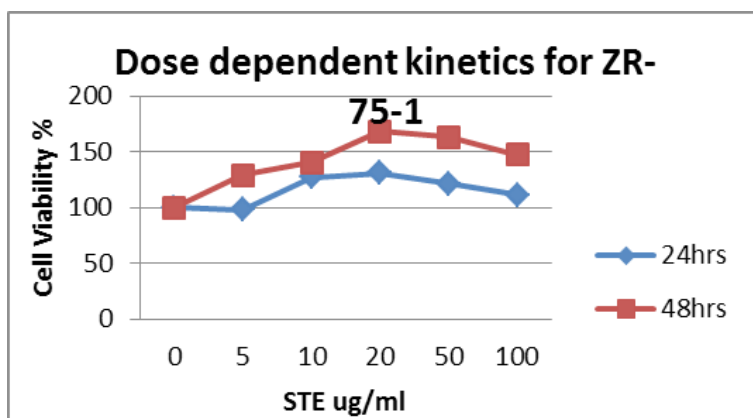


Fig. 4: Dose and Time dependent kinetics of STE treatment on ZR 75-1 cells using MTT assay

Effect of NNK and STE in AKR1B10 expression MCF-7, ZR 75-1, MB MDA 231 and T47D cells

Effect of NNK and STE was studied on AKR1B10 expression in MCF-7, ZR 75-1, MB MDA 231 and T47D cell lines.

NNK did not induce AKR1B10 expression in MCF-7, ZR75-1 and MB MDA 231 while T47D cell line showed induction of AKR1B10 at 48hrs and 72hrs of exposure. STE exposure modified AKR1B10 expression in MCF-7 and ZR75-1 cell lines and induced expression at 24, 48 and 72hrs in T47D cell line. It did not induce AKR1B10 expression in MB MDA 231. (figure 5)

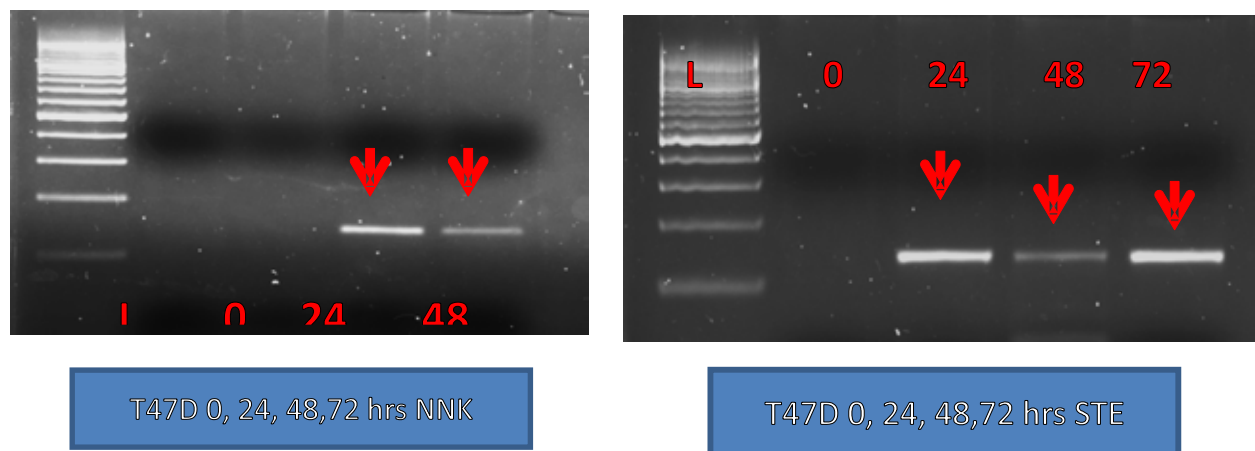


Fig. 5: RT PCR showing AKR1B10 expression when exposed to NNK or STE

AKR1B10 activity was suppressed using sulindac and oleanolic acid as inhibitors.

The inhibiting concentration of the drugs was estimated using NADPH as a substrate and measuring the difference of O.D at 340 nm. Using 12.8 μ M concentration of both the drugs MTT and FACS analysis was done to evaluate the effect of drug inhibiting AKR1B10 on the cells.

Sulindac suppressed proliferation and apoptosis to 50% ($p=0.09$) whereas Oleanolic acid inhibited proliferation and apoptosis to 50-60% ($p=0.01$).

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

Scientific staff : Dr. Shalu Jain, Dr. Anju Bansal, Dr. Sunita Saxena

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

Duration : 2013-15

Aims, Objectives and Background:

Carcinoma prostate is the fifth most commonly diagnosed cancer worldwide, and there has been significant increase in the incidence of prostate cancer over the last 25 years. Incidence of prostate cancer varies widely across the world, with South and East Asia showing lower incidence compared to Europe, and especially to the United States. In India, it is the fifth most common cancer among men. 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 has 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:

TMPRSS2:ERG (T2:ERG) and prostate cancer antigen 3 (PCA3) are the most advanced prostate cancer (PCa) early detection biomarkers. The recent identification of gene fusions of the 5'-untranslated region of TMPRSS2 (21q22.3) with the ETS transcription factor family members, like ERG (21q22.2), suggests a mechanism for overexpression of the ETS genes in the majority of prostate cancers. In the current study we identified the T2:ERG rearrangements in 58% of 55 prostate cancer biopsy samples by real time PCR (RT-PCR). We have also analysed matched urine samples from patients also to assess the potential of T2:ERG as a non-invasive marker and found a concordance level of 95.2% between tissue and urine sample results. Sensitivity of TMPRSS2: ERG fusion marker was 58 % whereas specificity was 100% as none of BPH sample was found to have the fusion transcript. The positive predictive value of fusion marker is 100% and negative predictive value of the marker is 71.25%. Expression analysis of ERG gene has shown a significantly marked upregulation of this gene in T2:ERG fusion positive PCa cases ($RQ=32.5$) as compared to fusion negative cases. The levels of PCA3 RNA were analysed in 75 cDNA samples including 40 prostate cancer cases (PCa) and 35 BPH controls. We observed that PCA3 gene is highly specific for prostate cancer and strong over expression of PCA3 gene in prostate cancer cases (35.5 fold) as compared to BPH ($p=0.034$). When we have compared the expression of PCA3 gene between T2:ERG fusion positive, fusion negative and BPH patients, expression of PCA3 gene was found to be 54.63 fold upregulated in fusion negative patients group as compared to 23.06 fold up regulation in fusion positive patient group ($p=0.05$) when BPH group was taken as control group. The present study shows that TMPRSS2:ERG and PCA3 are highly useful biomarkers for prostate cancer and has a great potential as a non invasive biomarker as well.

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

Scientific staff : Dr. Usha Agrawal, Ms. Nitu Kumari

In collaboration with : Dr. Anoop

Duration : 2014-16

Aims, Objectives and Background:

Urinary bladder cancer is the 7th most common cancer of males globally and 70% of them are superficial Transitional cell carcinomas. Treatment like TURBT appears simple once diagnosed, but management is difficult due to frequent recurrences. Superficial bladder tumors makes prognosis of the disease very difficult and provides a powerful rationale for adjuvant chemo/immunotherapy to TURBT treatment. This study has been undertaken to identify the differentially expressed tumour tissue proteins in urinary bladder cancers compared to normal bladder mucosa and to detect the presence of the differentially expressed tumour proteins in urine. Urine cytology is a non-invasive diagnostic tool to detect urinary bladder cancer but not sensitive for detection of early bladder cancer. Hence there is need of non invasive urinary biomarker which is useful in diagnosis of disease and recurrences.

Work done during the year:

During the year under report, the proteomic profile of 3 urinary bladder cancer and control samples was performed. Data analysis showed differentially expressed proteins between cancer and control.

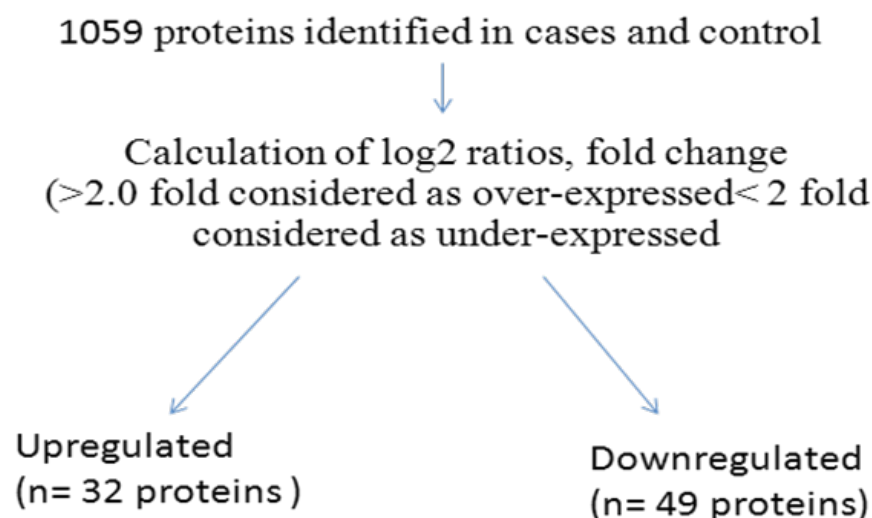


Figure. Flow chart showing identification of dysregulated proteins

The data obtained is at present being analyzed by bioinformatics tools to identify cancer specific proteins which can be used as non invasive biomarkers.

8. Genome-Wide Analysis of Genetic Alterations in Patients with Esophageal Cancer from Northeast India using Single Nucleotide polymorphism arrays

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

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

Duration of Project : 2011-14

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of death from cancer. In North east population esophageal cancer is the second leading cancer in males and third leading cancer in females. The predominant type of esophageal cancer is esophageal squamous cell carcinoma (ESCC).

Reports from Assam region of North East showed that the combination of wide spread consumption of tobacco, betel quid/betel nut along with genetic factors may be responsible for high incidence of ESCC in this region. In the current study we have studied the genetic variations mainly chromosomal changes, LOH and copy number alterations in case of ESCC in this region in association with tobacco and betel quid chewing habit through SNP Array. We have also done functional validation of FGF12 gene in kys410 cell line knocking it down by siRNA.

In this study, we performed Affymetrix® Genome-Wide Human SNP Array for 20 samples. To identify copy number among the chromosomal region we execute CEL files in GeneChip Operating Software (Affymetrix). To decode the common aberrations at various chromosomal regions we imported into the Copy Number Analysis Tool 4.0 Software (CNAT) from Affymetrix. SNPs with significant number of amplified and deleted regions of different arms of chromosome were further carried out. This analysis identified thirteen of 43 genes in amplified regions 50 genes in deleted regions. *In silico* analysis of amplified and deleted region genes were further promoted for pathway analysis, ontology analysis, network of cancer gene analysis, and differential expression cancer gene analysis in ethnic Chinese population. These analyses were able to identify five putative molecular marker (amplified region genes *COL11A1*, *FGF12*, *PAK1*, and deleted region genes *DLC1*, *NPHP4*). These makers further analyzed among the 22 type of cancer to know the mRNA level of these genes to find out suitable and appropriate esophageal cancer gene. FGF12 was only one suitable marker showed upregulated expression as a positive esophageal cancer in oncomine expression and Cancer Cell Line Encyclopedia (CCLE) databases (Figure 1) as well as found downregulated in normal tissue databases.

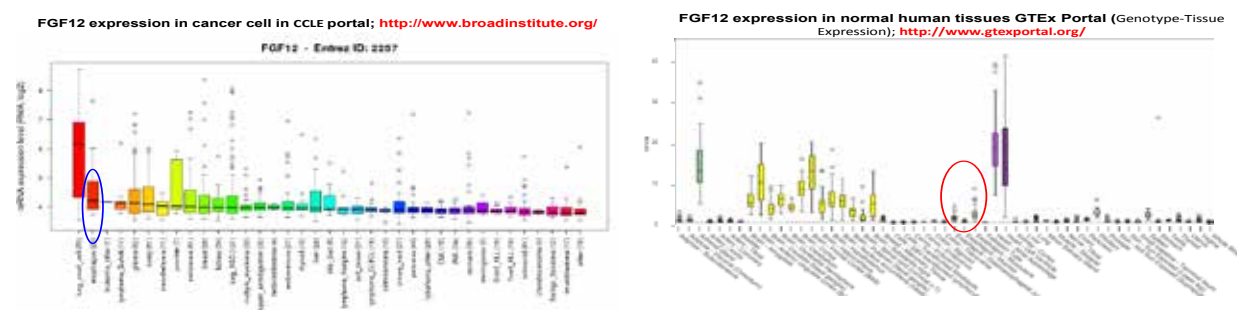


Fig. 1. FGF12 expression in Cancer cell line and normal human tissues databases

In addition to genomic alterations found in the current study, we have also reported *FGF12* to be significantly upregulated in our previous 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. With these leads we did functional characterization of FGF12 by knockdown studies in ESCC cell line KYSE410 since this cell line showed over expression of FGF12 compared to other cell lines. (Figure 2).

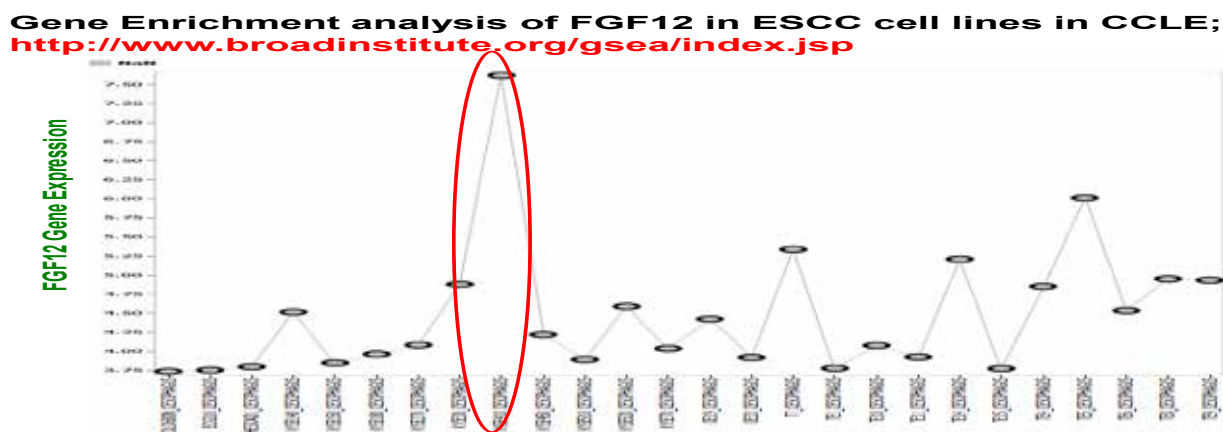
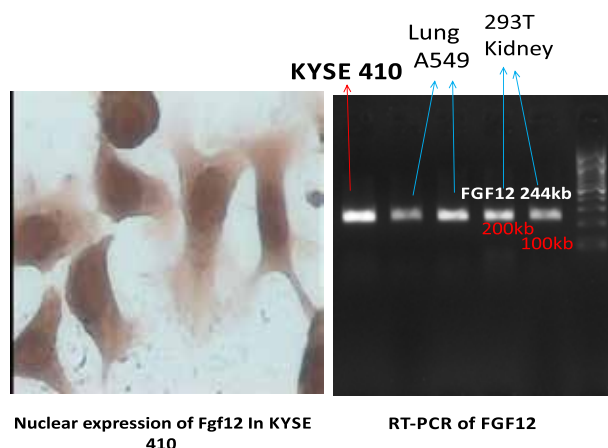


Fig. 2: FGF12 expression in among the ESCC cell lines

KYSE 410 cell line was transfected with siRNA to knockdown using Lipofectamine® RNAiMAX Reagent (Life Technologies™) followed by Proliferation assay, Wound healing and colony formation assays. These assays showed more than 50% of cancerous cells were inhibited their activity after knockdown of FGF12.

Expression of Fgf12 in KYSE 410



9. Epigenetic studies in esophageal cancer in high risk region of Northeast India

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Scientific staff	: Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. L. C. Singh, Dr. Virendra Singh
In collaboration with	: Dr. Amal C. Kataki, Director, BBICI, Guwahati, Assam Dr. J. Mahanta, Director, Regional Medical Research Centre, Dibrugadh Dr. Jagannath Sharma, Chief Consultant, Dept. of Pathology, BBICI, Guwahati, Assam Dr. B. B. Borthakur, Consultant, Dept. of Surgical Oncology, BBICI, Guwahati, Assam Dr. Avdesh Rai, Research Scientist, Dept. of Pathology, BBICI, Guwahati, Assam Dr. R. K. Phukan, Regional Medical Research Centre, Dibrugarh, Assam
Duration	: 2011-14

Aims, Objectives and Background:

Esophageal cancer incidence is reported in high frequency in northeast India. The etiology is different from other population at India due to wide variations in dietary habits or nutritional factors, tobacco/betel quid chewing and alcohol habits. Since

DNA methylation, histone modification and miRNA-mediated epigenetic processes alter the gene expression, the involvement of these processes might be useful to find out epigenetic markers of esophageal cancer risk in northeast Indian population. This study has been under taken to find out the contribution of epigenetic modifications on the development and progression of esophageal cancer in high risk population from north east India.

Work done during the year:

Genome wide methylation profiling was done by Infinium 450k array in paired tumor and normal tissue samples collected from patients with matched age, sex, ethnicity and grade of tumor. Integration of present methylation data with microarray expression data published earlier by our group was also done. To prepare a network of genes displaying enriched pathways together with list of genes exhibiting promoter hypermethylation or hypomethylation with inversely correlated expression, we did the Integrome analysis. Methylation Efficiency Index (MEI) was also calculated for genes resulted in Integrome analysis. The study resulted in 23 Integrome network enriched genes having relevance to tumor progression. These includes 4 genes with promoter hypermethylation and down regulation and 19 genes with promoter hypomethylation and up regulation. Top 5 genes with highest MEI score were COL1A1, TAC3, SERPINA4, TNFSF13B and IL22RA2. The methylation and expression status of circulatory proteins involved in immunoregulation (IL22RA2 and TNFSF13B), extra cellular matrix remodeling (SERPINA4) and contraction of the circular muscle of human esophagous (TAC3) could be further explored as non-invasive biomarker for esophageal cancer.

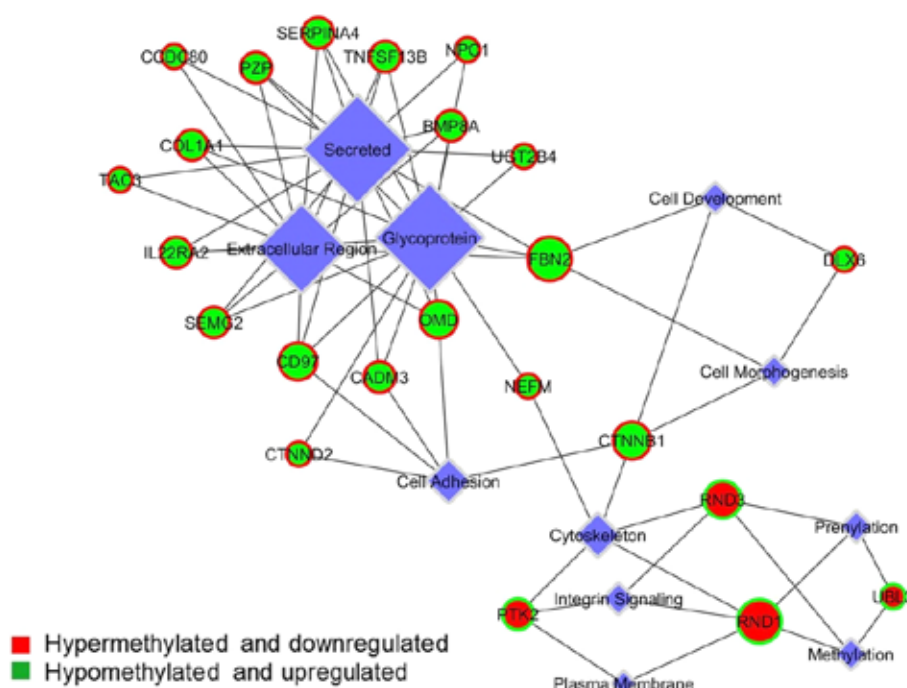


Figure. Integrome network analysis. It resolved 23 genes that correlated methylation status with gene expression status. Network encompasses biological categories, differentially expressed and methylated genes that were significantly enriched. Green and red color circles represent upregulated genes with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

10. 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
Collaborators	: Dr. A.C. Kataki, B. Barooah Cancer Institute, Guwahati, Assam Dr. Y. Mohan Singh, RIMS, Imphal, Manipur
Duration	: 2010-15

Aims, Objectives and Background:

Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world, but occurs at relatively high rates in some geographic regions and among certain ethnic groups, with the highest incidence worldwide being reported from southeast Asia

and southern Chinese. High incidence of NPC in Northeast (NE) region of India has been reported where it is the eighth most common cancer. The etiological factors of NPC include a complex interaction of genetic, viral, environmental and dietary factors. Antigenic presentation of EBV-derived peptides is suspected to be involved in the pathogenesis of EBV-associated diseases. In addition, polymorphisms in the HLA region, particularly in the class I region, is also known to be associated with the occurrence with the disease. The aim of the current study had been to analyze if Epstein Barr viral sequences in the tumor tissue along with host immunogenetic factors can explain the high prevalence of nasopharyngeal carcinoma in different ethnic groups of Northeastern States. During the year under report we have studied genetic alterations in HLA region among patients with nasopharyngeal carcinoma using Next generation sequencing.

Work done during the year:

The results of previous studies has demarcated a section on HLA class I region and EBV RNA sequences with susceptibility for NPC. Further to study additional genetic alterations the entire HLA super locus (3.8 Mb regions) using next-generation sequencing (NGS) technology was studied. The study was carried out in 4 NPC cases and 4 corresponding age and sex matched controls. The major pathways that were affected by the genes that involved variations were Antigen processing and presentation, Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Cell adhesion molecules (CAMs), Asthma, Systemic lupus erythematosus, Intestinal immune network for IgA production, Intestinal immune network for IgA production, Endocytosis and Natural killer cell mediated cytotoxicity. Analysis of results showed a high association of five SNP located in HLA region with the Nasopharyngeal cancer Of these five SNPs two SNPs were novel present in genes namely COL11A2 and MUC22 whereas three SNPs present in genes HLA DRB5, HLA-DPA1 and TAP2 were already known.

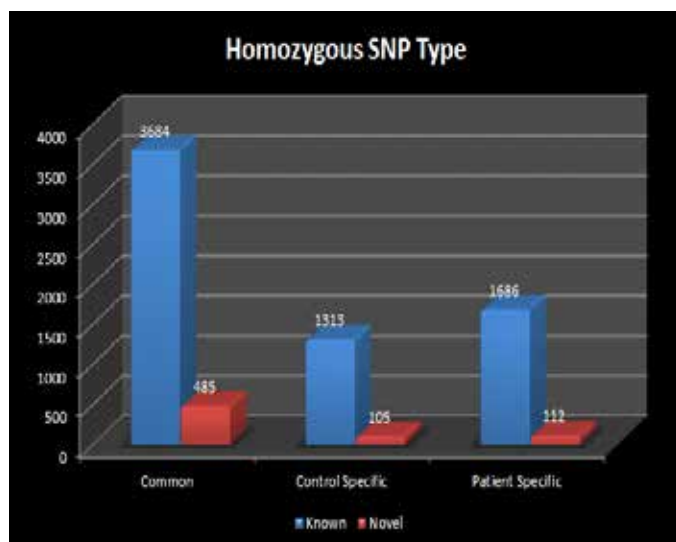


Fig. 1: Bargraph showing classification of homozygous SNPs in patients and controls

Table 1: Homozygous SNP Type classification

Homozygous SNP Type	Common	Control Specific	Patient Specific
Known	3684	1313	1686
Novel	485	105	112

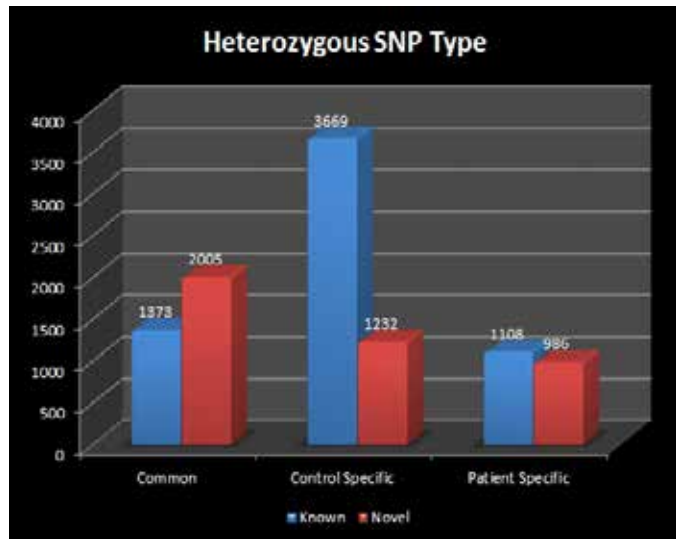


Fig. 2: Bargraph showing classification of heterozygous SNPs in patients and controls

Table 2: Heterozygous SNP Type classification

Heterozygous SNP Type	Common	Control Specific	Patient Specific
Known	1373	3669	1108
Novel	2005	1232	986

Further the validation of these SNPs is being done in large sample size. To conclude the results so far it can be said that variations in HLA region has an important role in occurrence of NPC in northeast India.

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

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

Duration : 2013-15

Aims, Objectives and Background:

A major issue in the treatment of acute myeloid leukemia (AML) is resistance to chemotherapeutic drugs. Different mechanisms of drug resistance, including ATP-binding cassette (ABC) transporters, are responsible for treatment failures. ATP-binding cassette (ABC) membrane proteins comprise a superfamily of transporters with a wide variety of substrates. Humans have 49 members in this superfamily. The majority of these proteins have not yet been examined in AML. Current study had been under taken 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

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Work done during the year:

TaqMan® Human ABC Transporter Arrays was used to analyze the expression of these ABC transporters in Adult AML and healthy PBMC. The expression of ABCA1 (4.9 fold), ABCD4 (1.56 fold), ABCF1 (1.61) and ABCG1 (3.25 fold) was higher in non responder as compared to responders patients in array results. The expression of the selected genes (ABCF1, ABCA1 and ABCG1) was then analyzed by real-time PCR in 30 Adults patients (Age > 18years) of AML including 16 patients with complete remission(CR), and 14

with non complete remission (NCR). In accordance with the results of the Tagman arrays, all the three genes were overexpressed in NCR patients compared to patients who responded to chemotherapy. The median expression in the non-responder patient samples was two to four times higher, and the differences were highly significant ABCF1 ($p=0.001$) and ABCG1 ($p<0.001$) (Figure 1).

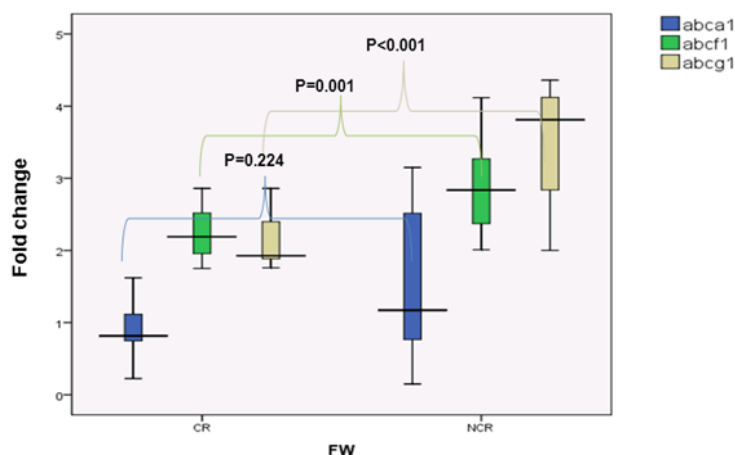


Fig 1: Expression of three ABC transporters in samples from 30 Adults with AML (CR-16, NCR-14)

Baseline expression of ABCA1, ABCF1, and ABCG1 in cell lines

Figure 2 shows the RT-PCR expression for ABCA1, ABCF1, and ABCG1 expression in K562 and THP-1 cell line compared to the normal samples (5). No mRNA expression was detected for ABCG1 (no amplification over 35 cycles) in K562 cells. ABCA1 expression was high in THP-1 cells compared to K562 cells. ABCF1 expression does not show much variation in expression level between two cell lines.

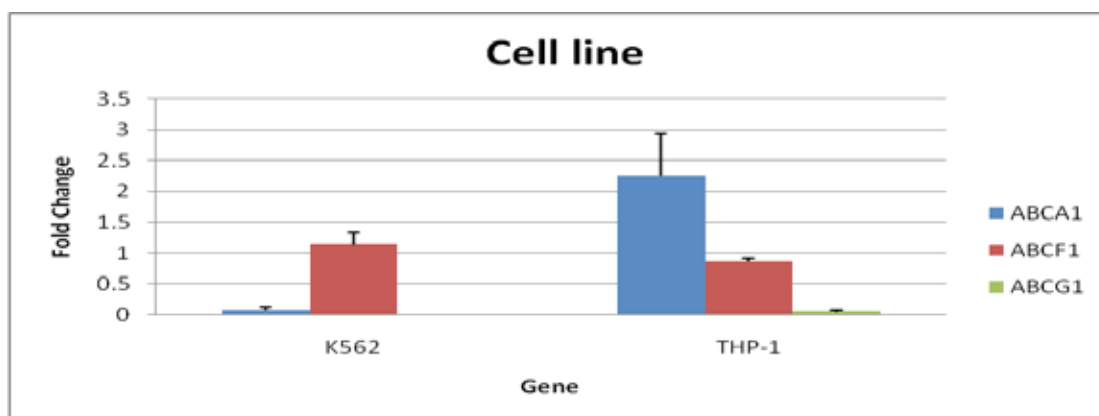


Fig.2: Expression of three ABC transporters in cell line K562 and THP-1 compared to five healthy people

In vitro Response to Cytosine arabinoside (Ara-C)

We checked whether these two genes are involved in the response of AML to cytostatic drugs. Two cell lines THP-1 and K-562 were incubated with Cytosine arabinoside (Ara-C) (**0.8 & 1.2ug/ml**). These concentrations were selected by determining the IC-50 for each drug in both cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. For Ara-C drug. After 24, 48 and 72hrs hours of incubation, the mRNA expression of ABCA1, ABCF1 and ABCG1 was analysed in treated and untreated cell samples by real time RT-PCR. Result shows that the drugs Ara-C led to an increase of ABCF1 expression in THP-1 and K-562 cells while highest expression of ABCG1 gene was at 24hrs exposure. Interestingly we did not find the expression of ABCG1 in K-562 cells (Figure 3).

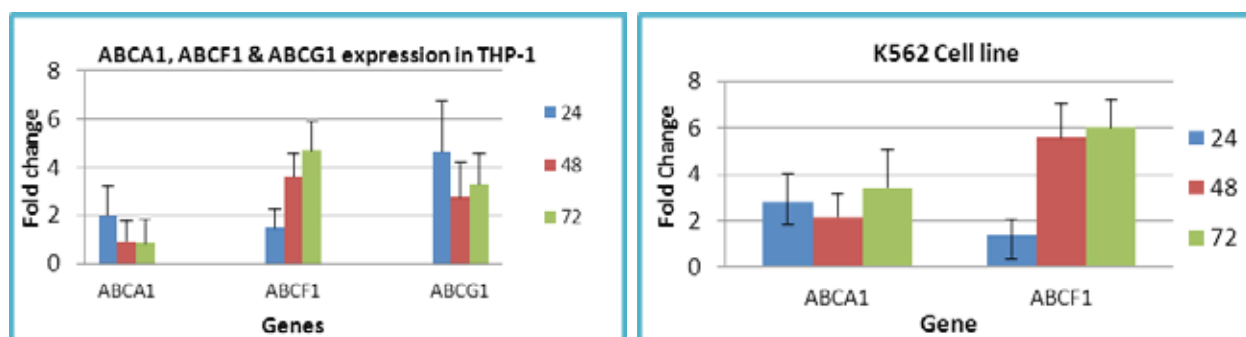


Fig. 3: Expression of ABCA1, ABCF1 and ABCG1 in K562 and THP-1 cells after treatment with Cytosine arabinoside. The expression after 24, 48 and 72 hours is given in relation to the expression before treatment.

These results indicate that both ABCF1 and ABCG1 genes may be involved in the mechanism of drug resistance in AML.

12. Proteomic Analysis of Plasma Exosomes to Identify Circulatory Biomarkers for Gallbladder Carcinoma

Scientific staff	: Dr. Sunita Saxena, Dr. Poonam Gautam, Dr. Usha Agrawal, NIOP Delhi
In collaboration with	: Dr. Puja Sakhuja, Dr. Anil Agrawal, GBPH, Delhi Dr. Ravi Sirdeshmukh, IOB, Bangalore
Duration	: 2014-15

Aims, Objectives and Background:

Gallbladder carcinoma (GBC) is the fifth most common and aggressive malignancy of the gastrointestinal tract, with **high prevalence and incidence rate** in Latin America and **Asian region, including northern and northeast India**. Five-year survival rates for advanced stages are drastically low ($\leq 5\%$) in comparison to early stages (70-90% in Stage I and 45-60% in Stage II, when treated with extended cholecystectomy). Therefore, in order to have increased survival of GBC patients, it would be important to diagnose the disease at early stages. However, till date, a panel of highly sensitive and specific biomarkers for diagnosis of GBC is not available for clinical applications. **Exosomes** are small membrane vesicles ranging from 40 to 100 nm in size that are secreted by a multitude of cell types as a consequence of fusion of multivesicular late endosomes/lysosomes with the plasma membrane. Several studies have reported the functional role of exosomes in disease initiation and progression. Tumor cells have been shown to secrete exosomes, often in increased amounts compared to normal cells, and these exosomes carry genomic and proteomic signatures representative of the tumor cells from which they were derived. While these unique signatures make exosomes ideal for cancer detection, exosomes derived from cancer cells have also been shown to play a functional role in cancer progression. Therefore, profiling of exosomes from different stages of cancer holds a great promise for early diagnosis of cancer. The present proposal aims to identify exosomal proteins with differential levels in early and advanced stages of gallbladder carcinoma to achieve a panel of biomarkers for early diagnosis and therapeutic monitoring of the disease.

Work done during the year:

Isolation and characterization of exosomes from human blood plasma

We have optimized the isolation and characterization of exosomes from pooled plasma of healthy individuals. An equal volume of plasma from five healthy individuals was pooled and performed ultracentrifugation for isolation of plasma-derived exosomes. SDS-PAGE analysis was done to study the exosomal protein profile. In order to characterize exosomes, the level of CD63, an exosomal protein marker, in exosomal and non-exosomal fraction was studied by dot blot and western blot analysis. Both the assays showed significant enrichment of CD63 in exosomal fraction. The study would be further extended to analyze differential plasma-derived exosomal proteins in gallbladder carcinoma samples.

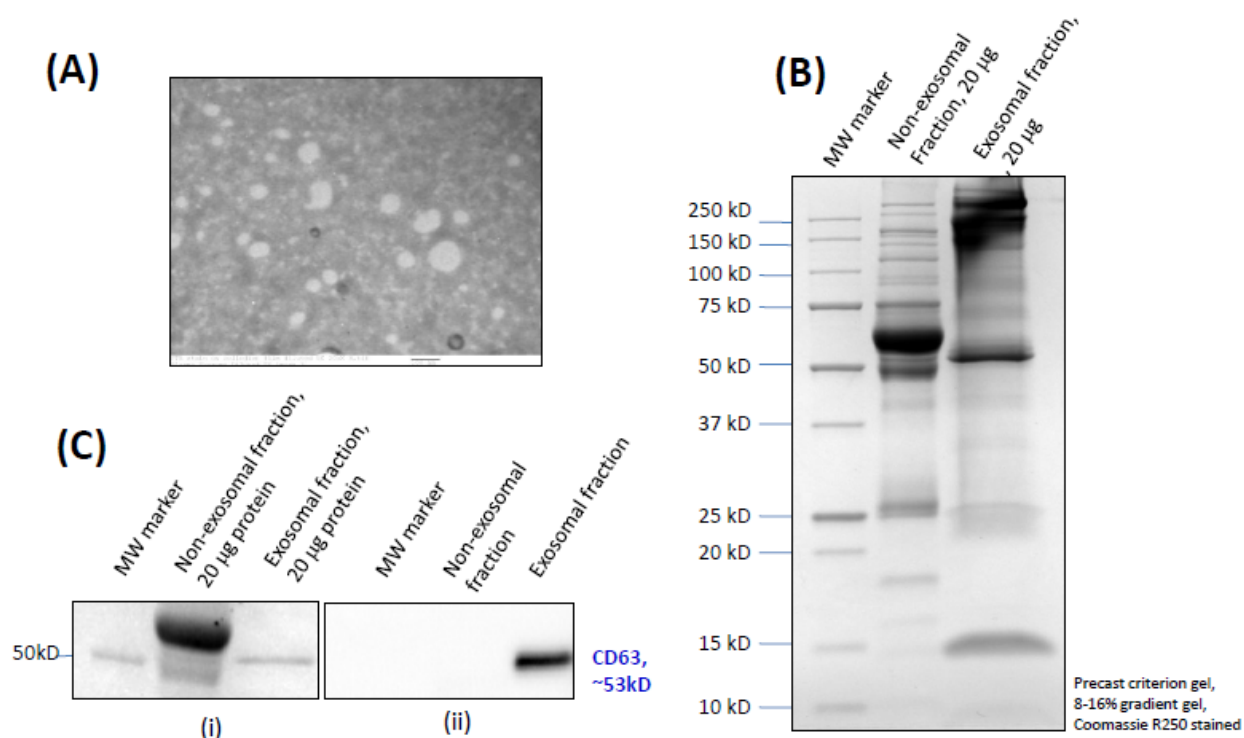


Figure: Isolation and characterization of exosomes from pooled plasma of healthy individuals. (A) Transmission electron micrographs of plasma derived exosomes. Exosomes from pooled plasma of healthy individuals isolated by ultracentrifugation method were resuspended in PBS and loaded on 2% collodion coated grids. Negative staining was performed using phosphotungstic acid (PTA). Images of exosomes (30-100 nm) were acquired using Hitachi TEM 7500 at 1,40,000X magnification, scale bar- 100 nm. (B) SDS-PAGE analysis showing protein profile of Exosomal and Non-Exosomal fraction of pooled plasma from healthy individuals. (C) Western blot analysis showing enrichment of CD63, an exosomal protein marker, in exosomal fraction of pooled plasma from healthy individuals (i) Ponceau S stained image after the blotting (ii) immunoblot showing significant enrichment of CD63, an exosomal protein marker, in exosomal fraction.

13. Analysis of Glioblastoma-Derived Exosomes for Identification of Circulatory Protein Biomarkers for Clinical Applications

Scientific staff : Dr. Poonam Gautam, Dr. Avninder Pal Singh, NIOP, Delhi

In collaboration with : Dr. R. K. Saran, GBPH, Delhi
Dr. Ravi Sirdeshmukh, IOB, Bangalore

Aims, Objectives and Background:

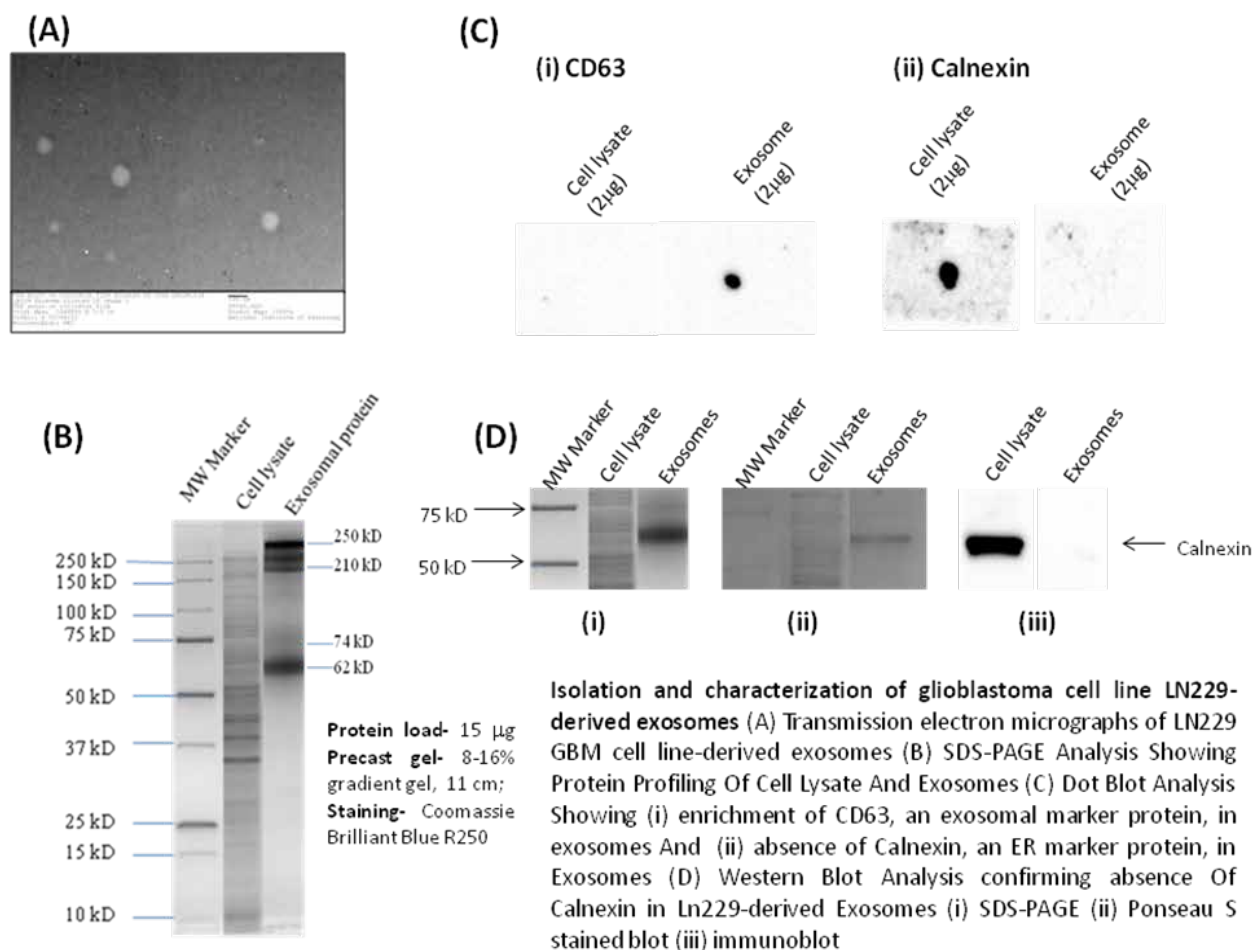
Despite major advances in high-throughput technologies and multi-dimensional profiling in the last decade, management of Glioblastoma Multiforme (GBM), one of the most malignant and aggressive forms of primary brain tumors, remains a clinical challenge. These aggressive tumors exhibit local metastasis and are resistant to current modalities of treatment, generally resulting in tumor recurrence. Effective methods for post-treatment surveillance of glioblastoma are still in the waiting and there is strong need for high confidence identification of blood-based biomarkers. Exosomes have recently emerged as a novel source of circulatory biomarkers for cancer. Tumor cells have been shown to secrete exosomes, often in increased amounts compared to normal cells, which carry genomic and proteomic signatures representative of the neoplastic condition. These are 30-100 nm microvesicles, secreted by all cell types and contain various bioactive molecules including proteins, miRNA, mRNA, DNA, lipids. Recently, cancer-derived exosomes have been shown to have a significant role in promoting tumorigenesis. These are also reported to be remarkably stable in biological fluids such as blood plasma, urine etc and considered to be an excellent resource for biomarker discovery for cancer detection and post-treatment surveillance. The present proposal is to identify exosomal proteins from GBM cell lines and explore their potential as circulatory biomarkers for post-treatment surveillance of GBM patients. Exosomes from five glioblastoma cell lines will be analyzed by applying high resolution mass spectrometry approach. Exosomal proteins common

among glioblastoma cell lines and reported to be differentially expressed at tissue level (based on available literature) with function relevant to tumorigenesis will be verified in blood plasma of control and GBM subjects (before and after surgery) using Enzyme Linked Immunosorbent Assay (ELISAs). The study may lead to identification of a panel of circulatory markers for post-treatment surveillance of GBM patients.

Work done during the year:

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

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



14. Understanding the role of chemokines and their receptors in growth and development of Glioblastoma

Scientific Staff : Ms Ira Sharma, Dr. Avninder Singh, Dr. BSA Raju, Dr. Sunita Saxena

In collaboration with : Dr. K.C. Sharma, Dept. of Neurosurgery, Safdarjung Hospital

Duration : 2013-18

Aims, Objectives and Background:

Glioblastoma multiforme (GBM) is the most common and aggressive malignant neoplasm of the CNS with poor survival rate. Due to limited success in currently available treatment modalities, new pathways are being explored which can arrest the

development of these tumors. Chemokine signalling pathways have been implicated previously in several cancers. They are involved in myriad of biological processes affecting different aspects of cancer and it is increasingly being unravelled that these could directly influence the tumour growth by activating pathways related to cell survival and cell proliferation or indirectly by promoting angiogenesis. Understanding their role could pave way for identification of specific chemokine-receptor axis that could be utilized for developing a targeted therapy to arrest the growth and development of these tumours.

This is planned to elucidate the differential gene expression of chemokines and their receptors in low grade and high grade glioma (GBM) with assessment of therapeutic potential of key target genes.

Work done during the year:

Gene expression study of entire panel of chemokines and their receptors had been under taken in low grade astrocytoma (diffuse astrocytoma DA) (n=10), high grade astrocytoma (gliomablastoma multifome, GBM) (n=10) and two normal post mortem brain tissues as control using RT² Profiler PCR array kit (Qiagen). Data was analysed using Qiagen's Data Analysis Centre. A dramatic increase in chemokines and receptor genes was evident in volcano plot of GBM in comparison to DA (Figure 1). We observed that 7 Genes were commonly up-regulated in both GBM and DA, while 20 were differentially expressed in GBM and only 1 was differentially expressed in DA. Among the down-regulated genes, 8 were commonly down-regulated in both GBM and DA, 4 were differentially up-regulated in GBM and only 2 were up-regulated differentially in DA. Only 1 gene, CXCL8/IL8 was up-regulated in GBM while it was down-regulated in DA (Figure 2)

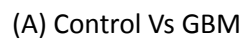


Fig. 1: (A) Volcano plot for GBM (B) Volcano plot for DA

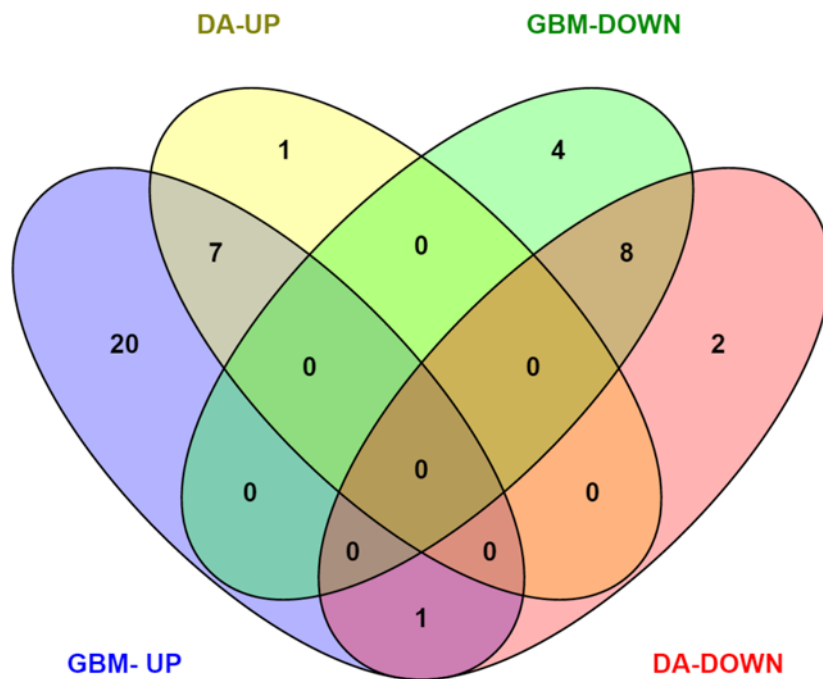


Fig. 2: Venn diagram showing differentially expressed up regulated and down regulated genes in between GBM and DA. DA-UP (up regulated genes in Diffused Astrocytoma), GBM-UP (up regulated genes in GBM), DA-DOWN (down regulated genes in Diffused astrocytoma), GBM-DOWN (down regulated genes in GBM).



INFECTIOUS DISEASES

LEISHMANIASIS

1. Mechanism of resistance to miltefosine in *Leishmania donovani*

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

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

Duration : 2012-16

Aims, Objectives and Background:

Miltefosine has been introduced in 2002 in Indian subcontinent for visceral leishmaniasis (VL) elimination. Long half-life of miltefosine 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 *Leishmania donovani* has become the top priority to rescue the efficacy and longevity of this drug.

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Work done during the year:

In the year under report we validated the selected genes from microarray results in clinical isolates as well as in lab generated miltefosine resistant *L. donovani* (LdM30) parasites. We also evaluated *in vitro* susceptibility of transfected parasites LdLip++ towards sodium antimony gluconate (SAG) and amphotericin B (AMB).

Validation of differentially modulated genes in clinical isolates of *L. donovani* and in lab generated miltefosine resistant (LdM30) parasites:

Genes which were preferentially modulated in relapse case isolates as well as having biological significance like transporters, involved in metabolic processes, antioxidant defense mechanism, cellular component and several hypothetical genes (proteins with unknown function) were chosen for real time validation to validate the microarray results. Q-RT-PCR was performed in clinical isolates of *L. donovani* comprising pretreatment group isolates (n=5) and isolates from relapse cases (n=5). We also analysed gene expression for the selected genes into LdM30 parasites. Results obtained by Q-RT-PCR were consistent with the microarray data for all the genes confirming the differential gene expression between pretreatment group isolates and isolates from the relapse cases (Fig1 and 2). Expression pattern of selected genes in LdM30 parasites (Fig 3 and 4) were found similar as in clinical isolates.

Gene expression analysis in clinical isolates:

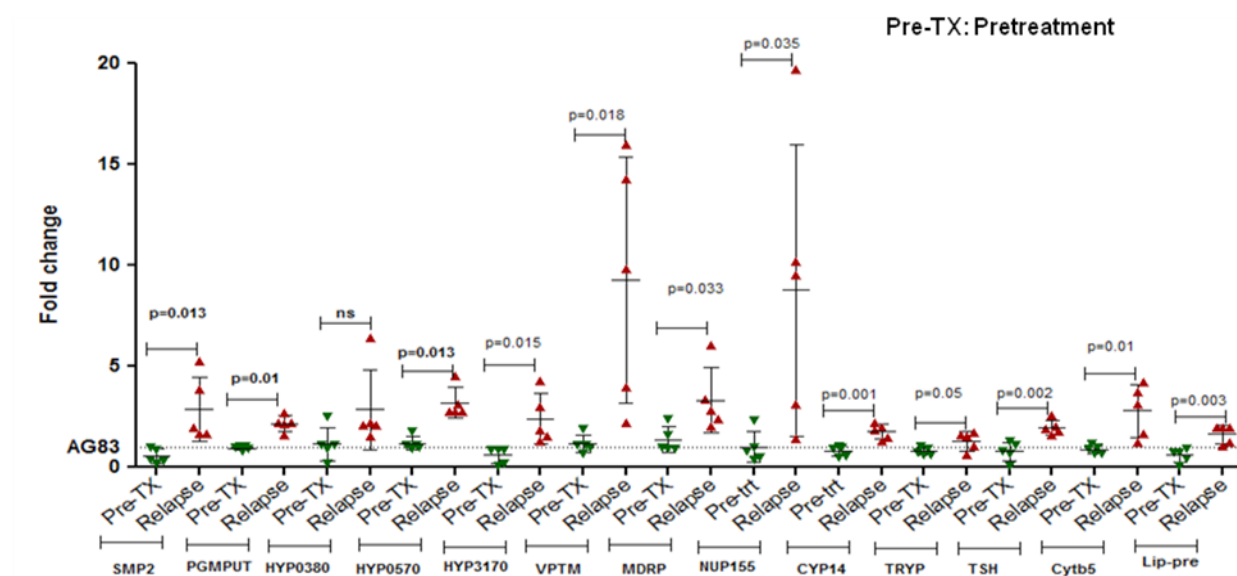


Fig. 1: Real time Q-PCR analysis of genes upregulated in *L. donovani* clinical isolates from relapse cases (n=5) and pretreatment group (n=5). Values are mean \pm SD of two independent experiments each in triplicate. Alpha Tubulin and GAPDH used as endogenous control, LdAG83 is the reference strain.

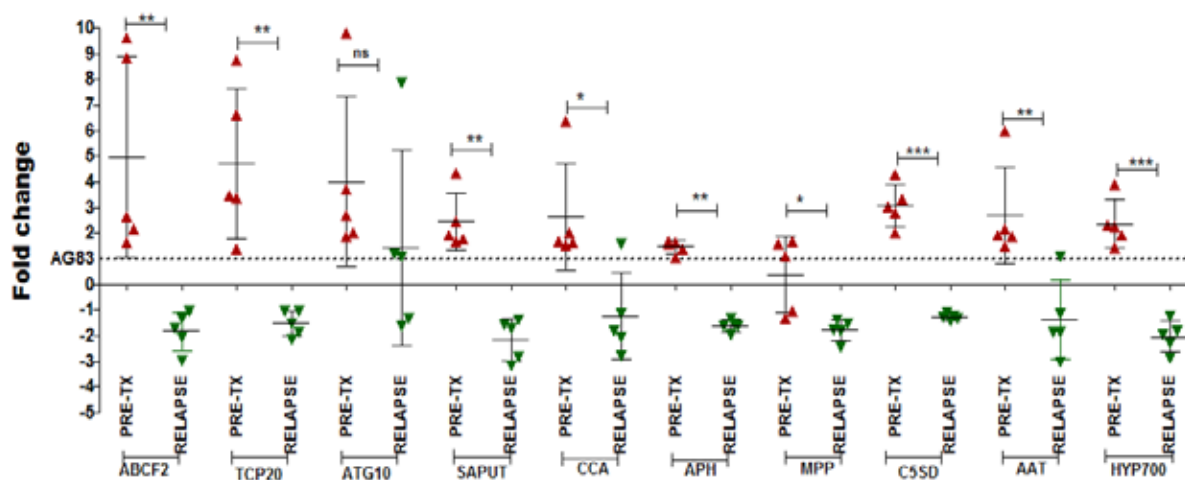


Fig. 2: Real time Q-PCR analysis of downregulated genes in *L. donovani* pretreatment group (n=5) and relapse group (n=5) isolates. Values are mean \pm SD of two independent experiments each in triplicate. Alpha Tubulin and GAPDH used as endogenous control, LdAG83 is the reference strain.

Gene expression analysis in LdM30 isolate:

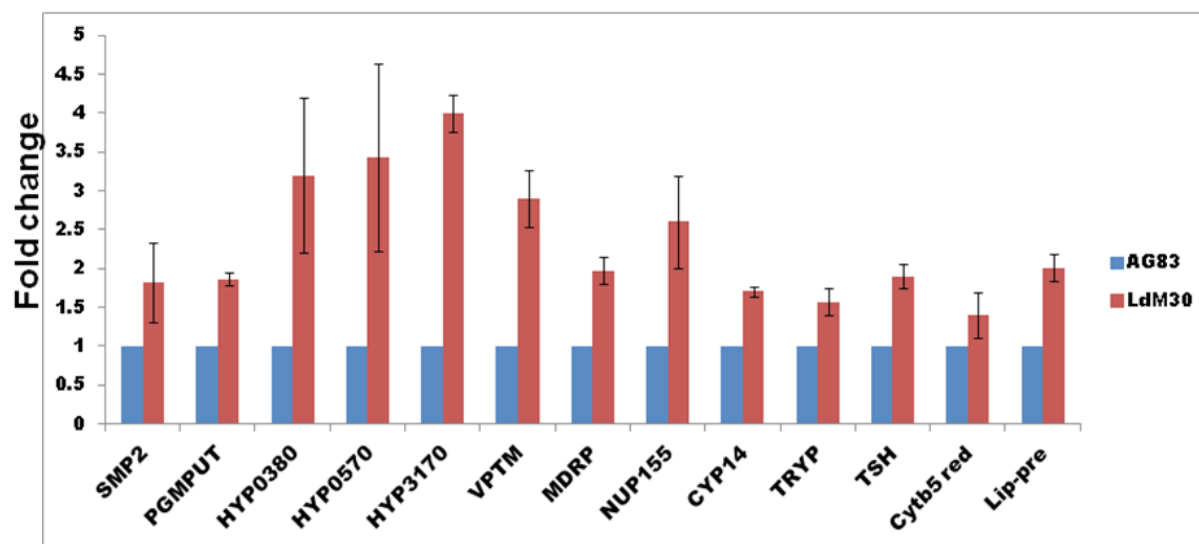


Fig. 3: Q-PCR analysis of selected upregulated genes in LdM30 parasites. LdAG83 used as reference strain. Alpha Tubulin and GAPDH were used as endogenous control. Values are mean \pm SD of two independent experiments each in triplicate.

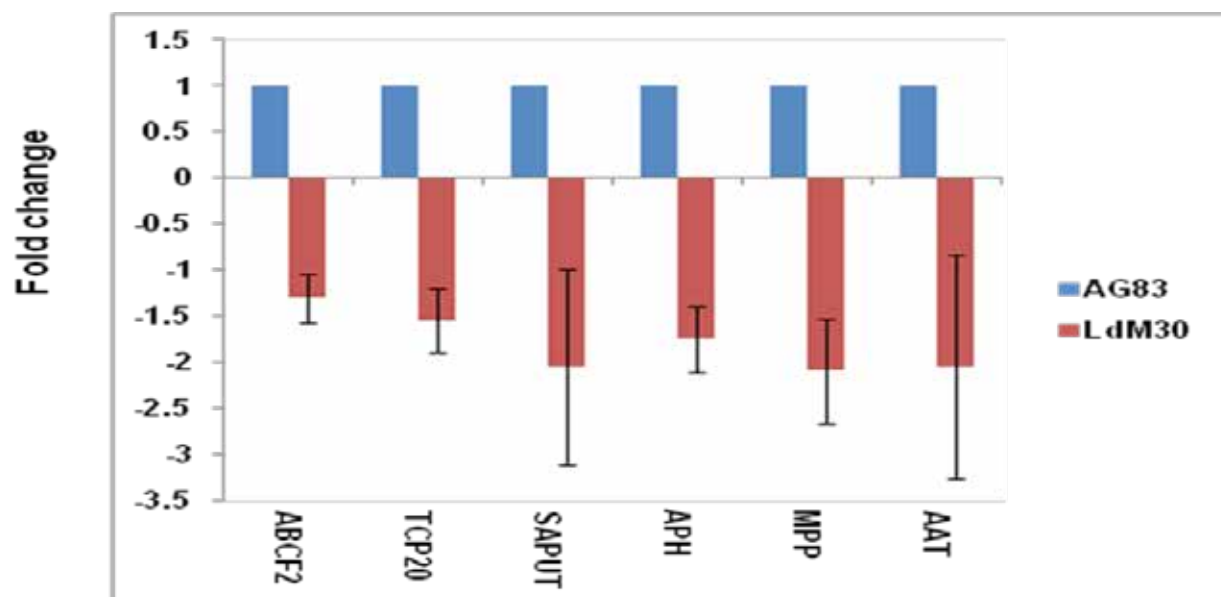


Fig 4: Expression analysis of selected downregulated genes in LdM30 parasites. Values are mean \pm SD of two independent experiments each in triplicate.

Susceptibility of LdLip⁺⁺ towards SAG and AMB:

In our previous year report we have shown that there is significant decrease in susceptibility towards miltefosine in transfectant parasites overexpressing lipase precursor like protein (LdLip⁺⁺). LdLip⁺⁺ parasites showed 3 fold decrease in miltefosine susceptibility both at promastigote and intracellular amastigote level when compared with LdWT and vector control LdNeo. We further evaluated susceptibility pattern of the LdLip⁺⁺ parasites towards other antileishmanial drugs like sodium antimony gluconate (SAG) and amphotericin B (AMB). We found there is no significant change in susceptibility pattern towards SAG and AMB in LdLip⁺⁺ parasites. The 50% inhibitory concentration (IC₅₀) of LdLip⁺⁺ was comparable to LdWT and LdNeo (vector control) parasites both at promastigote and at intracellular amastigote level in case of AMB and at amastigote level in case of SAG. (Table1)

Table1:

Isolates	MIL IC ₅₀ ±SD µM		AMB IC ₅₀ ±SD µM		SAG IC ₅₀ ±SD µg/ml
	Promastigote	Amastigote	Promastigote	Amastigote	Amastigote
LdWT	1.26± 0.4	2.66±0.67	0.78± 0.13	0.44±0.03	4.83±0.42
LdLip⁺⁺	3.9±0.68	9.1±0.60	0.89± 0.11	0.49±0.20	6.22±0.46
LdNeo	1.49±0.2	3.9±0.45	0.76± 0.16	0.42±0.16	5.33±0.57

Future work:

Studies on lipase activity, tolerance to reactive oxygen species (ROS) and miltefosine uptake will be carried out for LdLip⁺⁺ parasites. These parameters will also be analyzed in clinical isolates.

2. Studies on mechanism of resistance towards paromomycin in *Leishmania donovani* parasites

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

Duration : 2013–17

Aims, Objectives and Background:

Paromomycin (PMM) has recently been introduced for the treatment of VL in India as a monotherapy and in combination therapy. It is vital to understand the mechanisms of PMM resistance to increase the life span of this drug. Potential role of ATP binding cassette (ABC) transporters has already been established in drug resistance in *Leishmania*. We have reported earlier that there was increase in expression of ABC transporters in PMM resistant (PMM-R) isolates. In our previous report, we determined the susceptibility towards paromomycin in the three PMM-R isolates in presence of inhibitors, verapamil and amlodipine to ABC transporters, MDR1 and ABCG2 respectively, at promastigote stage and showed that there was increase in susceptibility towards paromomycin in presence of inhibitors to ABC transporters. In

continuation to that, during the period under study we analyzed the paromomycin susceptibility of the three PMM-R isolates in presence of inhibitor to ABC transporters at intracellular amastigote stage.

Work done during the year:

PMM susceptibility in presence of inhibitors to ABC transporters at intracellular amastigote stage:

We determined susceptibility of the three lab generated PMM resistant and their corresponding wild type isolates towards paromomycin in presence of inhibitors (Verapamil and Amlodipine) to ABC transporters at intracellular amastigote stage in order to further validate the role of ABC transporters in paromomycin resistance. A significant increase of up to 1.9 fold in presence of verapamil and >2 to 6 fold in presence of amlodipine, in susceptibility towards paromomycin was observed in PMM R strains. However, their corresponding wild type parasites did not show any significant difference in PMM susceptibility in presence or absence of inhibitors (Fig. 5). The data confirmed the role of ABC transporters in PMM resistance in *L.donovani*.

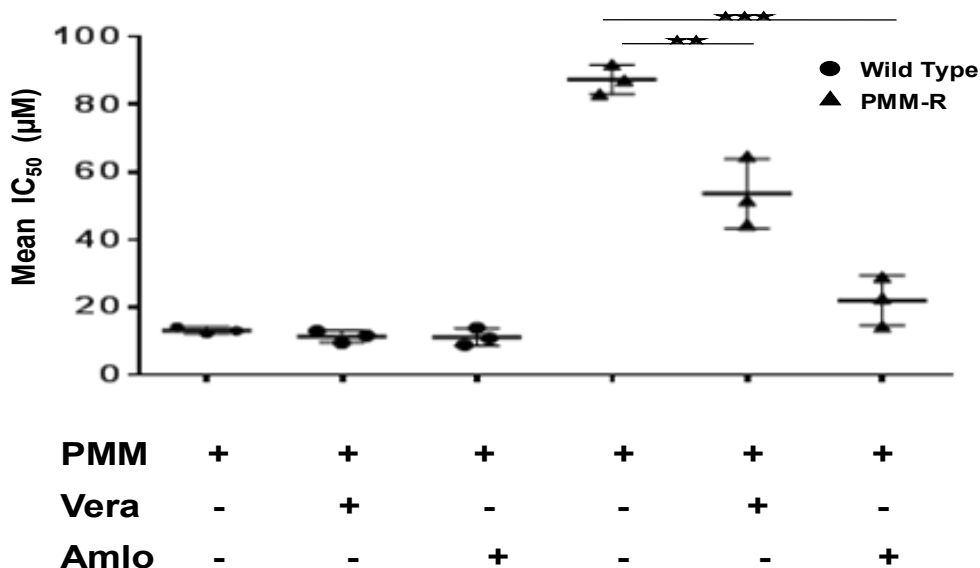


Fig 5: susceptibility of PMM-R isolates in presence of verapamil and amlodipin. *In vitro* susceptibility towards paromomycin in presence of verapamil (Vera) and amlodipine (Amlo) in three different PMM-R and WT isolates at amastigote stage. Each individual value represents mean fold change in IC₅₀ ± SD of the results from two separate assays. (** P value range 0.001-0.01, *** P value range 0.0001-0.001)

Future Work:

Comparative transcriptomic profiling of PMM resistant *L. donovani* parasites using genomic microarray technology will be carried out to identify genes/pathways showing modulated expression in PMM resistance. Selected genes will be taken up for functional characterization.

3. Analysis of clinical efficacy of oral miltefosine in treatment of post kala azar dermal leishmaniasis (PKDL) in India

Scientific staff	: Dr. Poonam Salotra, Ruchi Singh, Kumar Avishek , Aditya Verma , Deepak Kumar Deep , Sandeep Verma
In collaboration with	: Dr. V Ramesh
Duration	: 2014-15

Aims, Objectives and Background:

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Post kala-azar dermal leishmaniasis (PKDL) is a dermatosis that usually develops as a sequelae of visceral leishmaniasis (VL) or kala-azar (KA). Miltefosine (MIL) is the only orally effective antileishmanial drug, which was first approved for VL treatment in India in 2002. Standard treatment of PKDL remains unsatisfactory and increasing resistance to antimonials has paved the way for the oral drug miltefosine for PKDL treatment. However, recent studies show a significant decline in the final cure rate of VL after MIL treatment in the Indian subcontinent. This study analyses the efficacy of miltefosine in the treatment of post kala-azar dermal leishmaniasis (PKDL) patients recruited over a period of 5 years with 18 months of follow-up.

Work done during the year:

Clinical results after MIL treatment

We have evaluated response of MIL treatment in 86 cases of PKDL (females n=22 and males n=64), that reported to Department of Dermatology, Safdarjung Hospital (SJH),

over last 5 years. Confirmed PKDL cases were prescribed treatment with oral MIL, 50 mg twice daily for 3 months ($n=56$) and 2.5mg/kg/day for 3 months in children ($n=4$) referred as regimen I or 50 mg thrice daily for 2 months ($n=26$) for adults referred as regimen II. Out of 86 patients who were recruited for MIL treatment, 73 (57 in Regimen I and 16 in Regimen II) completed the treatment and achieved cure. Twelve patients failed to report regularly and 1 patient was unresponsive to MIL; these 13 patients were not included for further analysis (Fig 6).

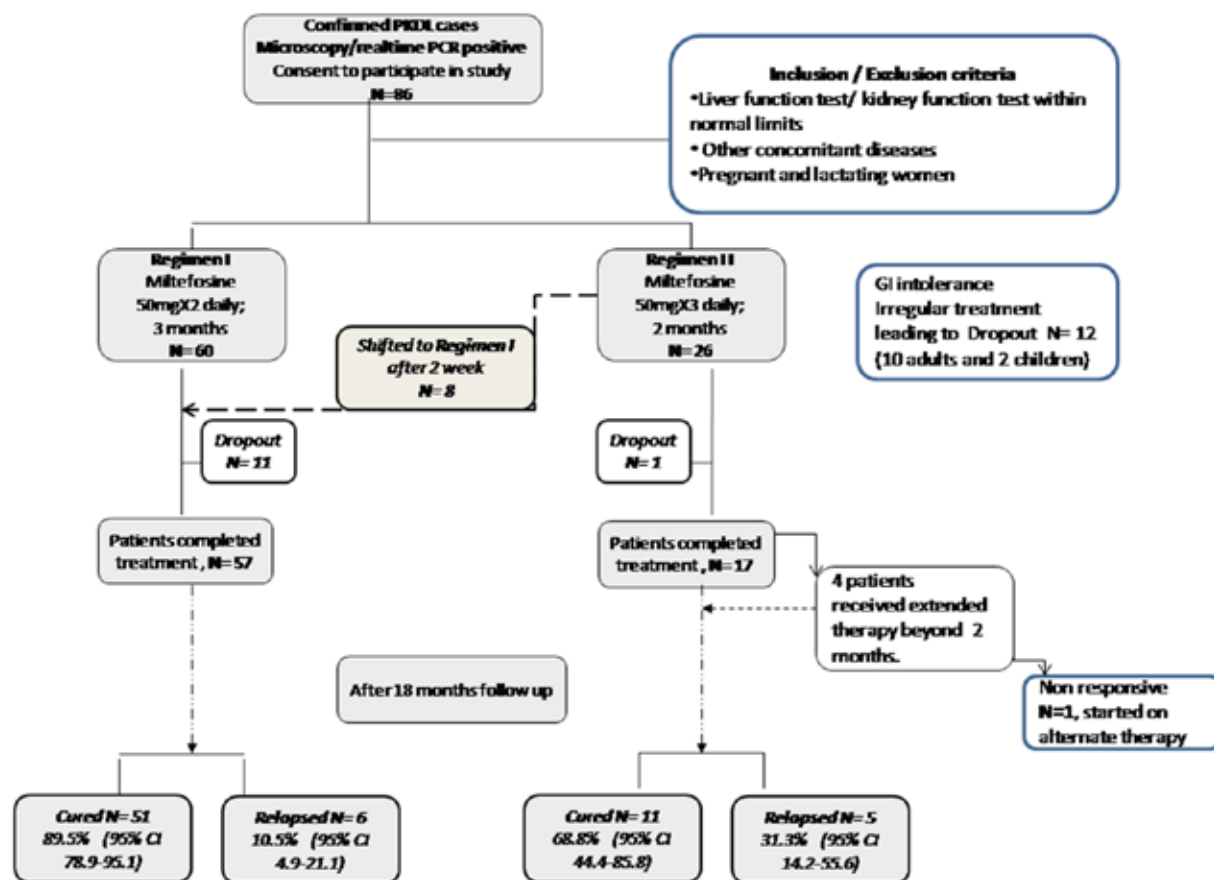


Fig 6: Recruitment, treatment and follow-up of PKDL patients.

At the end of the treatment, a marked improvement was noticed in all the 73 patients. Initial cure was achieved in seventy patients (57 from regimen I and 13 from regimen II). The remaining 3 patients with extensive lesions were cured only after extended treatment beyond 2 months at 150mg/day. The cure rate for regimen I was 89.5% (95%CI = 78.9 - 95.1) and for regimen II was 68.8% (95%CI = 44.4 - 85.8%).

In the 12 month follow up period, 3 of the 73 patients showed signs of recurrence of the disease, one at 5 months and another two at 8 months, while the remaining 70 patients completed 12 months follow up without signs of relapse. By the end of 18 months follow-up, yet another 8 patients showed signs of relapse. The relapse rate at 12 month follow up was 4.1% (3/11), while it increased to 15% (8/11) at 18 months follow up. A total of 5/16 (31.3%; 95% CI 14.2-55.6) patients relapsed after treatment in regimen II compared to 6/57 (10.5%; 95% CI 4.9-21.1) patients in regimen I. The relapse rates in the two regimens were significantly different ($p=0.0406$), indicating better efficacy of regimen I with cure rate of 89.5% (95% CI 78.9-95.1). The cases that relapsed were eventually treated with 11 AmB (1500mg) or Ambisome (30mg/kg body wt). Importantly, there were no further relapses at 24 months of follow-up.

Parasite load in final cured and relapse cases

Parasite load at the pre- and post- treatment stages and at the time of relapse was determined by Q-PCR. At the pre-treatment stage, parasite load was found to be significantly higher ($P<0.005$) in the cases that eventually relapsed ($n=11$, mean=11,842 parasite load/ μ l slit aspirate) compared to those that attained final cure ($n=62$, mean=2,302 parasite load/ μ l slit aspirate) (Fig. 7). For the assessment of cure, parasite load was determined in a few samples ($n=30$) at one month post treatment. No detectable parasites were found in slit aspirate in majority of the cases (26/30); residual parasite (< 10 parasite load/ μ l slit aspirate) was seen in 2 cases which relapsed and in another 2 cases which did not relapse during 18 months follow up, suggesting that the presence of residual parasite at one month post treatment may not be predictive of relapse.

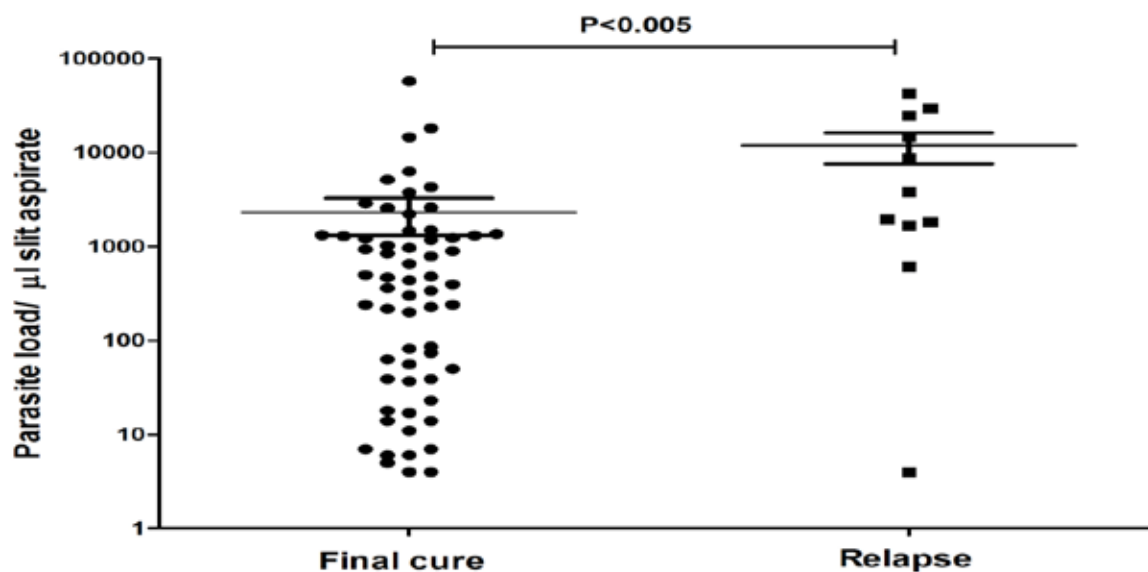


Fig 7: Scatter plot showing parasite load at the pre-treatment stage in the cases that eventually relapsed vs those that remained cured. Parasite load was determined by Q-PCR in slit aspirate sample at the time of diagnosis of PKDL and expressed as the number of *Leishmania* parasite/µl slit aspirate. P value was calculated using Mann-Whitney test. Horizontal bars indicate mean± SEM.

In vitro miltefosine susceptibility in relapse cases

MIL susceptibility was determined both at promastigote and intracellular amastigote stage for six cases that relapsed, including two double relapses, and compared with that of the pre-treatment isolates. Pre-treatment isolates showed a mean $IC_{50} = 4.07 \pm 1.36 \mu M$ (range $2.27 \pm 0.01 \mu M$ to $6.37 \pm 1.44 \mu M$) whereas isolates from relapse cases showed significantly ($P < 0.005$) reduced sensitivity (mean $IC_{50} = 8.53 \pm 0.98$, range $6.76 \pm 1.77 \mu M$ to $9.47 \pm 1.13 \mu M$) (Fig. 8A). Similarly at intracellular amastigote stage, the mean IC_{50} of pre-treatment isolates was $7.85 \pm 1.66 \mu M$ (range $5.65 \pm 0.7 \mu M$ to $9.6 \pm 0.89 \mu M$) which was significantly lower ($P < 0.005$) than that of isolates from cases that relapsed (mean $IC_{50} = 16.99 \pm 2.0$, range $13.26 \pm 0.89 \mu M$ to $18.5 \pm 1.1 \mu M$) (Fig. 8B). We observed increasing tendency in IC_{50} of isolates from double relapse cases ($18.35 \pm 0.13 \mu M$) as compared to the isolates after single relapse cases ($14.98 \pm 2.43 \mu M$) although this difference was not significant ($P = 0.18$).

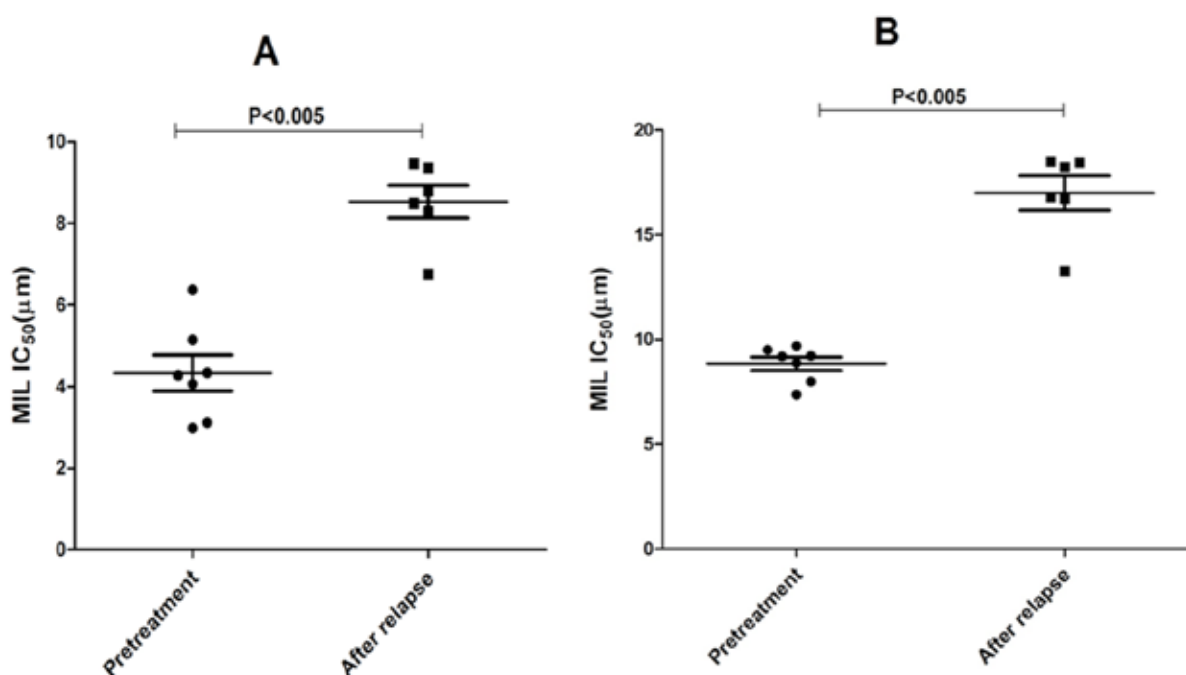


Fig 8: *In vitro* MIL susceptibility of parasite isolates from cured (n=7) and relapsed (n=6) PKDL patients. MIL susceptibility at (A) promastigote stage (B) amastigote stage. Each individual value represents mean IC₅₀ ± SD of the results from two separate assays. P value was calculated using Mann-Whitney test. Horizontal bars indicate mean ± SEM.

Outcome

In the present study, we found that the relapse rate in PKDL following miltefosine treatment has increased substantially. We also found that high parasite load at the pre-treatment stage in the PKDL cases that eventually relapsed as compared to the cases that did not, indicating that the patients with higher initial parasitic burden are at a higher risk of relapse. Further, parasites isolated from the patients after relapse displayed > 2 fold higher tolerance to MIL compared to isolates from pre-treatment stage, both at promastigote as well as amastigote stages. These MIL tolerant parasites observed here in relapse cases are a cause of concern as they may serve as a reservoir for circulation of drug tolerant parasites which may eventually give rise to drug resistant strains in VL. This study suggests that the efficacy of monotherapy with miltefosine is declining, indicating the need of introducing alternate drugs/ combination therapy with miltefosine.

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

Duration : 2011-2015

Aims, Objectives and Background:

Leishmania parasite has digenetic life cycle, flagellated promastigote form is found in vector sand fly and non-flagellated amastigote form in the human host. Amastigote-specific genes are likely to play central roles in survival of *Leishmania* parasite in the mammalian host. Based on our previous studies, we have selected a gene A1 which is highly expressed at the amastigote stage of the parasite. The aim of this study is to characterize A1 gene and evaluate its effect on the growth and survival of the parasite by over-expression and gene deletion. In the last year report we had shown that over-expression of A1 gene did not alter the growth and phenotype of the parasite. In the year under report, we have demonstrated up-regulated expression of A1 protein at amastigote stage and prepared A1 gene single allele knock out parasites.

Work done during the year:

Up regulated expression of A1 in amastigotes at protein level

Previous studies carried out at RNA level by northern blot analysis have shown up-regulated expression of A1 at amastigote stage compared to the promastigote. In the present study, we have compared the expression of A1 at protein level at amastigote and promastigote stages by western blot analysis. The data showed that the expression of A1 is up regulated at the protein level by approx 3 fold at amastigote stage compared to the promastigote (Fig. 9).

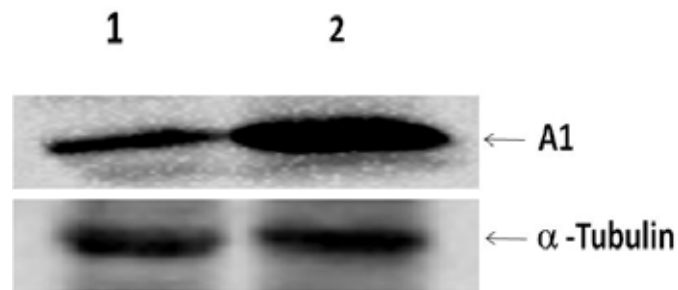


Fig. 9: Western blot showing the up regulated expression of A1 at amastigote stage. Membrane was probed with anti A1 and rabbit IgG conjugated with HRP was used as secondary antibody and membrane was developed by using ECL. **Lane 1:** Lysate of parasite at promastigote stage. **Lane 2:** Lysate of parasite at amastigote stage. α - tubulin was used as an endogenous control.

Generation of A1 gene single allele knock out *Leishmania* parasite (*LdA1*^{+/-})

For the deletion of A1 gene homologous recombination method was used (Fig. 10). In the previous year report, we had shown that the knock-out construct with 5' and 3' flanking regions (5FLK and 3FLK) of A1 and neomycin (NEO) antibiotic resistance gene was generated (5FLK/NEO/3FLK). Now this construct was used to transfect the *Leishmania* parasite for deletion of A1 gene. Transfected parasites were selected under NEO pressure.

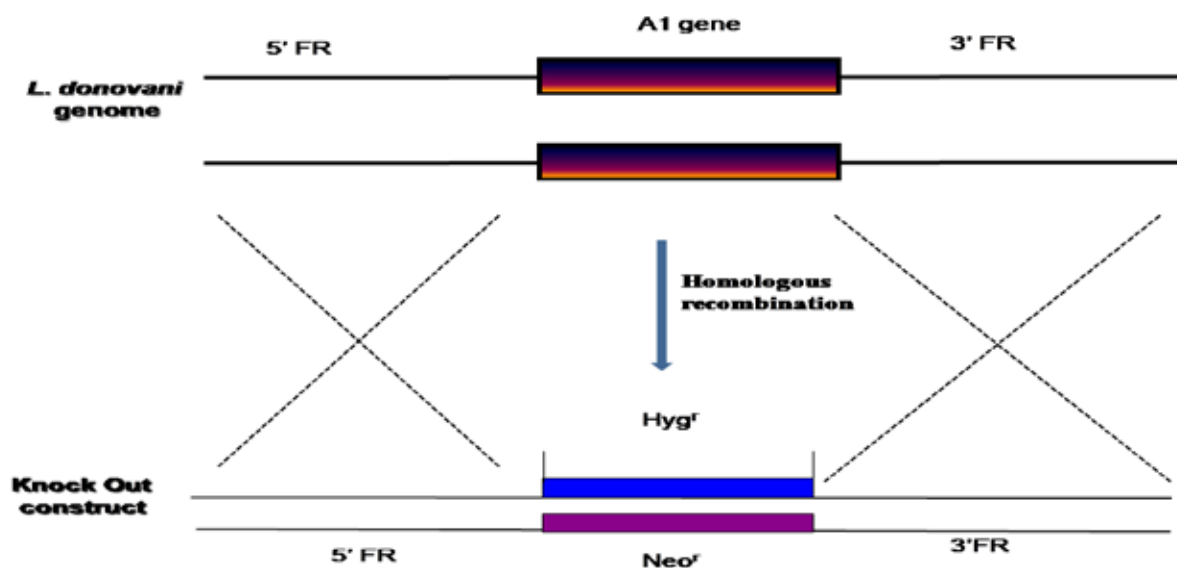


Fig. 10: Diagram showing design and use of constructs for A1 gene disruption in the *L. donovani* genome

Once the transfectant started growing in the antibiotic pressure, genomic DNA was isolated and insertion of knock-out construct to the *Leishmania* genome was confirmed by PCR using primers of NEO. A desired fragment of ~ 500bp was amplified by PCR (Fig.11).

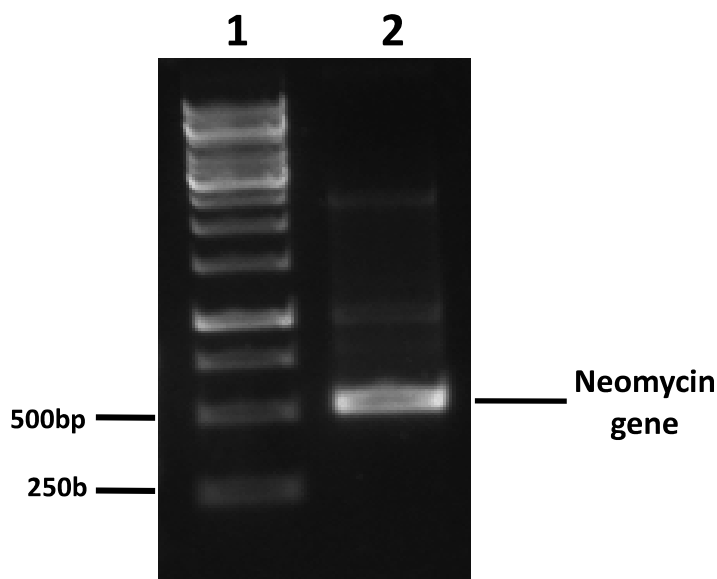


Fig.11: PCR amplified product
Lane1: 1kb ladder
Lane2: 500bp neomycin gene

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Further integration of knock-out construct at the right place was confirmed by sequencing as well as by PCR using forward primer for the gene present just before A1 and reverse primer for the NEO gene of knock-out construct. An expected fragment of ~ 1.4 kb amplified after PCR, confirmed integration of knock out construct at the right place (Fig. 12). Thus generation of A1 gene single allele knock out *Leishmania* parasite (LdA1+/-) was confirmed.

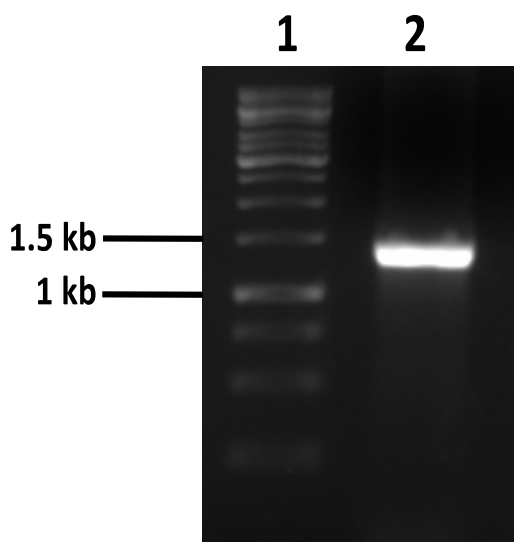


Fig.12: PCRconfirmation of integration of knock-out construct in place of A1
Lane1: 1kb ladder
Lane2: 1.4kb amplified PCR product

Construction of DNA for the deletion of 2nd allele of A1 gene

Knock-out construct with HYG antibiotic resistance gene was generated for the deletion of 2nd allele of A1 gene. Plasmid with 1st allele knock-out construct (pTOPO-5FLK/NEO/3FLK) and another plasmid having HYG gene (pTOPO-HYG) was digested with *SpeI* and *SalI* to release the NEO (Fig. 13) and HYG gene (Fig.14) respectively. HYG insert was then subcloned into the pTOPO-5FLK/NEO/3FLK plasmid in place of NEO. Positive clones (pTOPO-5FLK/HYG/3FLK) were confirmed by PCR as well as by restriction digestion.

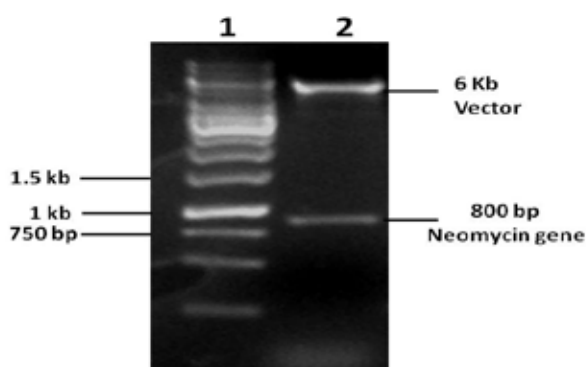


Fig. 13: *SpeI* and *SalI* digestion of Plasmid with 1st allele knock-out construct
Lane1: 1kb ladder
Lane2: 6kb vector back bone and 800bp fall out of neomycin gene

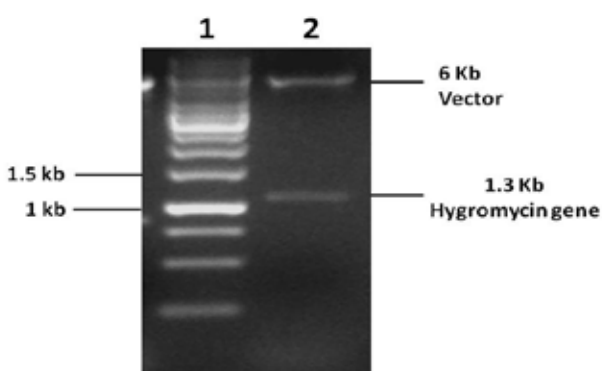


Fig. 14: *SpeI* and *SalI* digestion of Plasmid with hygromycin gene
Lane1: 1kb ladder
Lane2: 6kb vector back bone and 1.3kb fall out of neomycin gene

Finally the clone (pTOPO-5FLK/HYG/3FLK) was digested with *SWaI* and *KpnI* restriction enzymes to get 2nd allele knock-out construct (2.5 kb) having 5' and 3' flanking regions of A1 and HYG antibiotic resistance gene (5FLK/HYG/3FLK) (Fig.15). This construct is now being used for transfection to delete 2nd allele of A1.

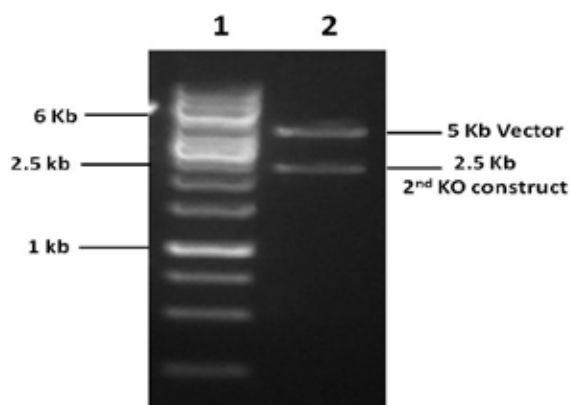


Fig. 15: *SwaI* and *KpnI* digestion of 2nd allele knock-out clone
Lane1: 1kb ladder
Lane2: 5kb vector back bone and 2.5 kb fall out of knock out construct

Future plan:

Double knock out mutants of A1 (*LdA1*^{-/-}) will be generated by transfection using the construct (5FLK/HYG/3FLK) and further analysis will be carried out to evaluate the growth behaviour and phenotype of the *LdA1*^{-/-} parasite.

5. Ubiquitin related modifier1: A post translational modification machinery in *Leishmania donovani*

Scientific staff : Dr. Poonam Salotra, Ms Vanila Sharma

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

Duration : 2012-15

Aims, Objectives and Background:

Protein modification by ubiquitin (Ub) and ubiquitin-like molecules (Ubls) is a diverse biological process that regulates the activity of the target proteins. Systematic studies of Ubls in trypanosomatids like *Leishmania*, the causative organism of potentially fatal visceral leishmaniasis, would yield a better understanding of the disease pathogenesis and identify novel therapeutic targets. Earlier our lab has demonstrated the existence of ubiquitin fold modifier-1 (Ufm1) and its conjugation pathway in *L. donovani*, which was the first report in any Trypanosomatid parasite.

Last year we had reported the cloning, expression, purification, immunolocalisation of LdUrm1/LdUba4 proteins and identified the interacting target proteins of LdUrm1 by mass spectrometry.

Work done during the year:

Episomal expression of LdUrm1^{WT} and LdUrm1^{ΔG} in *Leishmania*

During the year under report we overexpressed LdUrm1 and its non-conjugatable form to understand the significance of LdUrm1 for the growth and survival of *L. donovani*.

To achieve the above objective we generated three different *L. donovani* mutants: (i) parasite that over- expressed LdUrm1 viz LdUrm1⁺⁺; (ii) parasite that expressed non-conjugatable form of LdUrm1 viz LdUrm1^{ΔG} (iii) and control strain transfected with pKSNeo empty vector (LdUrm1^{KS}). Schematic presentation for construction of mutants is depicted in Fig 16A. We amplified LdUrm1 with *SpeI* restriction site and N terminal HA tag using primers; OEUrm1F/R and OEUrm1ΔGF/R for LdUrm1⁺⁺ and LdUrm1^{ΔG} respectively as mentioned in Table 2. The amplicons were subsequently subcloned into *SpeI/SpeI* backbone of *Leishmania* expression vector pKSNeo and *L. donovani* cells were transfected with these constructs. Transfectants were selected for resistance in G418. Overexpression was confirmed on western blots using anti HA antibody (Fig. 16B).

Table 2: Primer sequence used to amplify LdUrm1 and LdUba4 from *L. donovani*

Construct	Primer Name	Primer sequence	Restriction enzyme
LdUrm1 ⁺⁺	OEUrm1F	5' <u>GGactagt</u> TACCCATACGATGTTCCAGATTACGCTA TGCAGATGACGCACAGAAAAATC 3'	<i>SpeI</i>
	OEUrm1R	5' <u>Gactagt</u> GCCGCCGTGCAGAGTAGATACAAACTC' 3'	<i>SpeI</i>
LdUrm1 ^{ΔG}	OEUrm1ΔGF	5' <u>GGactagt</u> TACCCATACGATGTTCCAGATTACGCT ATGCAGATGACGCACAGAAAAATC 3'	<i>SpeI</i>
	OEUrm1ΔGR	5' <u>Gactagt</u> GCCGTGCAGAGTAGATACAAACTC 3'	<i>SpeI</i>
Restriction sites included in the primers are underlined. HA tag is marked bold in the primers designed for transfection studies.			

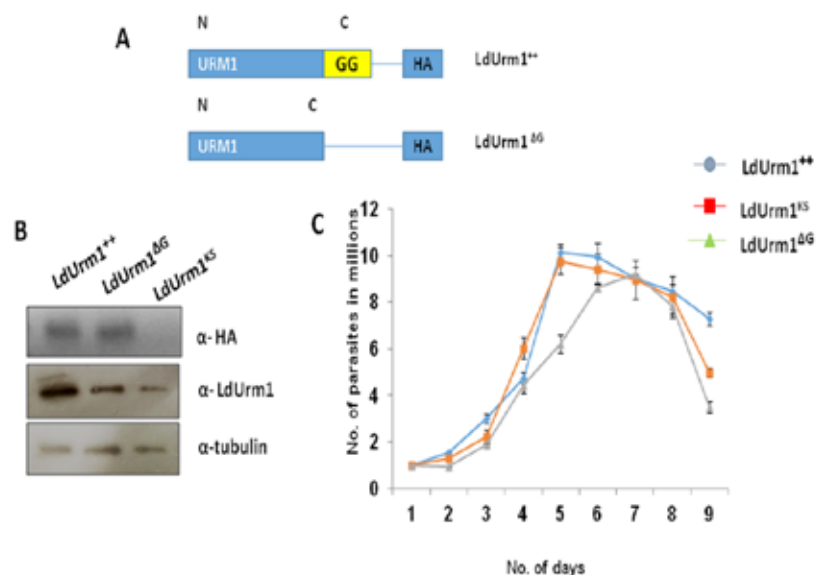


Fig. 16: Characterisation of *L. donovani* cells overexpressing LdUrm1 (LdUrm1⁺⁺) compared to *L. donovani* expressing nonconjugatable LdUrm1, (LdUrm1^{ΔG}). **A.** Schematic presentation for the LdUrm1⁺⁺ and LdUrm1^{ΔG} construct cloned in pKSNeo. **B.** Appearance of LdUrm1⁺⁺ and LdUrm1^{ΔG} on Western blot when probed with α- HA antibody confirming transfection (*upper panel*; Lane 1 and 2). The blot when re-probed with α-LdUrm1 antibody showed elevated expression of LdUrm1 in LdUrm1⁺⁺ (*middle panel*; Lane 1) compared to LdUrm1^{ΔG} (*middle panel*; Lane 2). The amount of protein loaded onto gel was normalized using α-tubulin antibody (*lower panel*). **C.** Growth curve of LdUrm1⁺⁺; LdUrm1^{ΔG} and pKSNeo transfected *L. donovani* cells. Each data point on the curve represents the mean± standard deviation of three separate assays.

LdUrm1^{KS} (mock) and LdUrm1⁺⁺ showed normal and comparable growth, however LdUrm1^{ΔG} showed slower growth (Fig. 16C) confirming negative dominant effect of the mutant protein. Despite reduced growth rate, the culture expressing truncated LdUrm1 (LdUrm1^{ΔG}) survived for normal growth period probably due to the rescuing effect of the endogenous LdUrm1 over LdUrm1^{ΔG}. LdUrm1^{ΔG} also showed aberrant morphological phenotypes in liquid culture, displaying variant sizes, forming cells with shortened length and often remaining attached at one end near flagellar pocket. Hence, LdUrm1^{ΔG} cells displayed heterogeneous behaviour with respect to cell separation indicating that LdUrm1 is essential for the normal cell division in *L. donovani*.

Outcome of the study

The present study is the first to demonstrate *Leishmania* specific ubiquitin related modifier-1 conjugation pathway(LdUrm1- LdUb4) and its target proteins in *Leishmania donovani*. The identification of LdUrm1 mediated protein modification pathways in *Leishmania*, with its distinct subset of diverse substrate proteins identified in this report present themselves as potential drug targets.

CHLAMYDIASIS

Immunopathogenesis of Reactive Arthritis Induced by *Chlamydia Trachomatis*

Scientific Staff : Dr. Sangita Rastogi, Mr. Praveen Kumar

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

Duration : 2010-15

Aims, Objectives and Background:

Sexually transmitted *Chlamydia trachomatis* infection is a widespread public health concern because of its prevalence and potentially devastating consequences, including Reactive Arthritis (ReA). The immunopathogenic mechanisms involved in *C. trachomatis*-induced ReA development are still not clear: an altered immune response has been reported in ReA. The joint inflammation results from a continuous production of pro-inflammatory molecules such as chlamydial hsp60, lipopolysaccharide, altered gene expression in infected host cells and from stimulation of the adaptive immune system which is not sufficient for bacterial clearance. It is likely that cytokines play a critical role in the immunopathogenesis of ReA as a predominant Th1 cytokine profile has been reported.

Circulatory chsp60 is an inflammatory protein which plays protective role during *C. trachomatis* infection, however, it is still unknown how this protection takes place in synergism of cytokine regulation. Furthermore, cytokine imbalance and the interaction between bacteria and HLA B27 might also play major roles in the failure to eliminate the triggering microbial *C. trachomatis* antigen, leading to the disease manifestations and chronicity. An earlier study by Bas and co-workers demonstrated low IFN-gamma concentration in HLA B27-positive patients with *C. trachomatis*-induced ReA and related this to the tendency of these patients to have more severe or chronic arthritis.

The aim of the study was to evaluate the role of inflammatory proteins, viz.: chsp60, hsCRP during the immunopathogenesis of *C. trachomatis* and to elucidate the effect of the genetic factor, viz.: HLA B27 on few Th1/ Th2/ Th17 cytokines in *C. trachomatis*-induced ReA/ uSpA.

Work done during the year:

The present report summarizes the results obtained in a total of 115 arthritic patients (ReA/ uSpA (n- 45), Rheumatoid Arthritis (RA)/ Osteoarthritis (OA) (n- 70). These patients were enrolled under the guidance of the collaborating rheumatologist from Department of Rheumatology & Clinical Immunology, Army hospital (R&R), New Delhi. ESSG (European Spondyloarthropathy Study Group) 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).

Effect of chlamydial heat shock protein 60 (chsp60) on cytokines:

IgG antibodies to chsp60 were found in the serum of 09 uSpA patients. Level of IFN-gamma, IL-4, IL-6, IL-17A cytokines in the serum were compared with the chsp60-negative patients. IFN-gamma was upregulated ($p > 0.05$; not significant) in the chsp60-positive patients, however, no differences were found in IL-4/ IL-6/ IL-17A cytokines levels in both groups. IFN-gamma, IL-4, IL-6, IL-17 cytokines were further compared individually to each other in the chsp60-positive uSpA patients and it was observed that levels of IFN-gamma ($p = 0.04$) and IL-17A ($p = 0.0001$) were increased significantly than IL-6. IL-17 was also found to be significantly higher ($p = 0.01$) than the IFN-gamma level (**Table 1; Fig. 1**).

Table 1: Influence of chlamydial heat shock protein 60 on cytokines in the serum of undifferentiated spondyloarthropathy patients

	chsp60 +ve (n- 09) patients	chsp60 -ve (n- 32) patients	'p' value
IFN-gamma (pg/ ml)	2 - 95 32.5 10.3 - 56.2	2 - 48.1 19.5 11.2 - 29.9	0.23 ^{ns}
IL-4 (pg/ ml)	7.3 - 33 14.17 10.4 - 22.2	3.4 - 14.1 10.5 8.3 - 11.7	0.42 ^{ns}
IL-6 (pg/ ml)	1.7 - 36.2 8.3 6.1 - 11.2	1.5 - 78.9 6.96 3.5 - 21.5	0.77 ^{ns}
IL-17A (pg/ ml)	16.1-160 73.0 50.8 - 114.6	3.07 - 424.9 58 39.2 - 152.3	0.95 ^{ns}

Note: Data shows range (minimum to maximum) followed by median value and inter-quartile range.

Abbreviation: ns – not significant

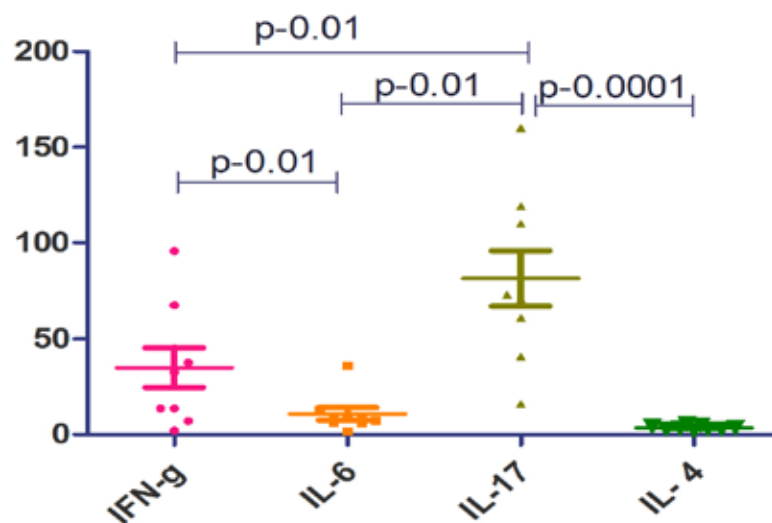


Fig. 1: Serum cytokines in chlamydial heat shock protein 60-positive undifferentiated spondyloarthropathy patients

Effect of HLA B27 gene on cytokines:

A total of 40% (18/45) ReA/ uSpA patients were positive for the HLA B27 gene in the synovial fluid. Among these, 38.8% (7/18) patients were found to be infected with *C. trachomatis*. Comparison was made between pro-inflammatory and anti-inflammatory cytokines and High Sensitive C-Reactive Protein (hsCRP) in the HLA B27-positive (*C. trachomatis*-infected) versus HLA B27-positive (*C. trachomatis*-uninfected) patients. The level of hsCRP was significantly higher ($p = 0.007$) in HLA B27-positive (*C. trachomatis*-infected) patients in comparison to the uninfected patients having HLA B27 gene. Levels of cytokines, viz.: IFN-gamma, IL-4, IL-6, IL-17A were compared between both groups, however, no significant differences were obtained (Table 2).

Table 2: Differential expression of synovial fluid cytokine profile in HLA B27-positive *Chlamydia trachomatis* infected/ uninfected ReA/ uSpA patients

	HLA B27 +ve <i>C.trachomatis</i> infected patients (n- 07)	HLA B27 +ve <i>C.trachomatis</i> uninfected patients (n- 11)	'p' value
IFN-gamma (pg/ ml)	2.3 - 34.3 14.2 10.3 - 24.2	2 - 39.1 19.5 9.2 - 45.9	0.54 ^{ns}
IL-4 (pg/ ml)	6.3 - 32.6 13.17 10.4 - 21.2	2.4 - 13.1 10.1 8.3 - 12.7	0.39 ^{ns}
IL-6 (pg/ ml)	1.4 - 39.2 8.9 5.4 - 11.2	1.5 - 39.9 7.46 3.5 - 19.5	0.9 ^{ns}
IL-17A (pg/ ml)	14.1 - 163 63.0 49.8 - 109.6	3.07 - 233.9 58 39.2 - 152.3	0.2 ^{ns}
hsCRP (µg/ ml)	10 - 73 49 20.6 - 62.3	10.2 - 23.2 14.2 12.2 - 16.2	$p < 0.007$

Note: Data shows range (minimum to maximum) followed by median value and inter-quartile range.

Abbreviation: ns – not significant

Based on the above findings, it was concluded that chsp60 plays protective role during the course of uSpA as evident by the upregulated pro-inflammatory/ protective cytokines, viz.: IFN-gamma and IL-17A. In *C. trachomatis*-infected and HLA B27-positive ReA/ uSpA patients *versus* the uninfected HLA B27-positive patients, no significant differences were observed in cytokine levels.

TUBERCULOSIS

1. Understanding the structural flexibility and functional diversity of PE/PPE protein family of *Mycobacterium tuberculosis*: Identification and role of unstructured/disordered regions in this family using *in-silico* tools

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

In Collaboration with : Dr. Seyed E. Hasnain, IIT Delhi; Dr. A. Srinivasan, AIIMS

Duration : 2012-2018

Aims, Objectives and Background:

The acid rich PE/PPE protein family is exclusive to *Mycobacterium* and abundant particularly in pathogenic strains. This group of proteins is potential source of antigenic variation and has critical roles in pathogenesis. Many PE/PPE proteins are shown to have membrane anchor regions and are surface localized. Few of these PE/PPE proteins are involved in host-pathogen interactions and can also function as immune modulators thus, modifying the host immune response. Members of PE/PPE protein family also have been linked to virulence. The molecular three-dimensional structure of a protein is vital to its function; therefore, understanding protein structure is a very important for assigning and understanding its function.

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Work done during the year:

All the PE/PPE gene family of *Mycobacterium tuberculosis* were selected and included in this study. *In-silico* analyses for disordered region, protein binding sites in disordered region and domain, motif for these proteins were carried out using RONN, ANCHOR and Prosite-ExPasy, respectively.

(1) Prediction of disordered region in PE/PPEs:

RONN analysis was used to predict the percent Disorder in proteins:

The percentage of their disordered profile of the proteins of PE, PPE and PE_PGRS family range between 20-80%.

(2) Disorder and secondary structure: comparison of disorder and secondary structure are shown below,

(a) PE family: PE proteins are the most structured in the whole family

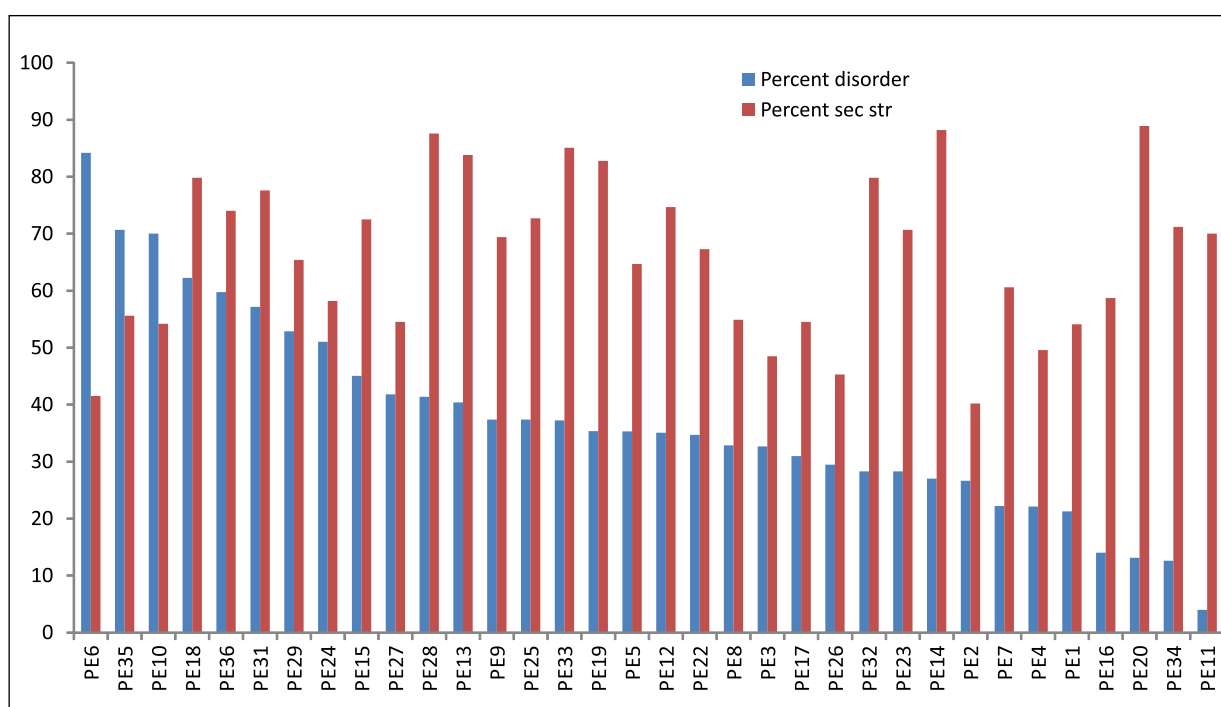


Fig 1a. Vertical lines signify the percent secondary structure and disorder content, horizontal line protein name. Blue bars shows percent disorder content and red bars percent secondary structure in each protein.

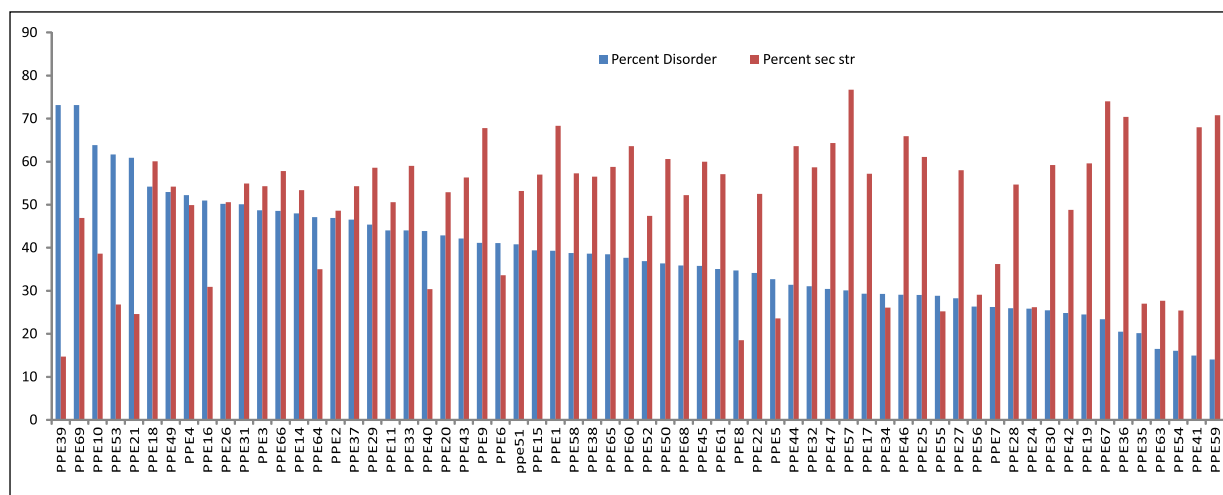
(b) PPE family: is highly enriched with partially disordered proteins

Fig 1b. Vertical lines signify the percent secondary structure and disorder content, horizontal line protein name. Blue bars shows percent disorder content and red bars percent secondary structure in each protein.

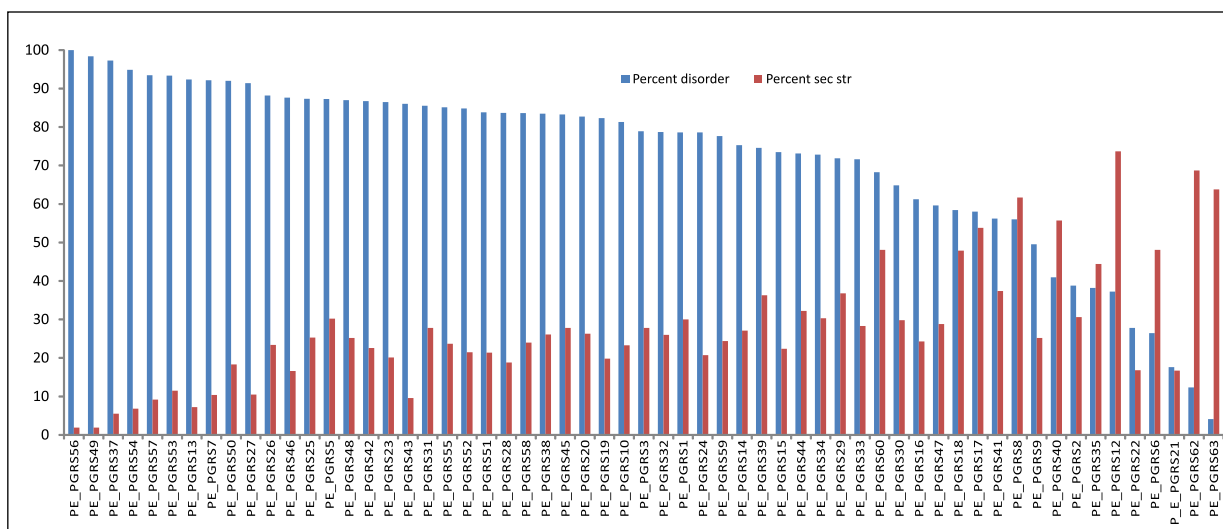
(c) PE_PGRS family: most unstructured proteins are found in this subfamily among whole family.

Fig 1c. Vertical lines signify the percent secondary structure and disorder content, horizontal line protein name. Blue bars shows percent disorder content and red bars percent secondary structure in each protein.

(3) PE PPE family have biased amino acid composition:

The amino acid composition comparison of PE PPE protein family with the structured protein in NCBI revealed high glycine and alanine content, basic amino acid lysine, arginine and cysteine frequency is less than the other proteins in NCBI database.

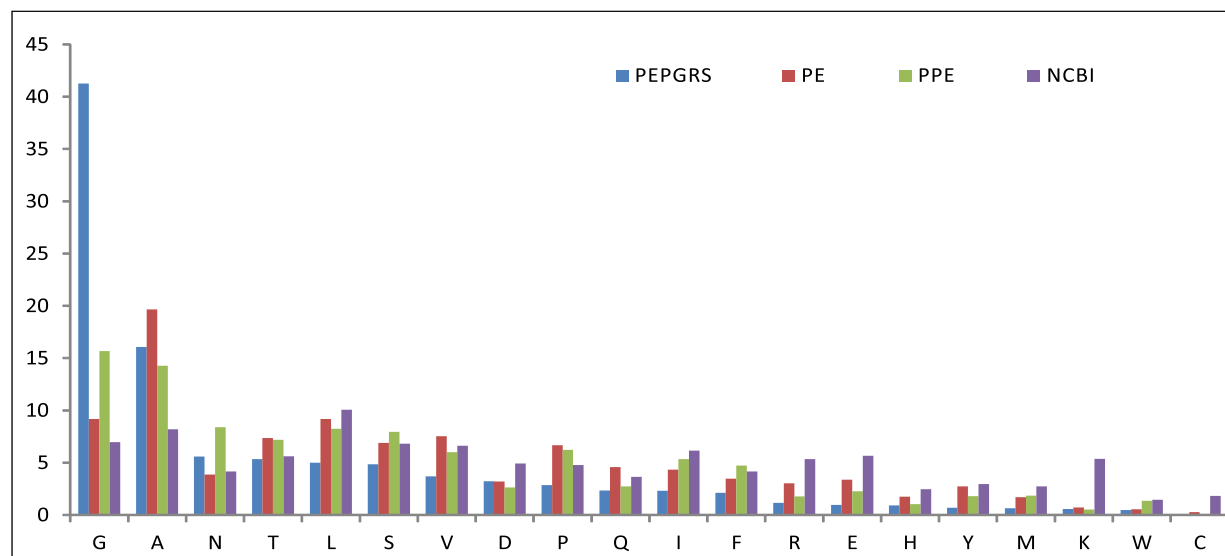
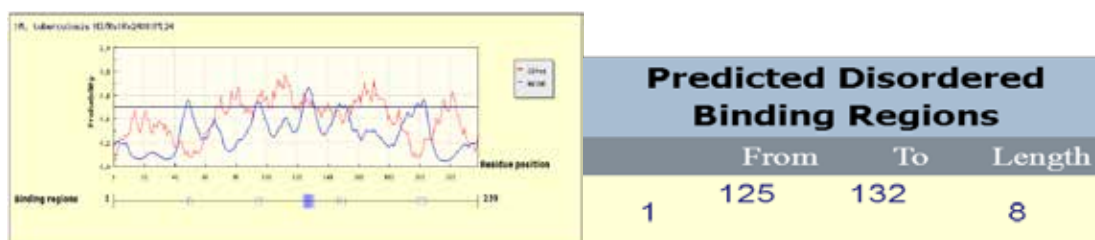


Fig 2. Percent amino acid frequency comparison of PE PPE family with ordered proteins of NCBI database.

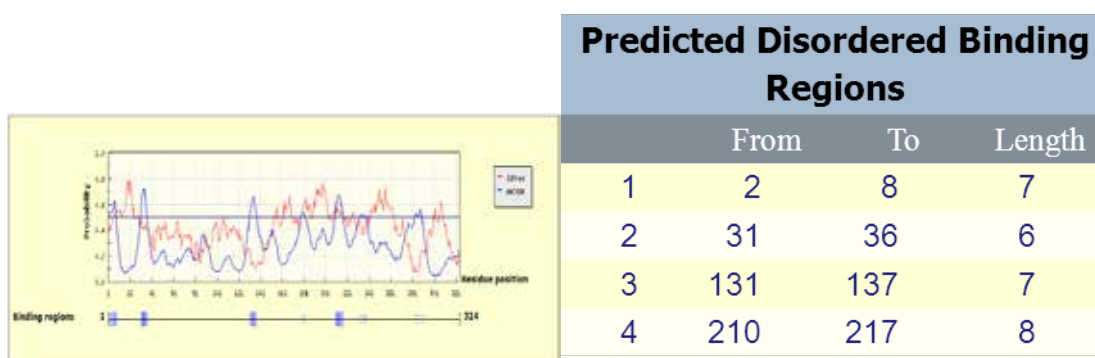
(4) Prediction of protein binding sites in disordered regions:

ANCHOR was used for predicting protein binding sites in disordered regions of PE/PPE protein family. These analyses showed the presence of putative protein binding sites in disordered regions.

Comparative ANCHOR analysis of PE/PPE family of H37Rv (Virulent strain) with that of H37Ra (avirulent strain) revealed loss and gain of protein binding sites in disordered regions. Comparative analysis of PE24 of H37Rv showed gain of some protein binding sites in disordered regions in PE24 of H37Ra strain as is evident from Fig (3).



PE24 (H37Rv)



PE24 (H37Ra)

Fig 3. Blue regions in the figure shows protein binding sites in disordered regions

(5) PE PPE Protein Family is Rich in Low Complexity Regions (LCRS):

Low-complexity regions in protein sequence are regions having biased amino acid composition and little diversity in their amino acid composition. Moreover, LCRs have position dependent functions, centrally located LCRs are mostly involved in transcriptional related functions while as terminal (t-LCRs) are involved in translational and stress-response related functions. Our computational analysis of PE PPE protein family revealed that the family is highly enriched with alanine and glycine rich LCRs.





















Table 2: Showing number of LCRs in PE PPE protein family

Protein family	Alanine rich LCRs		Glycine rich LCRs	
	t-LCRs	C-LCRs	t-LCRs	C-LCRs
PE	26			1
PE_PGRS	57		4	57
PPE	58	11	2	22

t-LCRs- terminal low complexity regions

c-LCRs- central low complexity region

- (6) Motifs and Signature sequences found in PE PPE family: - Pro site analysis revealed presence of different motifs and signature sequences in disordered regions of PPE and PE_PGRS proteins.

Accession number	Domain, motif or Signature type	Description
PS50079	NLS_BP Bipartite nuclear localization signal profile	
PS01076	ACETATE_KINASE_2 Acetate and butyrate kinases family signature 2	
PS00136	SUBTILASE_SER Serine proteases, subtilase family, serine active site	
PS00012	PHOSPHOPANTETHEINE Phosphopantetheine attachment site	
PS00017	ATP_GTP_A ATP/GTP-binding site motif A (P-loop)	
PS51125	NHL NHL repeat profile	
PS00016	RGD Cell attachment sequence	
PS01287	RTC RNA 3'-terminal phosphate cyclase signature	
PS00016	RGD Cell attachment sequence	<div>626 - 628: RGD</div> <div>778 - 780: RGD</div> <div>1636 - 1638: RGD</div> 
PS00227	TUBULIN Tubulin subunits alpha, beta, and gamma signature :	
PS50079	NLS_BP Bipartite nuclear localization signal profile	
PS00583	PFKB_KINASES_1 pfkB family of carbohydrate kinases signature 1	
PS00316	HMG_COA_REDUCTASE_2 Hydroxymethylglutaryl-coenzyme A reductases signature 2	
PS00029	LEUCINE_ZIPPER Leucine zipper pattern	
PS00029	LEUCINE_ZIPPER Leucine zipper pattern	
PS00178	AA_TRNA_LIGASE_I Aminoacyl-transfer RNA synthetases class-I signature	
PS00016	RGD Cell attachment sequence	
PS00016	RGD Cell attachment sequence	
PS00016	RGD Cell attachment sequence	
PS00016	RGD Cell attachment sequence	
PS00626	RCC1_2 Regulator of chromosome condensation (RCC1) signature 2 :	
PS00501	SPASE_I_1 Signal peptidases I serine active site :	
PS00449	ATPASE_A ATP synthase a subunit signature :	
PS00879	ODR_DC_2_2 Orn/DAP/Arg decarboxylases family 2 signature 2	
PS00016	RGD Cell attachment sequence	
PS00016	RGD Cell attachment sequence	

Future Work Plan:

Validation of these *in-silico* results will be performed by characterizing few targets from the above analysis experimentally.

2. *Mycobacterium tuberculosis* Unique Signature Protein Binds DNA Non-Specifically and Protects against Reactive Oxygen Species

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

In collaboration with : Dr. Seyed E. Hasnain, IIT Delhi; Dr. A. Srinivasan, AIIMS

Duration : 2012-2017

Aims, Objectives and Background:

TB is not only caused by *M.tb*, but in a few cases *Mycobacterium bovis* has also been reported to be the causal organism. *Mycobacterium tuberculosis* is a highly successful intracellular pathogen that has evolved by successive genome reduction events. More than 100 mycobacterium species are known, however, only few are pathogenic for humans. Therefore, elucidating the role of unique proteins of pathogenic species will help in understanding the basic biology of pathogenesis of this deadly pathogen. We used an *in-silico* approach, first identifying the unique proteins followed by their characterization using different wet lab experiments.

Work done during the year:

Unique Proteins of *Mycobacterium tuberculosis* (*M.tb*):

Comparative Proteomic analyses of 13 *Mycobacterium* species revealed 25 protein sequences unique to *M.tb* which include, 8 from toxin-anti-toxin category, 9 are hypothetical proteins, 3 as possible prophages, 2 are acid and phagosome regulated proteins and 2 belong to PE_PGRS family of Proteins. Our nucleotide blast of the 25 selected targets nucleotide sequences showed that 19 have their homologs in *M.bovis* BCG.

Two Unique Proteins are Signature to *M.tb.*:

BLASTp of Signature Protein 1 (SP-1) with all in the NCBI revealed SP-1 does not have any significant sequence coverage and similarity with other proteins of non-tuberculosis mycobacterium. Signature Protein-2 (SP-2) shares 100% identity and similarity with homolog in one of the strain of *M.Bovis*. There is no other protein in the database which shares more than 50% identity and 100% coverage with SP-2 signature.

Cloning, Over-Expression and Purification of SP-2:

The open reading frame encoding signature protein-2 of *M.tb* was amplified by PCR from H37Rv genomic DNA using forward and reverse primers. The PCR product was directly cloned in the expression vector pET28a. Poly histidine –tagged recombinant protein was purified from *E.coli* BL21 (DE3) expression strain using Ni-NTA affinity chromatography. SDS-PAGE analysis of purified protein revealed 37kda SP-2 as homogenous protein.

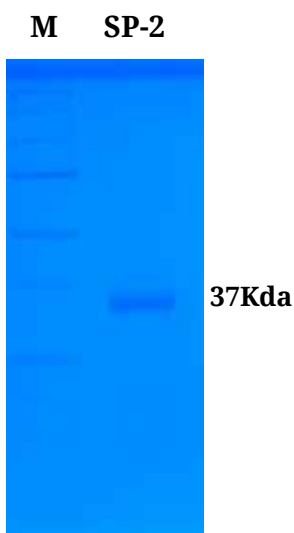


Fig 1: SDS-PAGE analysis of purified Signature Protein-2

Signature Protein-2 Protects DNA against Hydroxyl Radical Damage:

In order to validate anti-oxidant property of Signature Protein-2 (SP-2).A linear 893-bp fragment of DNA was incubated *in-vitro* with H_2O_2 and Fe (II) ($FeSO_4$) in the presence or absence of recombinant *M.tb* SP-2 or a control Protein BSA. The DNA

was completely degraded by $\text{FeSO}_4/\text{H}_2\text{O}_2$ treatment and substantially protected from degradation by pretreatment with recombinant SP-2. In contrast, pretreatment with equivalent concentration of BSA did not result in any protection. Fig (2)

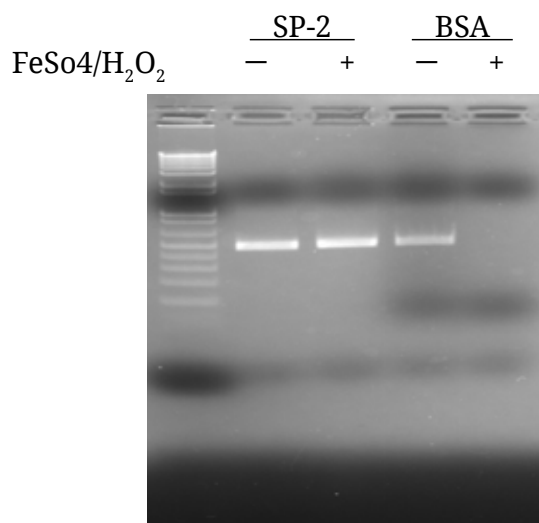


Fig 2: Linear DNA (230ng) was used alone or incubated with equal concentration (4 μg) of SP-2 or BSA for 30 minutes. Samples were treated for 7 minutes with $\text{FeSO}_4/\text{H}_2\text{O}_2$ or left untreated as indicated in Fig (2). Samples were analyzed on ethidium bromide stained 1% agarose gel. Lane 1, DNA ladder; Lane 2, linear DNA + SP-2; Lane 3, Linear DNA + SP-2+ $\text{FeSO}_4/\text{H}_2\text{O}_2$; Lane 4, BSA + Linear DNA; Lane 5, BSA + Linear DNA + $\text{FeSO}_4/\text{H}_2\text{O}_2$.

Signature Protein-2 Protects DNA from DNase Digestion:

DNA protection against DNase was assessed in vitro using 230ng of 843-bp PCR amplified linear DNA amplicon or 900ng pET28a plasmid DNA. Both linear and plasmid DNA were allowed to interact with SP-2 protein or BSA equal concentration for 30 minutes at RT. DNase I was then added and the DNase treatment was carried out for 10 min at 37°C. The reaction was terminated by incubation at 65°C for 10 min.

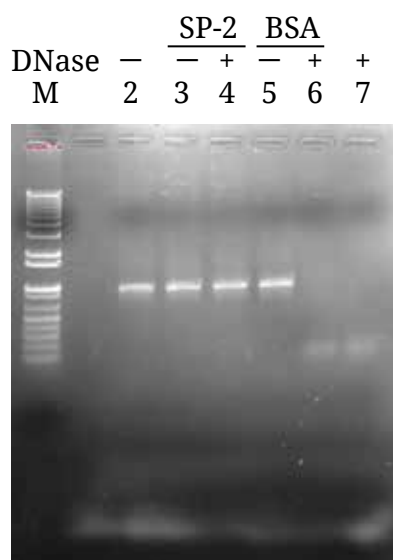


Fig (3a)

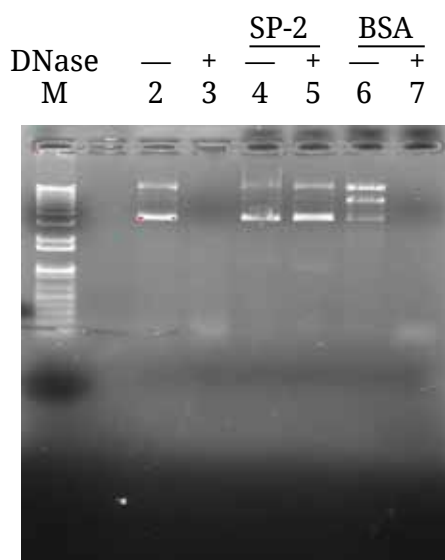
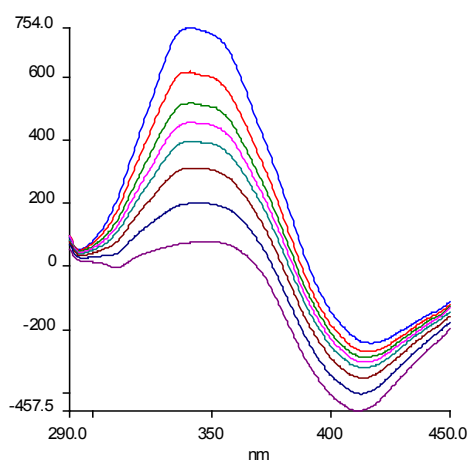


Fig (3b)

Figure 3: DNA protection assay. (a) PCR amplified linear DNA amplicon(843bp) was incubated with equal concentrations of SP-2 or BSA (4.8µg) for 30 minutes at room temperature. Lane 2, DNA + NFW; Lane 3, DNA + SP-2; Lane 4, Lane 4; DNA + SP-2 + DNase, Lane 5; DNA + BSA, Lane 6; DNA + BSA + DNase; Lane 7, DNA + NFW + DNase. (b) Plasmid DNA pET28a (900ng) was incubated with equal concentrations of SP-2 or BSA for 30 min at RT. Lane 2, DNA + NFW; Lane 3, DNA + DNase; Lane 4, DNA + SP-2, Lane 5, DNA + SP-2 + DNase, Lane 6, DNA + BSA, Lane 7, DNA + BSA + DNase.

Signature Protein-2 Binds both Ferric (II) and Ferrous (III) forms of Iron:

Florescence binding experiments were carried out using PerkinElmer LS55 spectrophotometer. All measurements were made at 21⁰C. SP-2 was used at the concentration of 200ng/ml and stock solutions of iron (FeSo₄ and FeCl₃) were made in degassed MQ water and added to 3ml of protein to give the final concentration of FeSo₄ 5.5nM-215nM and FeCl₃ 1.16nM-73.92nM. Changes in fluorescence maxima were observed using an excitation wavelength of 290 nm and an emission detection wavelength of 420 nm.

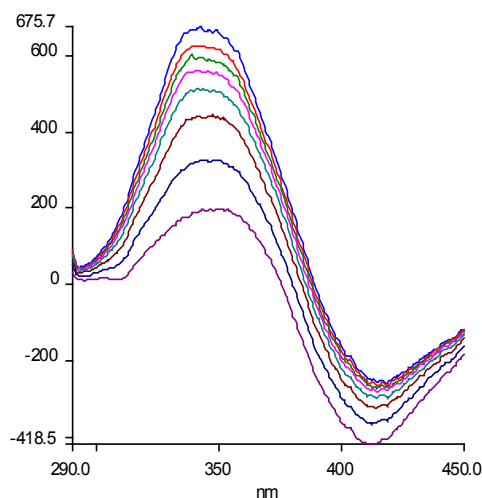


Iron (II) sulphate Binding

1. Protein alone
2. 5.5 nM
3. 11 nM
4. 16.5 nM
5. 27.5 nM
6. 55 nM
7. 165 nM
8. 215 nM

Fig 4: Iron (II) Binding assay of signature protein at different concentrations of Iron sulphate showing decrease in the fluorescence intensity with increasing Iron (II) concentration.

Iron (III) Binding assay using FeCl_3 : Iron (III) binding assay was done in the similar manner as for Iron (II) using fluorescence spectrophotometer.



Iron (III) Binding

1. Protein alone
2. 1.16 nM
3. 2.32 nM
4. 4.64 nM
5. 9.2 nM
6. 18.42 nM
7. 36.96 nM
8. 73.92 nM

Fig 5: Iron (III) Binding assay of signature protein at different concentrations of Ferric chloride showing decrease in the fluorescence intensity with increasing Iron (III) concentration.

Future work plan:

Study of Anti-Oxidant property of signature protein-2 in vivo by transforming into *M. smegmatis*.

3. Investigating the role PE35/PPE68 genes coded by the RD-1 region of the *Mycobacterium tuberculosis* genome in immune modulation and antigenicity

Scientific Staff : Dr. Nasreen Z. Ehtesham, Mr. Mohd Khubaib

In collaboration with : Prof. Seyed E. Hasnain, IIT, Delhi and Dr Noorudin Khan, Hyderabad University

Duration : 2013-2017

Aims, Objectives and Background:

Genome sequence of different mycobacterial species had revealed the presence of two unique families of proteins namely PE and PPE. Coded by 10% of total genome, these proteins are dispersed in the genome some times in operon or in close proximity of ESX proteins. ESX proteins had been linked to the virulence of *Mycobacterium tuberculosis* (*M.tb*). PE/PPE proteins have been found to increase in number during the course of evolution of mycobacterium species from non-virulent to extremely virulent species suggesting their role in *M.tb* virulence. Comparative genome analysis of different species of mycobacterium revealed the absence of some regions of genome in different species. These are known as regions of differences (RD) and are named as RD1-RD16. Among different RDs, RD1 had been directly associated with virulence, as strains lacking it are non-virulent. Complementation of RD-1 mutant genotype with RD-1 restores virulence.

Co-operonic PE35 and PPE68 genes are suspected to have role in virulence as they belong to RD1 region. *In-vitro* studies on PE35/PPE68 proteins have shown their role in immune modulation.

Work done during the year:

B-cell response against PE35 and PPE68 proteins in immunized mice

Hydropathy analysis using *DNASTAR* software showed PE35 to be less antigenic while PPE68 had several antigenic patches making it to be strongly antigenic. Mice were immunized with recombinant PE35, PPE68 and PE35+PPE68 proteins. Direct ELISA was

performed for the presence of different IgG subtypes in the serum of immunized mice against respective proteins. Results of ELISA revealed that PE35 was a weak B-cell antigen while PPE68 was a strong B-cell antigen (Figure 1 A, B, C, D). Combination of PE35 and PPE68 elicited significant B-cell response. Higher level of IgG2a against these proteins showed that B-cell response against these proteins is not influenced by Th-2 cytokines.

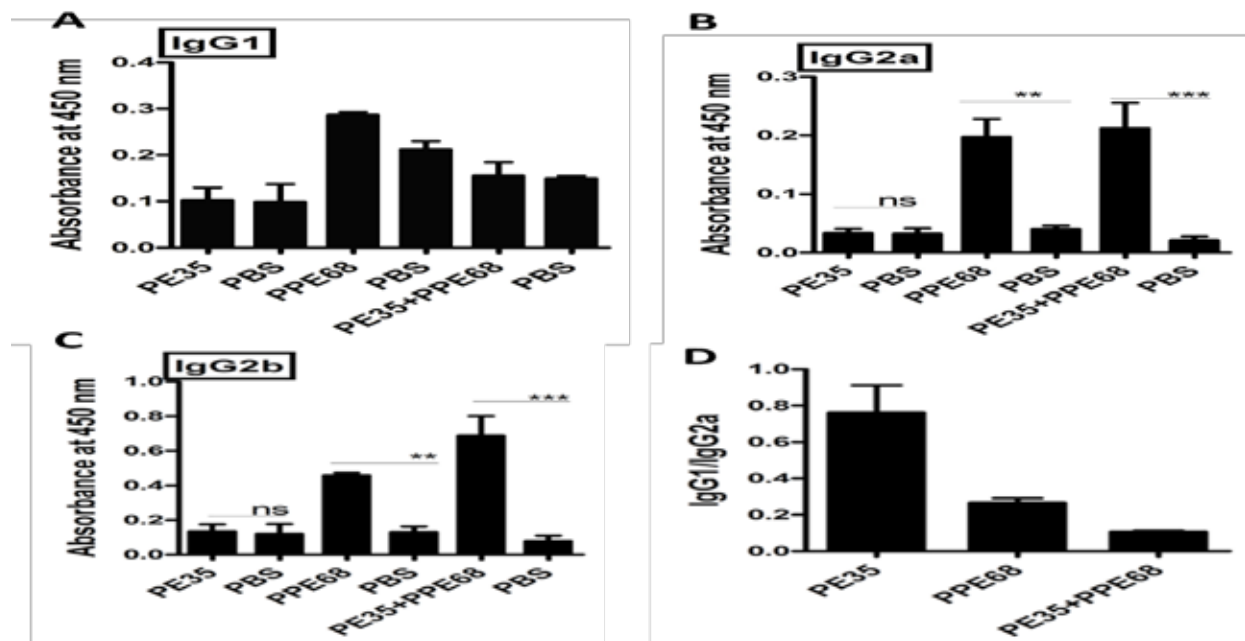


Fig.1: PE35 does not induce IgG1, IgG2a and IgG2b response in comparison with PPE68 alone and PE35+PPE68 in mice. Sera from immunized mice was used to perform ELISA to measure IgG1, IgG2a and IgG2b. (A) All immunized mice showed low IgG1. IgG2a and IgG2b were high in sera of PPE68 and PE35+PPE68 immunized mice (B, C). IgG1 vs IgG2a ratio determines whether the response is Th-1 or Th-2, $\text{IgG1/IgG2a} < 1$ revealed that B-cell response against PE35/PPE68 was not influenced by Th-2 cytokines. Error bar represents SD of average of 3 experiments in biological triplicates. ** $p < 0.005$, *** $p < 0.000$

PE35/PPE68 proteins down-regulate Th-1 cytokines in splenocytes:

Splenocytes isolated from immunized mice were re-stimulated with respective proteins in culture in presence of Golgi-plug/Golgi-stop solution for 8 hours. Cultured splenocytes were then labeled with fluorescent antibodies for CD4⁺ and CD8⁺ cells and intracellular IFN- γ and IL-2 cytokines. Frequency of CD4⁺ and CD8⁺ cells positive for IFN- γ and IL-2 was measured through Flow-cytometry. Results showed that there was significant decrease in number of CD8⁺ cell positive for IFN- γ and IL-2 for the

splenocytes re-stimulated with PE35+PPE68 proteins (Figure 2 A, B, C, D and E). However, no significant change was observed in CD4⁺ cell population. Recombinant PE35, PPE68 proteins when used in combination shifted immune response towards Th-2 type thus helping in survival of bacteria in the host.

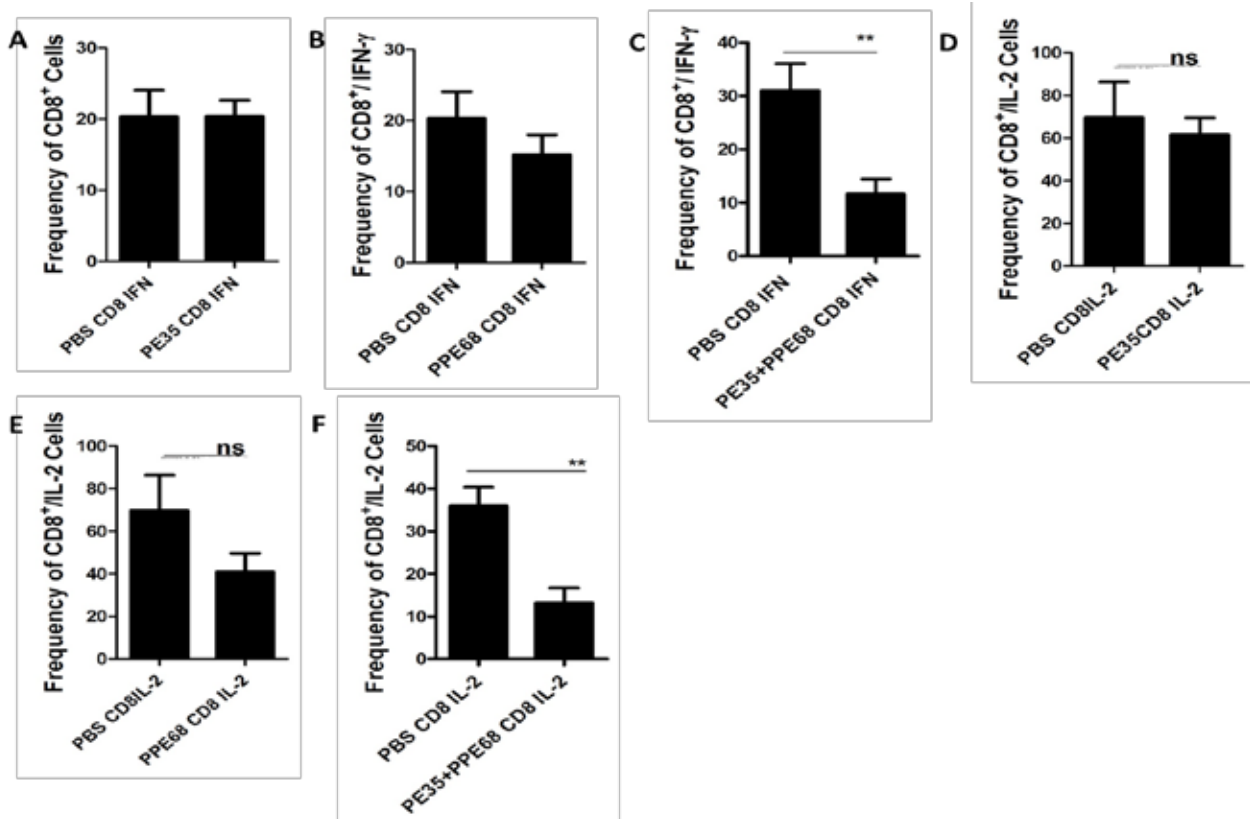


Fig. 2: PE35/PPE68 proteins reduce IFN- γ and IL-2 level in splenocytes. Splenocytes isolated from immunized mice were again challenged with same proteins in culture for 72 hours. Panels A, B, C) show graphical representation of frequency of CD8⁺ cells positive for IFN γ . Panels D, E, F show graphical representation in frequency of CD8⁺ cells positive for IL-2. Error bars represent SD from mean. **p<.005 ***p<.0005.

PE35/PPE68 proteins increase the frequency of CD4⁺ cells and decrease CD8⁺ cell population

Recombinant proteins were used to re-stimulate splenocytes in culture for 72 hour. Cells were labeled for the presence of CD4⁺ and CD8⁺ cell surface markers. Change in population size of CD4⁺ and CD8⁺ cells measured using Flow cytometry, show that there was decrease in population of CD8⁺ cells and increase in population of CD4⁺ cell

in treated splenocytes when compared with naïve cells (Figure 3 A, B). Change was significant for splenocytes re-stimulated with recombinant PE35+PPE68.

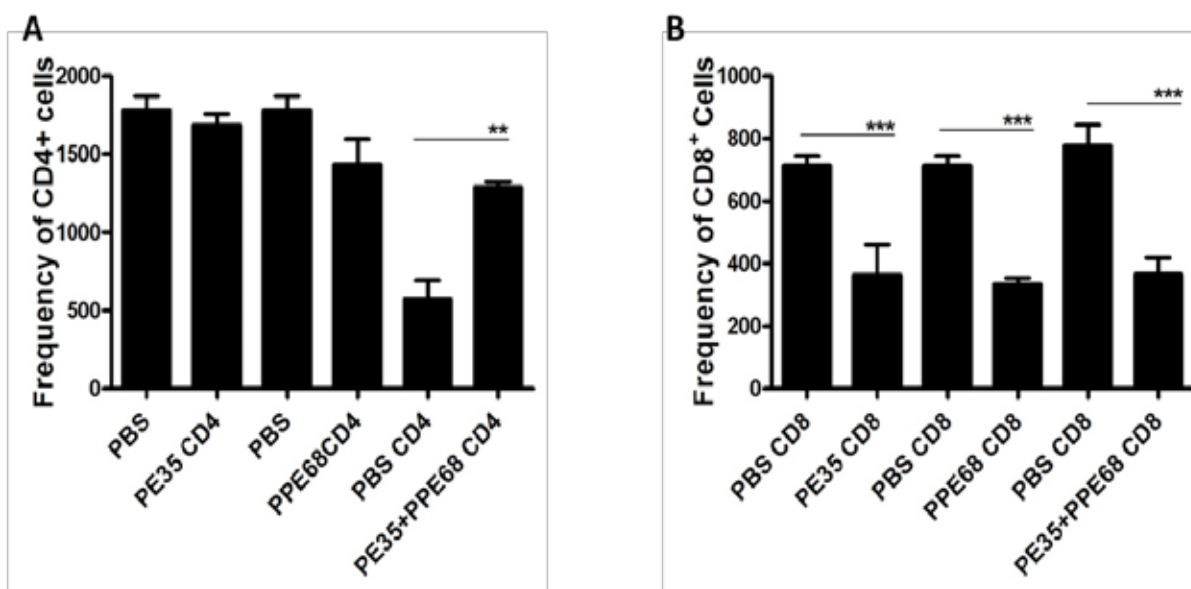


Fig.3. PE35/PPE68 proteins decrease CD8⁺ cell population and increase in CD4⁺ cell population in splenocytes of immunized mice. Splenocytes were isolated from mice immunized with PE32/PPE65 proteins alone or in combination, and treated with 3µg/ml of proteins in culture for 72 hours. Flow cytometer was used to (A) calculate the frequency of CD4⁺/CD8⁺ cells; (B) Change in frequency of CD4⁺ and CD8⁺. Error bars represent SD from mean. **p<.005, ***p<.0005.

Future perspective

Co-operonic PE35/PPE68 proteins had been shown to have role in immune modulation of macrophage cell line. Our data also suggest that they modulate immune response by shifting it from Th-1 to Th-2 type which may help in survival of bacilli inside the host cells. Signaling cascade involved in this mechanism is unknown. Investigations to understand the details of pathway(s) involved in the mechanism of action of these proteins in manipulating host defense mechanism will throw light on *M.tb* pathogenesis.

4. Role of RipA and its interaction with chaperonic protein of *M. tuberculosis* H37Rv strain

Scientific Staff : Dr. Nasreen Z. Ehtesham, Dr. Manish Bhuwan

Duration : 2014-17

Aims, Objectives and Background:

Functionally, Reactivation promoting factor Interacting Protein A (RipA), of *M. tuberculosis* H37Rv is a secretory protein that also has endopeptidase activity. The MoxR1 protein belongs to ATPases and is associated with various cellular activities. AAA+ proteins have been reported as novel molecular chaperone in bacteria and are involved in maturation or refolding of specific protein complexes.

Work done during the year:

I) Bimolecular fluorescence complementation assay

HEK293T cells cultured in Dulbecco's modified Eagle's medium (DMEM) were seeded in 2 ml of DMEM media in 6-well plates, containing poly-l-lysine treated cover slip. Plasmid DNA for RipA and MoxR1 cloned in pBiFC-VC155 and pBiFC-VN173 respectively, were mixed and transfected using PEI reagent. The 6-well plates were incubated at 37°C under humidified air containing 5% CO₂ for 6 h. The incomplete media was removed and complete DMEM was added and incubated for 16 h. Transfected HEK293T cells were fixed on cover slip by treatment with formaldehyde in 1X PBS for 15 min at room temperature and mounted on glass slide. Interaction was confirmed using confocal microscopy (Figure 1)

II) Aggregation prevention assay of MalZ protein in the presence of MoxR1

MoxR1 was expressed and purified from *E.coli* and recombinant protein was characterized. To determine the chaperone activity of MoxR1 protein the aggregation prevention assay for MalZ was performed. The MalZ (0.4 µM) protein both in the absence or presence of 5 µM and 10 µM of MoxR1 was mixed at room temp and

light scattering was measured at 500 nm OD. The experiment was carried out at 47°C. Similar experiment was also performed with MalZ (0.4 μ M) in the presence of 5 μ M and 10 μ M MoxR1 and 1mM ATP (Figure 2). This chaperone activity was further enhanced in presence of ATP (Figure 3)

I). RipA interacts with MoxR1 protein *in vivo*

Schematic representation for BIFC interaction

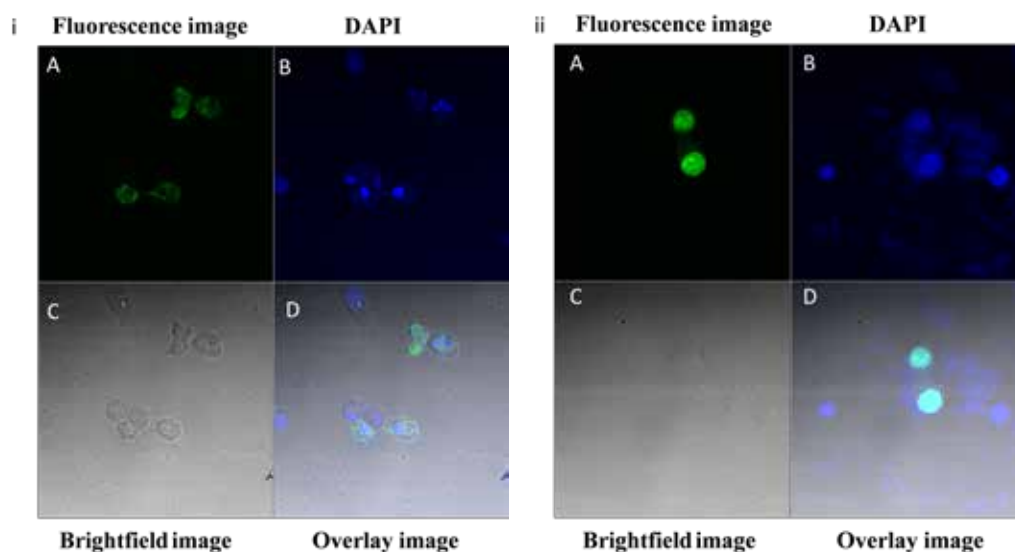
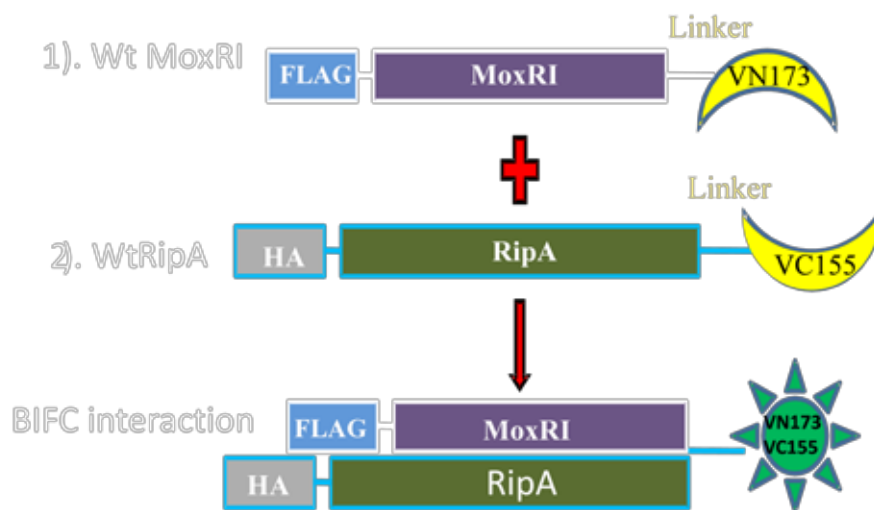


Fig. 1. RipA interacts with MoxR1 protein *in vivo* as shown by Bimolecular fluorescence complementation assay. BiFC assay was performed for visualization of RipA and MoxRI interaction in HEK293T cells. The fluorescence was detected in VC155-RipA + VN173-MoxRI (Image I). The highest fluorescence was seen in positive control VC155-*bjun* + VN173-*bfos* (Image II).

MoxR1 possesses a chaperonic activity

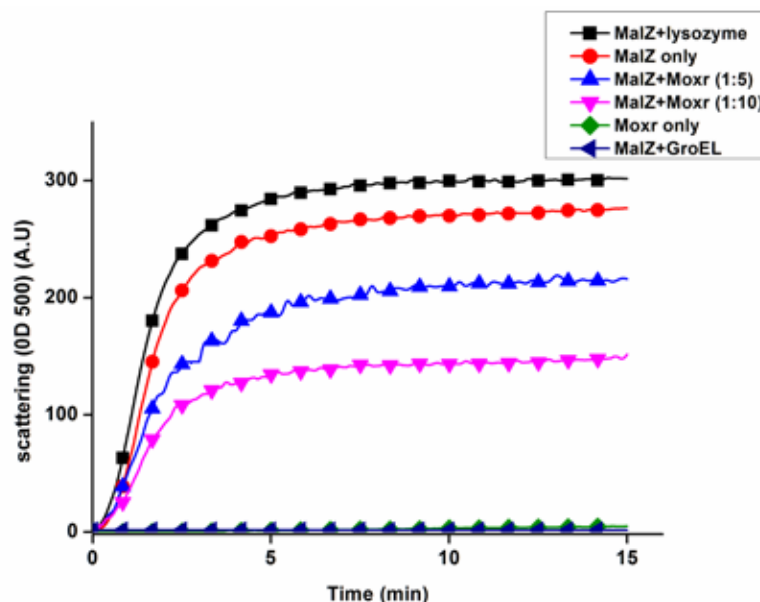


Fig. 2. MoxR1 prevents the thermal aggregation of MalZ protein which is a characteristic of a chaperonic protein. Lysozyme was used as a negative control and GroEL was used as a positive control.

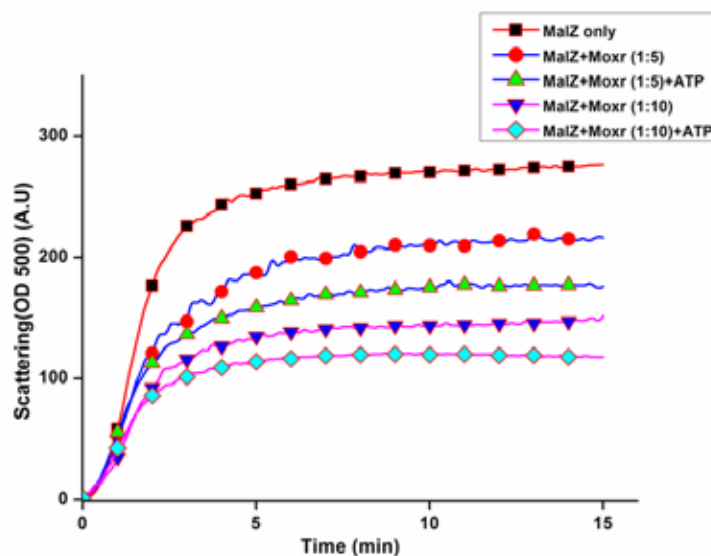


Fig. 3. MoxR1 possesses chaperonic activity. The chaperonic activity of MoxR1 was enhanced in the presence of ATP suggesting the role of AAA+ ATPase domain.

Future work:

Since, the MoxR1 protein has been shown to have a chaperonic activity, it will be used to evaluate the impact of MoxR1 on other mycobacterial proteins. Further, the regulatory mechanism of MoxR1 protein interaction with RipA will be elucidated.

5. Host signal transduction modulation by *M. tuberculosis*: Moonlighting functions of *M.tb* PPIases?

Scientific Staff : Dr. Nasreen Z. Ehtesham, Saurabh Pandey

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

Duration : 2011-2016

Aim, Objective and Background:

Peptidyl-prolyl *cis/trans* isomerases of *Mycobacterium tuberculosis* (*M.tb*), though primarily belong to foldases class of enzyme, isomerize the peptide bond preceding the proline bond. *M.tb* has two isoforms of prolyl isomerases, named PpiA and PpiB. These are ubiquitously expressing proteins, in absence of which serious folding bottlenecks are created. PpiA of *Mtb* is known to be structurally and phylogenetically related to eukaryotic cyclophilins. This enzyme being a component of the secretome of the pathogen is speculated to play a vital role in survival of *Mtb* in the harsh environment of macrophage.

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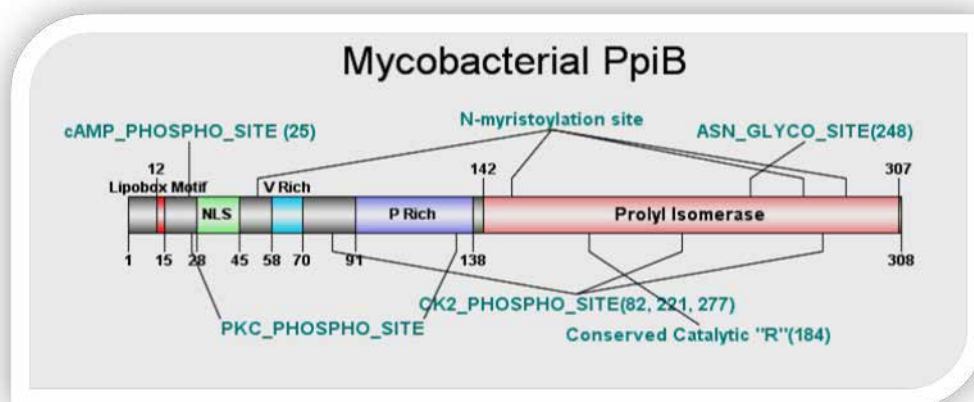


Fig. 1: Domain architecture of PpiB of *M.tb*. Signal sequence of PpiB comprises lipobox motif, nuclear localization sequence, valine rich and proline rich motifs and minor sites along with enzymatic moiety.

Work done during this year:

Role of peptidyl Prolyl isomerases (PpiB and PpiA) as chaperone:

In an attempt to functionally characterize recombinant PpiA and PpiB for its potential to act as chaperone, its ability to sustain heat induced aggregation was assessed. Having shown its structure stability its ability to prevent the temperature induced aggregation of heat labile protein, Maltodextringlucosidase (MalZ) was measured by fluorometer (Fig 2 A & 3A). Binding of ANS dye to PpiA and PpiB shifts the peak to the Blue region with very significant with increase in fluorescent intensity (Fig 2B & 3B), measure of surface hydrophobicity, which is known to be important for proteins with chaperone like function.

Further, to investigate the chaperone like function of rPpiB and rPpiA under physiological conditions, we investigated if *E. coli* expressing PpiB and PpiA of *M.tb* exhibit resistance to thermal shock as compared to the control strains (vector control). Our results showed that *E. coli* cells transformed with *PpiB* and *PpiA* gene of *M.tb* showed approximately ten folds more survival in comparison to the *E.coli* transformed with vector alone. These results provide conclusive evidence that rPpiB and rPpiA show chaperone like function both in in-vitro conditions and under physiological conditions (Fig 2C & 3C).

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PpiB: Chaperone Activity Assay

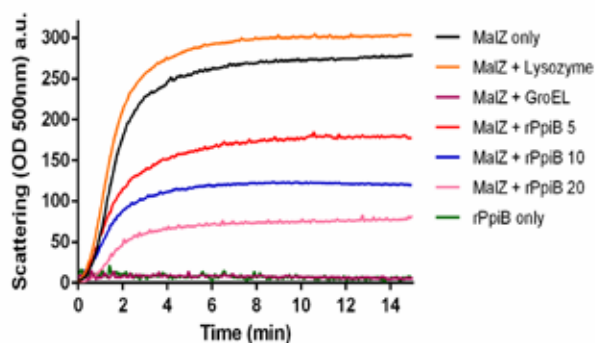


Fig. 2A: Suppression of MalZ aggregation by rPpiB. Light scattering at O.D. 500 mM with excitation and emission slit width 5 and 2.5, respectively was recorded to monitor the aggregation pattern. GroEL was used as a positive control. Lysozyme was used as a negative control.

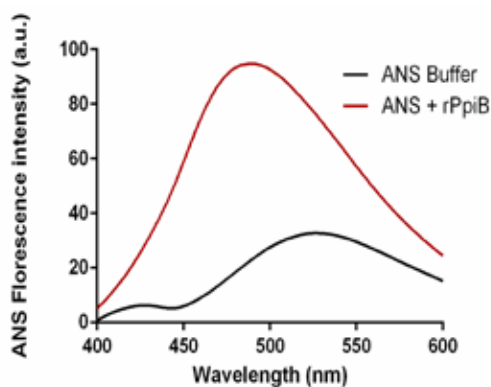


Fig. 2B. ANS Fluorescence spectra reveals surface hydrophobicity in *M.tb* rPpiB: Concentration of ANS and rPpiB used were 20 μ M and 0.1 mg/ml respectively. Blue shift in the position of peak and increase in the intensity of peak was observed on addition of rPpiB. The ANS emission was scanned in the range of 400 to 600 nm.

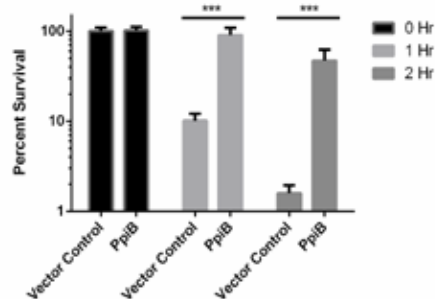


Fig.2C: rPpiB can rescue *E. coli* cells from thermal shock: *E. coli* BL21 cells were transformed with pGEX6p1 only and pGEXppiB. After heat treatment, at one hour interval at 50°C, *E. coli* transformed with *ppiB* exhibited approximately 10 fold more survival compared with vector control.

PpiA Chaperone activity Assay:

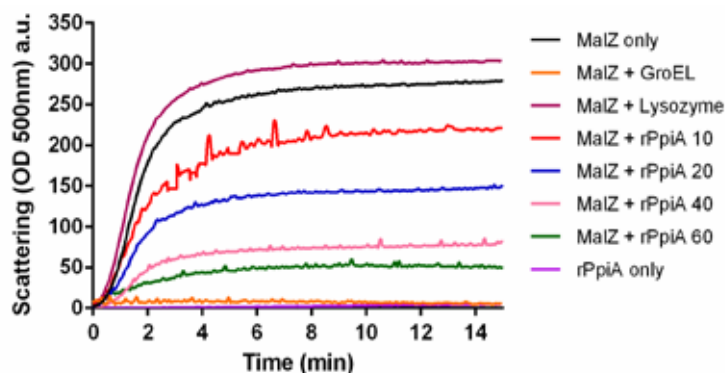


Fig. 3A. *M.tb* rPpiA suppresses aggregation of MalZ protein: The aggregation pattern was monitored by light scattering at O.D. 500 nm with excitation and emission slit width 5 and 2.5, respectively was recorded to monitor the aggregation pattern. GroEL was used as a positive control. Lysozyme was used as a negative control.

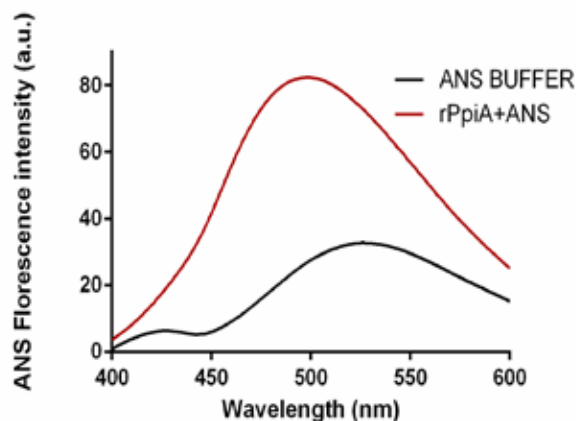


Fig. 3B: ANS Fluorescence spectra reveals surface hydrophobicity in *M.tbr*PpiA: Concentration of ANS and rPpiA used were 20 μ M and 0.1 mg/ml, respectively. Blue shift in the position of peak and increase in the intensity of peak is observed on addition of rPpiA. The ANS emission was scanned in the range of 400 to 600 nm

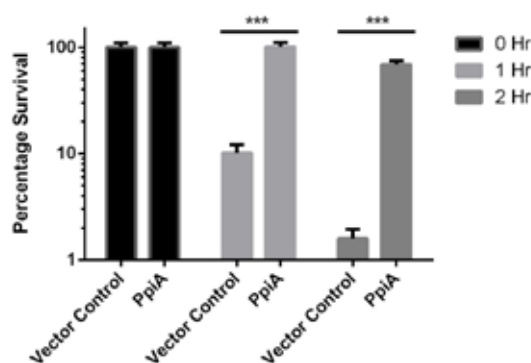


Fig. 3C: Cells overexpressing PpiA shows more tolerance to heat stress than vector only control: Time points were taken at the interval of 1hour when temperature switched from 37°C to 50°C. Protein induction is done at 37°C with 1mM IPTG. Figure is representative of three individual experiments; percentage error is shown in bar graph.

Expression of PpiA in HEK cell line imparts resistance against hypoxia and oxidative stress

HEK cells were transfected with vector alone or containing gene for PpiA. HEK cells expressing PpiA were exposed to oxidative and hypoxic stress, cell viability assay using MTT reveals significant increase in cell viability in the cells expressing PpiA as compared to vector control. These observations clearly validate a critical role of PpiA, in aiding the intracellular survival of the pathogen amid the hostile environment of infected cells.

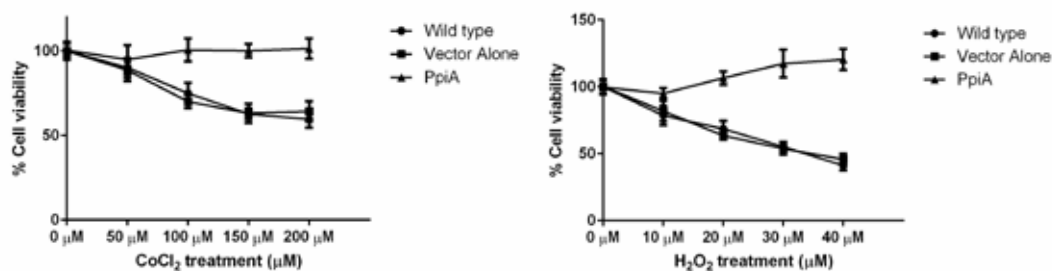
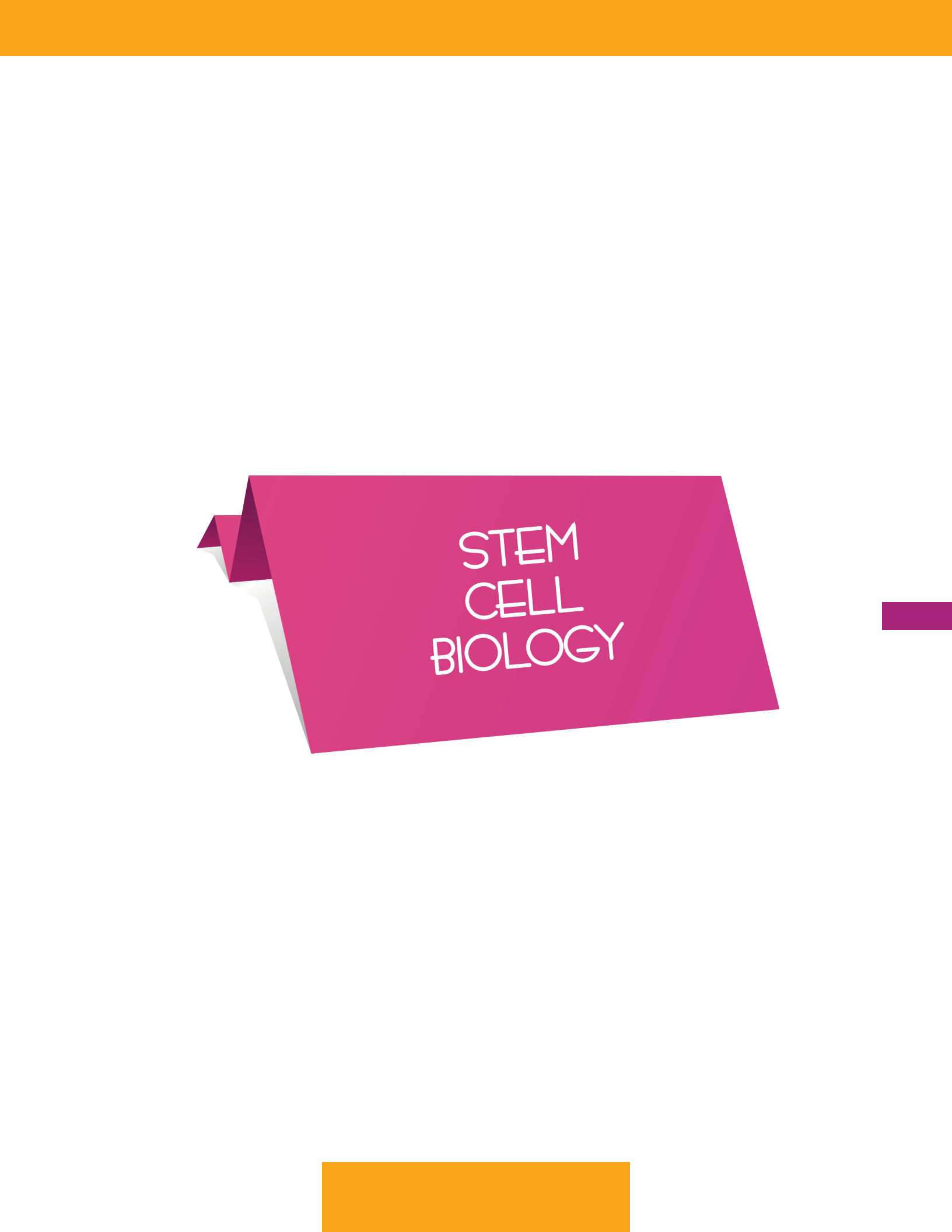


Fig. 4: PpiA imparts resistance to the HEK293t cells against Hypoxic and Oxidative stress. A, MTT assay was performed to score the % cell viability of HEK293t cells transiently expressing PpiA under the oxidative stress (H₂O₂ treatment dosage was given in 0, 10, 20, 30 and 40 μM concentration). B, Under same conditions, hypoxic stress was given and % cell viability was scored (CoCl₂ dosage given for hypoxia was 0, 50, 100, 150 and 200 μM concentration). The data is representative of three independent experiments. Error bar represents mean ± S.D

The image features a minimalist design with a white background. At the top, there is a solid orange horizontal bar. In the center, a pink parallelogram is tilted to the right, with the words 'STEM CELL BIOLOGY' written in white, uppercase, sans-serif font. To the left of this pink shape, a smaller, darker pink parallelogram is partially visible, also tilted. To the right of the pink shape, a thin, dark purple horizontal line extends towards the right edge. At the bottom center, there is a solid orange rectangular block.

STEM CELL BIOLOGY

STEM CELL BIOLOGY

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

Scientific Staff : L.K. Yerneni, Rishi Man Chugh, Madhusudan Chaturvedi, Bijendra Kumar

Duration : 2014-16

Aims, Objectives and Background:

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

Therefore, the objectives of the study included estimation of mitomycin C residues in the final product, finding out chromosomal stability in the cultured keratinocytes by G-Banding and demonstration of expansion potential of human epidermal keratinocyte cultures to be setup from human skin Biopsy employing the in-house culture technique.

Work done during the year:

We have completed estimation of traces of mitomycin C in the final product by HPLC-MS and the chromosomal stability in the cultured keratinocytes by G-banding.

I. Detection of traces of mitomycin C in cultured cells and tissues

Methods:

Sample Collection: Solutions were aspirated from triplicated samples from various steps of washing and lysed preparations from feeder cells and cultured epithelia generated from co-cultures of various feeder batches and human epidermal keratinocytes (Figure 1) as per the details given in Table 1. Trace levels of Mitomycin C (MC) were estimated in these samples by HPLC–MS/MS as described by Nozal et al. 2006 with certain modifications.

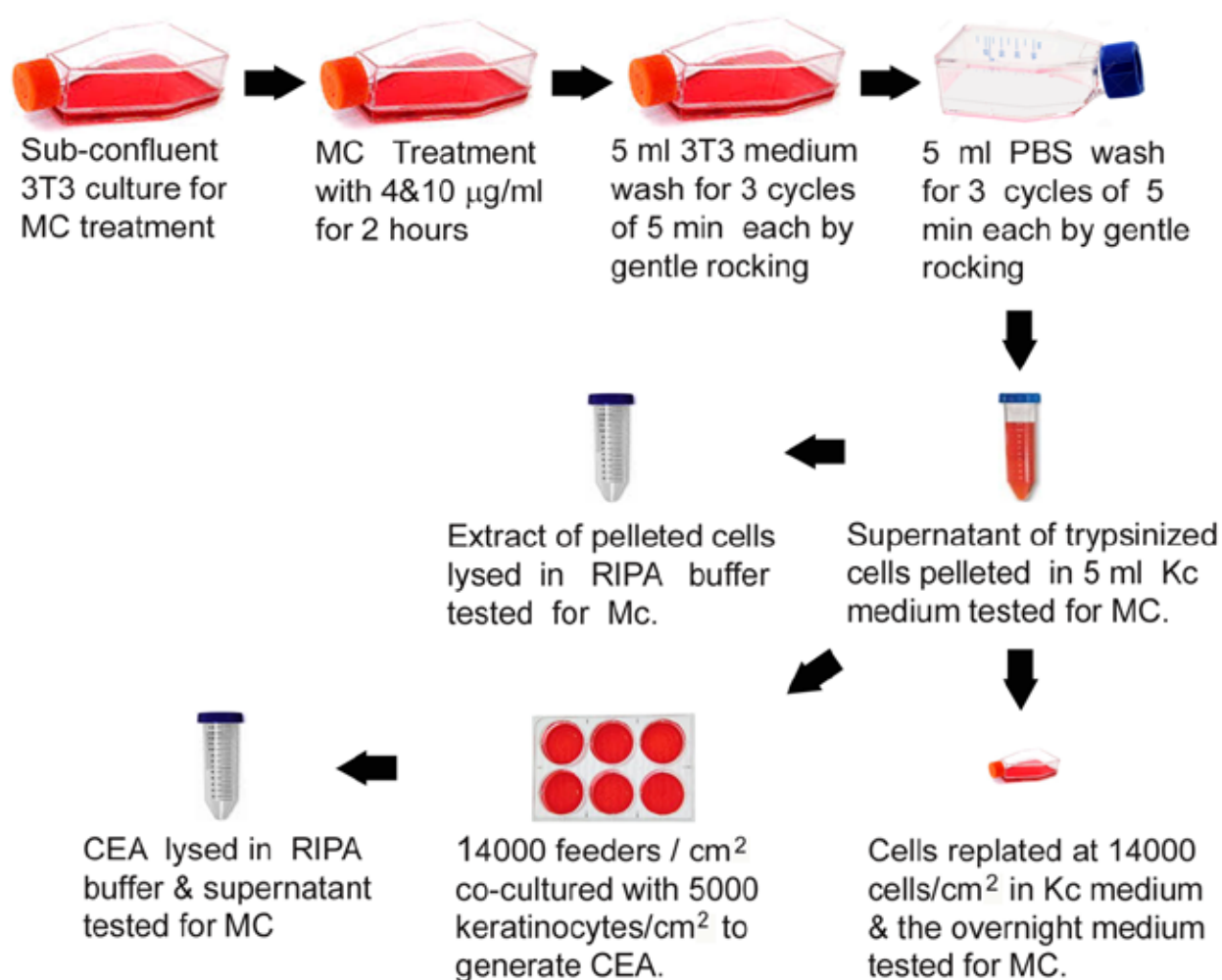


Fig.1: Flow-chart showing sequence of steps of washing and lysis for MC estimation.

The cultures intended for growth arrest were treated with MC for two hours with permutations of concentrations (µg/ml) and doses (µg/million cells) of 4-15, 4-150 and

10-30. The cultures were washed 3 times each with 3T3 final medium and PBS for 3 cycles of 5 minutes each by gentle rocking and each 3rd wash was tested for MC detection. In order to assess the impact of trypsinization on the release of membrane bound MC, both the supernatant of detached cell suspension and the extract of pelleted cells lysed in RIPA buffer followed disruption by three freeze-thaw cycles were tested for MC estimation. An overnight spent Kc-medium from cultures seeded with feeders alone and two-days spent medium from keratinocyte co-cultured with 4-150 MC feeders were additionally tested to see if cells released any trypsinization resisting traces of MC. The final product of cultured epidermis (CEA) generated in the presence of various feeders in 6 well-plates was further assayed for the traces of MC by similarly lysing in RIPA buffer.

Table 1. Details of all the post-exposure steps of washing and lysis

Step 1	Medium wash 1:- 5ml 3T3 medium that contained 10% Donor Calf Serum was added to T75 flask after exposure to MC and decanting the MC-medium and incubated at 37°C for 5 minutes in incubator with constant shaking at the rate of 12 strokes per minute.
Step 2	Medium wash 2:- 5ml 3T3 medium was added after decanting the 1 st medium-wash and incubated as in step 1.
Step 3	Medium wash 3:- 5ml 3T3 medium was added after decanting the 2 nd medium-wash and incubated as in step 1.
Step 4	PBS wash 1:- 5ml PBS was added after decanting the 3 rd medium-wash and incubated as in step 1.
Step 5	PBS wash 2:- 5ml PBS was added after decanting the 1 st PBS wash and incubated as in step 1.
Step 6	PBS wash 3:- 5ml PBS was added after decanting the 2 nd PBS wash and incubated as in step 1.
Step 7	Trypsinized Cell Supernatant:- 8ml of medium supernatant after centrifuging (500 g) the cells that were detached by 0.25% Trypsin + 0.03% EDTA.
Step 8	Lysis supernatant:- 2 x 10 ⁶ cells were lysed in 1.5 ml of RIPA buffer (composition: 150nM NaCl, 1% Igepal, 0.5% Sodium deoxycholate, 0.1% SDS & 50 nM Tris; Sigma R0278) repeatedly freeze-thawed in LN2 for 3 cycles; each consisted of snap freezing for 20 sec followed by thawing by vortex for 60 seconds. The high speed (14000 g at 4°C) supernatant assayed for MC.
Step 9	4-150 feeders - spent medium:- The MC exposed cells were replated alone at a density of 14,000 cells/cm ² in T25 flask and incubated with 5 ml Kc Medium. The overnight medium was assayed for MC.

Step 10	4-150 feeders & Kc co-culture - spent medium:-Two-days old spent Kc Medium from co-cultures initiated with feeders (14,000/cm ²) and 3 rd passage Kc (5000/cm ²).
Step 11	CEA lysate supernatant:- CEA generated from P3 keratinocytes after 8 days of co-culture with γ -IRR feeders or MC feeders of 4-15 or 4-150 group (plated as in step 10) were lysed in RIPA buffer and freeze thawed as per step 8. The high speed supernatant (as per step 8) were assayed for MC.

The underlined Steps represent those samples used for Mitomycin C estimation by HPLC/MS.

Chromatographic Conditions: Mobile phase consisted of water-methanol at 40:60 ratio, in a column of Purosper Star 55 x 4.6 mm, 3 μ m with a 3 minute run time at a flow rate of 0.4 ml/min in an LC/MS using ESI Positive Ion Source. The Ion Spray voltage Delta EMV(+) used was 400 at a gas temperature of 350°C, gas flow of 8 Liters/min and a nebulizer pressure of 50 psi. The Multiple Reaction Monitoring (MRM) methodology was used for scanning and acquiring MS data and their relevance to MS quantitation.

Calibration: Briefly, a set of MC calibration solutions with different concentrations i.e. 100, 75, 25, 10, 7.5, 1, 0.5, 0.25 η g/ml were made from a standard stock solution of 200 μ g/ml MC by dilution with methanol:water (1:1, v/v) solution followed by centrifugation at 13,000 rpm for 10 min, supernatants (400–500 μ l) were analyzed. All chromatographic experiments were carried out at room temperature using Agilent 1290 Infinity HPLC attached with Agilent Triple Quadrupole LC/MS 195. The parent ion with 357 m/z and the product ions of 313.9, 295.9, 280.8, 274, 242 m/z were found in method development process and the product ions with m/z 274 (Zhou et al., 2014) was selected for MC estimation and was monitored via ion trap mass analyzer. A detection accuracy of 99.99 \pm 3.43 was achieved while preparing a standard curve using MC standard solutions of 40, 25, 10, 5 & 1 η g/ml (Fig. 2).

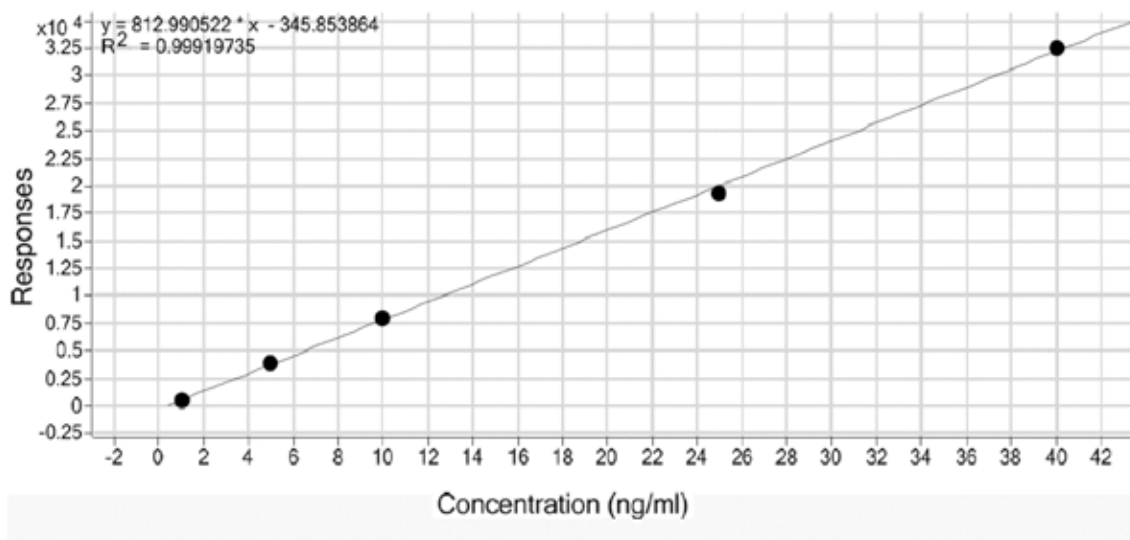


Fig. 2. Standard curve of Mitomycin C standard solutions of 40, 25, 10, 5 & 1ng/ml

Significant Results: The standardized method detected 3879.25 ng of MC per ml in the solution of 4µg/ml used for inactivating feeder cells (Figure 3). The residual MC concentrations in the 3rd feeder medium wash were 1.40±0.08, 3.14±0.12 and 4.12±0.06 ng/ml for 4-15, 4-150 and 10-30 feeder groups respectively. Upon the subsequent PBS wash at step 6, a residual MC of 0.40±0.01 ng/ml was detected only in 10-30 feeders while it was undetectable in the other feeder groups. But the trypsinization at step 7 resulted in the reappearance of MC traces in the supernatant of the pelleted cells and corresponded to 0.48±0.03, 0.68±0.02 and 0.78±0.03 ng/ml for 4-15, 4-150 and 10-30 feeder groups, respectively. On the other hand, no detectable traces were found in the lysed feeder cells at step 8 indicating that the molecules of MC bound to cell membrane possibly by loose physical interactions were released by trypsinization. Further, neither the 4-150 feeder cells plated alone nor those co-cultured with P3 keratinocytes have contributed to any detectable residues of MC in the spent media suggesting attainment of absolute washing in the previous step. Subsequently, the Cultured Epidermal sheets generated from the 3rd passage keratinocytes using any of the feeder groups showed no detectable MC residues.

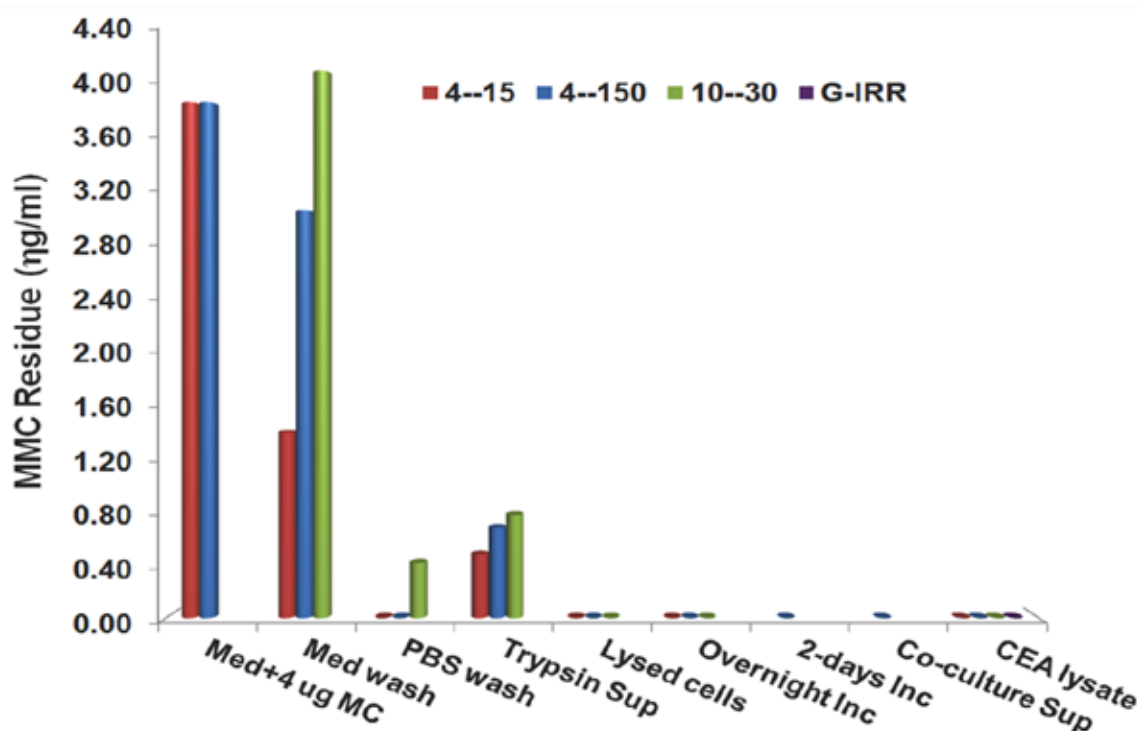


Fig. 3. Amounts of mitomycin C detected in various experimental groups.

II. Chromosomal stability in the cultured keratinocytes

Methods:

The keratinocytes supplied after passaging two times in feeder free conditions at the supplier end were further passaged two more times in the presence of the optimal 4-150 feeders and tested for chromosomal integrity.

Preparation of chromosomal spreads: The sub-confluent keratinocytes were exposed to 0.1 $\mu\text{g/ml}$ demecolcine for 4 hours followed by differential trypsinization to first remove the feeders and then to isolate keratinocytes. The cells were exposed to hypotonic solution of 0.075M KCl for 40 minutes followed by fixation in cold methanol-acetic acid (3:1) fixative. The cell suspension in fixative was dropped on to the acid clean slides and air-dried. Slides meant for chromosomal counts were stained in a final working solution of 0.08% Giemsa stain containing 5% glycerol and methanol in PBS. Slides meant for assessing structural aberrations were processed for G-banding which comprised of overnight incubation at 60°C, digestion in 0.25% trypsin in PBS for 2 minutes and staining in Giemsa for 15 minutes. Slides were scored as per the International System for Human Cytogenetic Nomenclature 2005.

Significant Results: All the 38 good metaphases counted have yielded normal chromosomal number in 4th passage keratinocytes grown in the presence of 4-150 feeders (Figure 4). Five metaphases analyzed for structural integrity by G-banding showed all 46 chromosomes with normal structure (Figure 5).

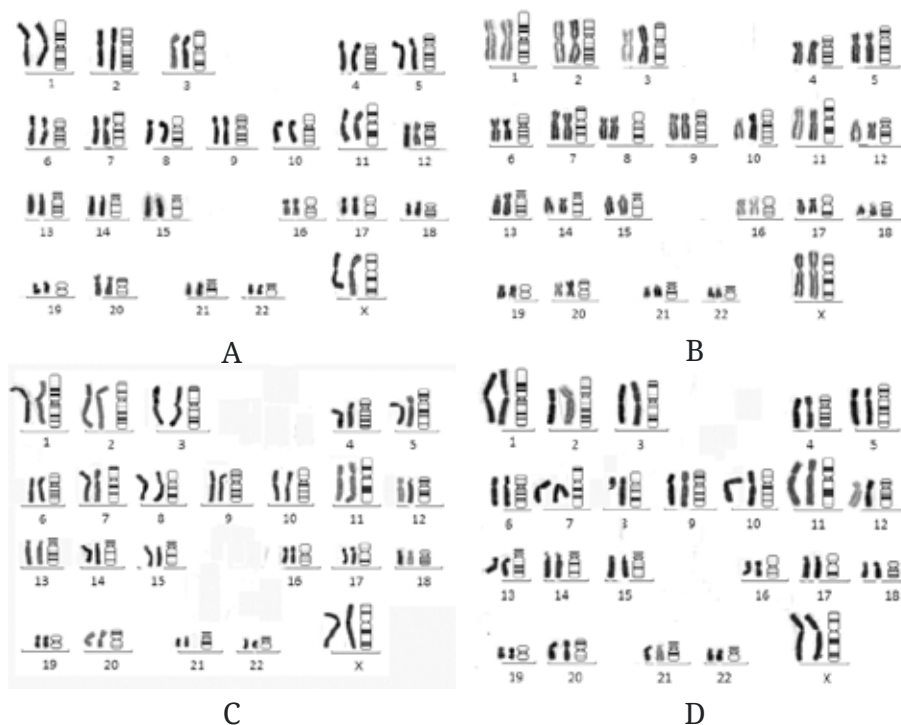


Fig. 4. Giemsa stained metaphases from cultured keratinocytes

Conclusion:

The culture process has resulted in no detectable residues of mitomycin C in the final product and normal Karyotype; therefore, may not pose serious concerns from the clinical application viewpoint.

Future course of action:

Karyotyping of human keratinocytes to be cultured up to 4 passages in the lab from a skin biopsy will be undertaken. Ethical permission has been obtained for collecting Human skin biopsy. The cultured keratinocytes were devoid of anchorage independent growth as shown in an *in vitro* tumorigenesis assay, but additional *in vivo* tumorigenesis assessment in Nude mice is initiated.

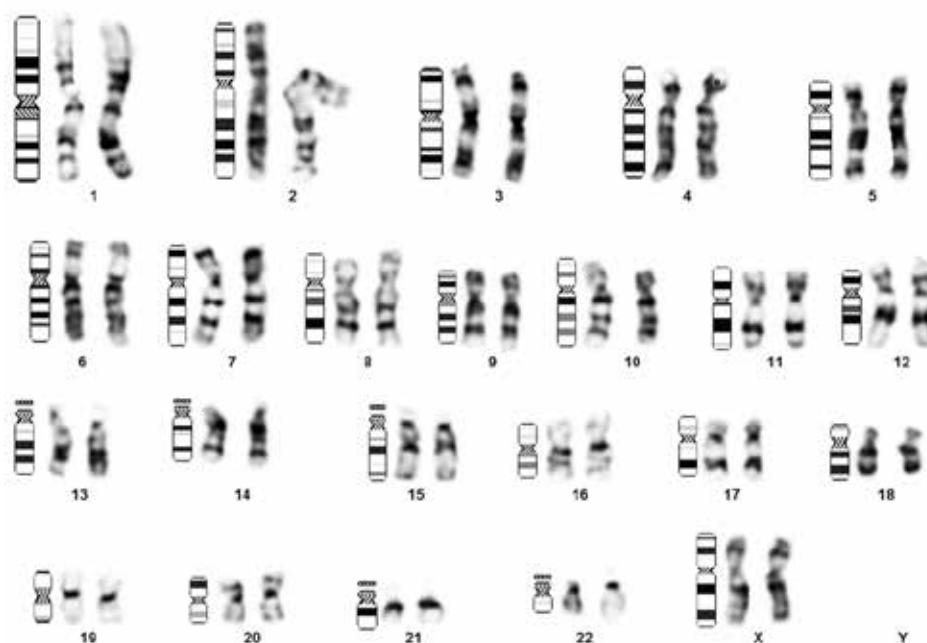


Fig.5. G-banded Karyotype of cultured keratinocytes showing normal chromosomes

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

Scientific Staff : L.K .Yerneni, Bijendra Kumar
Ms. Hemlata Chauhan (CSIR-SRF until July 2015)

Duration : 2013-16

Aims, Objectives and Background:

This project has been initiated to work out a detailed manufacturing protocol for cultured epithelial Autograft using Human dermal fibroblasts by replacing the xenogeneic Swiss 3T3 cells. A full ad hoc project has been submitted to ICMR under the translational research cell for funding in 2013. The project was initially suggested to expand to 4 years scope but subsequently reduced to 3 years. It has recently been approved but financial sanction is awaited. In the mean time, the work has been initiated through a CSIR direct fellow. It was proposed to standardize a working protocol to treat the human fibroblasts with Mitomycin C by applying the same

approach that was adopted in producing a fool-proof and efficient growth arrest and to produce & characterize the cultured epidermis.

Therefore, the objectives of the study includes, finding out a least toxic but effective Mitomycin C induction of human dermal fibroblast cell attenuation by titrating with exposure cell densities and volumes; verification of optimal influence of differentially growth arrested fibroblasts on proliferation & the basic characteristics of human epidermal keratinocytes; characterization of the human epidermal sheets cultured using such variedly growth arrested human dermal fibroblast cells to identify the best outcome and to grow epidermal sheets using human serum to simulate their production in clinical setting attaining total non-xenogeneic conditions.

Work done during the year:

Methods & significant Results: So far, identification of a suitable banking procedure so as to contain the sporadic failure of growth arrest on lines of our recently published concept (Chugh et al 2015) has been accomplished. However, the validation for absolute extinction of the growth arrested dermal fibroblasts using the minimal possible concentration of Mitomycin C (MC) and the method of isolating the feeders from the co-cultured keratinocytes have not yet been completed due to considerable technical limitations. Limitation of research funds has also been an additional impediment. Attempts were made to translate the doses derived from the cell density titration experiments into workable volumes through arithmetic derivation using the following formulae wherein v and ϵ are kept constant in (1) and (2), respectively.

$$\Delta = \frac{Cv}{\Sigma} \quad \text{--- (1)}$$

$$v = \frac{\Delta \Sigma}{C} \quad \text{--- (2)}$$

Δ = dose / cell (pg/cell or $\mu\text{g}/\text{million cells}$), C = concentration of MC ($\mu\text{g}/\text{ml}$), v = volume of treating solution (ml), Σ = exposure cell number (millions).

Volumetric titrations: The preliminary long term experiments spanning 35 days of post- MC exposure time to a range of volumes under a given concentration *per se* hinted at likely success towards achieving differential extinction.

Conclusion:

The preliminary volumetric experiments completed so far yielded leads for attaining differential feeder extinction and keratinocytes stimulation. Elaborate screening of a larger range of concentration-dose permutations of MC is necessary in this direction.

Future course of action:

The following outstanding tasks will be undertaken by dedicated lab personnel and funds from the newly sanctioned project. The tasks include screening & short-listing of probable feeder batches by volumetric titrations, standardizing the isolation of keratinocytes ensuring minimal feeder contamination and identification of the most preferred feeder batch by keratinocyte colony forming efficiency, mass cultures & characterization of epidermal sheets produced thereby.



ENVIRONMENTAL TOXICOLOGY

ENVIRONMENTAL TOXICOLOGY

Human Environmental Biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and Intrauterine Growth Restriction (IUGR)

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-110029

Duration : 2012-2015

Aims, Objectives and Background:

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Air toxics or 'hazardous air pollutants' (HAP) are organic pollutants present in the environment in low/trace concentration (other than criteria pollutants) that are known, or suspected to be as toxic and persistent. These include Volatile Organic Compounds (VOCs) including Benzene, Toluene, Xylene (BTX) and Polycyclic Aromatic Hydrocarbons (PAHs). Some PAHs are semi volatile in nature and therefore, these can be present in particulate as well as vapour phase while others are mostly adsorbed onto particles in the environment.

Annual PAH emissions of India were estimated to be 90 Ggy^{-1} by Zhang and Tao (2009). 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). Links between PAH exposure and elevated levels of DNA adducts, mutations, and reproductive defects

have been widely reported. 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 IUGR 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 year:

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 Safdarjung Hospital, New Delhi, have been enrolled as cases after screening for inclusion and exclusion criteria. Age and socio-economic status matched women with who delivered appropriate for gestational age (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 and urine have been collected from a total of 70 IUGR and 70 AGA (control) cases. Details of residential history (Location and duration of residence), home characteristics including heating and cooking sources and ventilation, and dietary habits of PAH-containing foods (i.e., fried, boiled, barbecued meat etc.) have been 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.

Since the controls and cases were age matched, no significant difference was observed between the two groups with respect to age. The average age of mothers of AGA babies was 24.31 ± 3.38 (range - 18 to 32 years) while the average age of mothers with IUGR deliveries was 23.43 ± 3.01 (range - 19 to 30 years). 42% AGA subjects and 56.5% IUGR subjects belonged to low socio-economic status. On the other hand, 42% IUGR and 53.6% AGA subjects belonged to middle income group.

Average maternal weight and BMI in IUGR deliveries (21.3 ± 2) was significantly

lower ($P < 0.05$) as compared to AGA deliveries (22.4 ± 1.9). The mean weight of placenta collected from IUGR subjects was 385.2 ± 48.2 g in comparison to 502 ± 53.2 g in AGA subjects ($p < 0.05$). The weight of baby ranged from 0.8-2.5 kg (Mean 2.0 ± 0.3) in IUGR subjects and 2.25-3.75 kg ($2.85 \pm .4$) in non-IUGR subjects.

Selection of Polynuclear aromatic hydrocarbons for analysis by HPLC:

According to US EPA priority list the following 16 PAHs were selected for the study: 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. HPLC conditions for best chromatographic separation of mixed PAHs were optimized by trying 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

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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 (RT) 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 included Phenanthrene, Flurenthene, cotinine (Fig. 1), Pyrene, Benzo(a)anthracene, Naphthlene, Benzo(a)pyrene, Acenaphthene, Chrysene, Acenaphthylene, etc. (Table 1).

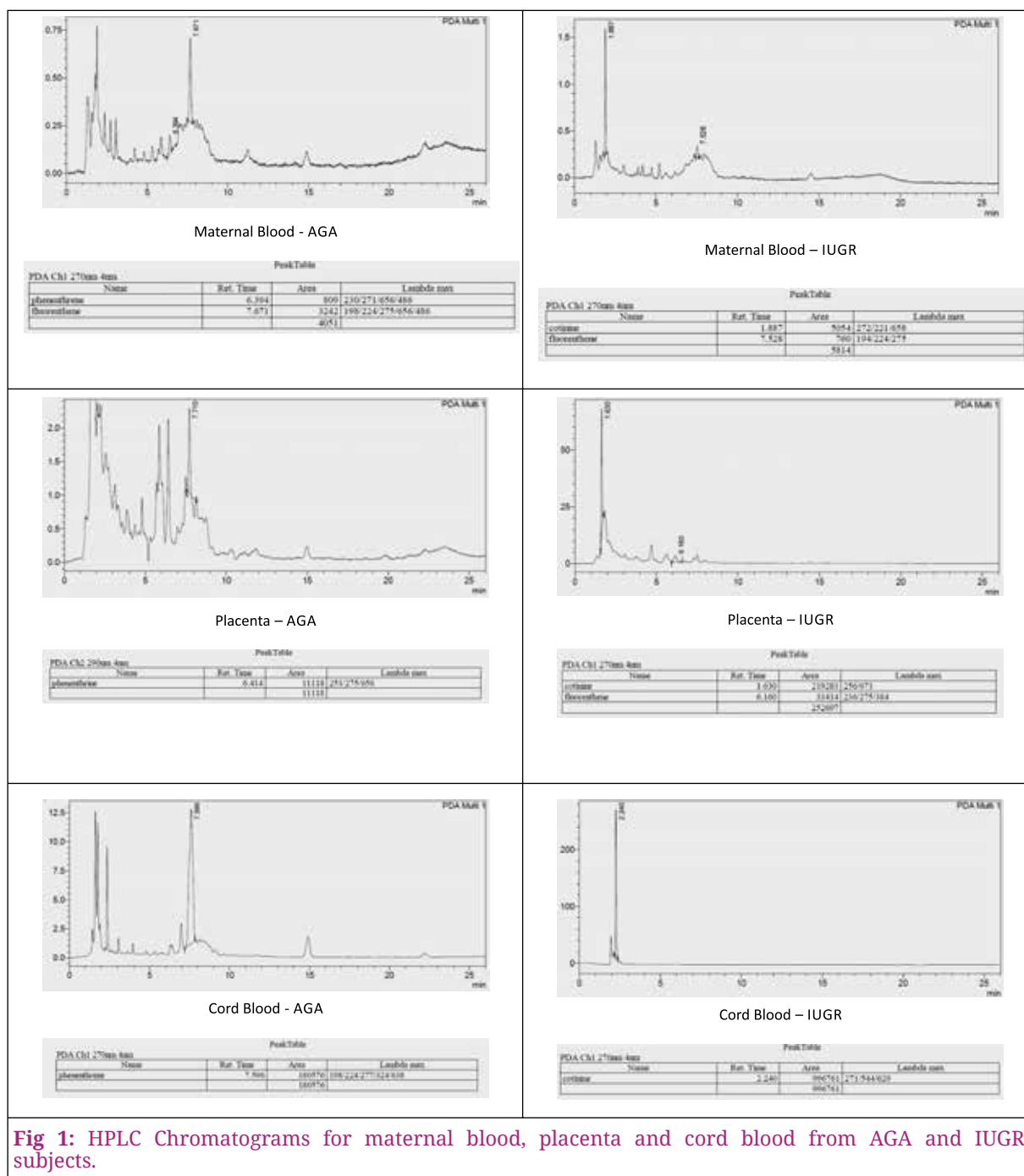


Fig 1: HPLC Chromatograms for maternal blood, placenta and cord blood from AGA and IUGR subjects.

Table 1:

	Control			IUGR		
	MB	P	CB	MB	P	CB
Acenaphthene	4	3	2	6	10	2
Acenaphthylene	3	2	0	6	3	2
Anthracene	0	2	0	0	3	0
Benzo(a)anthracene	2	2	0	2	1	1
Benzo(b)fluoranthene	0	4	1	3	6	5
Benzo(k)fluoranthene	0	1	1	0	4	0
Benzo(ghi)perylene	0	1	0	0	1	0
Chrysene	3	2	1	4	10	4
Dibenz(ah)anthracene	1	0	0	2	4	4
Fluoranthene	4	2	2	10	19	10
Fluorene	1	2	0	3	7	5
Indeno(123-cd)pyrene	0	0	0	4	2	0
Naphthalene	16	9	14	19	15	7
Nicotine	1	0	1	0	0	0
Phenanthrene	9	14	4	17	12	6
Pyrene	4	7	5	4	12	3

The study has revealed that placenta acts as efficient barrier only for a few of poly-aromatic hydrocarbons, viz., anthracene, benzo(ghi)perylene and indeno(123-cd)pyrene. Remaining PAH were able to cross the placental barrier to a variable extent.

Another interesting observation is presence of naphthalene and phenanthrene in about 18 to 20 percent and 13 to 17 percent subjects, respectively. The quantitative assessment of PAH exposure has been complicated by the large number of individual compounds in a given mixture and by the presence of PAHs in both the gas phase (2-ring and 3-ring compounds) and the particulate phase (4-ring to 6-ring compounds). While certain particulate-phase PAHs (notably benzo(a)pyrene) have been classified

as known or probable human carcinogens, air concentrations of these 4–6 ring PAHs tend to be very low and difficult to measure. Recently, attention has focused upon the more abundant gas-phase PAHs, notably naphthalene (Nap, two rings) and phenanthrene (Phe, three rings), as possible surrogates for PAH exposure. Naphthalene is typically the most abundant PAH measured from a given source and air levels of Nap tend to be highly correlated with the sum of all measured PAH levels (hereafter ‘total PAHs’) in workplaces. Since Nap is a known carcinogen of the lung in rodents, it is important to characterize human exposure to Nap per se. Phenanthrene is also present at high concentrations in PAH emissions and, while not classified as a carcinogen, is the smallest PAH to contain a bay region, a feature closely associated with carcinogenicity. Pregnant women may be exposed through indoor air pollution, moth balls and tobacco smoke.

Pyrene was found to be present in about 9% of IUGR cases while about 8% controls also showed the presence of pyrene. They may be exposed through dietary sources (grilled and smoked food) and second hand cigarette smoke. Another PAH, fluoranthene was about 19% of IUGR subjects as compared to only 4% of control subjects. It has been reported that fluoranthene is a major environmental chemical, which permeates the apical membrane, impaired cAMP-mediated HCO_3^- secretion and potentiated Cl^- secretion, distinct from the other PAHs benzo(a)pyrene and anthracene. The modulation of anion transport appeared to be mediated by the hIK-1 channel, whose sensitivity to PKA may be up-regulated by the sustained $[\text{Ca}^{2+}]$ elevation produced by fluoranthene. Thus, constant exposure to fluoranthene from the airway surface might be involved in airway surface acidification, resulting in exacerbation of various airway inflammatory diseases. Such respiratory disturbances are also likely to effect the intra-uterine development of the foetus.

A number of PAHs have been reported to cause tumour formation in laboratory animals on exposure through food, from breathing contaminated air and when it was applied to their skin. When pregnant mice ate high doses of a PAH (benzo(a)pyrene), they experienced reproductive problems. In addition, the offspring of the pregnant mice showed birth defects and a decrease in their body weight. There is no information available from studies about health effects in humans on being exposed to PAH. Quantification and further data analysis is in progress.



BIOINFORMATICS

BIOINFORMATICS

Second Phase of Task Force Biomedical Informatics Centre's of ICMR

Scientific Staff	: Dr. Arun Kumar Jain, Dr. Dibyabhaba Pradhan, Dr. Shweta Aggarwal, Mr. Arnab Nayek
Technical staff	: Mr. Prince Gautam
Duration	: Five years (01 April 2013 to 31 Mar 2018)

Aims, Objectives and Background:

The aim of Biomedical informatics Centre at National Institute of Pathology to promote and support informatics in biomedical research through National level workshops, training and research facility for biomedical scientists, research scholars and students. The following activities are in progress in the Biomedical informatics centre to achieve major objectives of the taskforce project.

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Objective 1: To identify genetic loci associated with diseases

Integrated catalogue on susceptible genes of psoriasis

Human associated studies related to psoriasis have received great attention from experimental researchers; however, there is no single comprehensive database to address psoriasis associated gene information. We have developed a new-web based database interface called psoriasis susceptibility gene database which provides detailed information for researchers interested in analyzing the involvement and association of human genome in psoriasis.

Overall, this database is divided into five tables: 1) the gene detail table, which stores information such as gene name, Gene ID, OMIM ID, UNIGENE ID, ENSEMBL ID etc., with the Gene ID defined as primary key, (ii) the homology table, which stores the

orthology relationships derived from Homologene database, (iii) Gene ontology details table which contains information such as HPRD ID, molecular class, molecular function, biological processes, sub-cellular location, tissue expression (iv) the Genetic marker table, and (v) 3D protein structure information table.

The current version of database contains 180 genes and 350 genetic markers involved in different types of psoriasis. The graph shows chromosome-wise distribution of genes (Fig 1). Psoriasis-related genes were classified according to the molecular function of each protein and the biological process in which it is involved. The later versions of the database is also proposed to integrate with an in-house psoriasis electronic patient record with patient specific data from our institute and collaborators; to improve patient data management, research, disease diagnosis and therapy at the point of care.

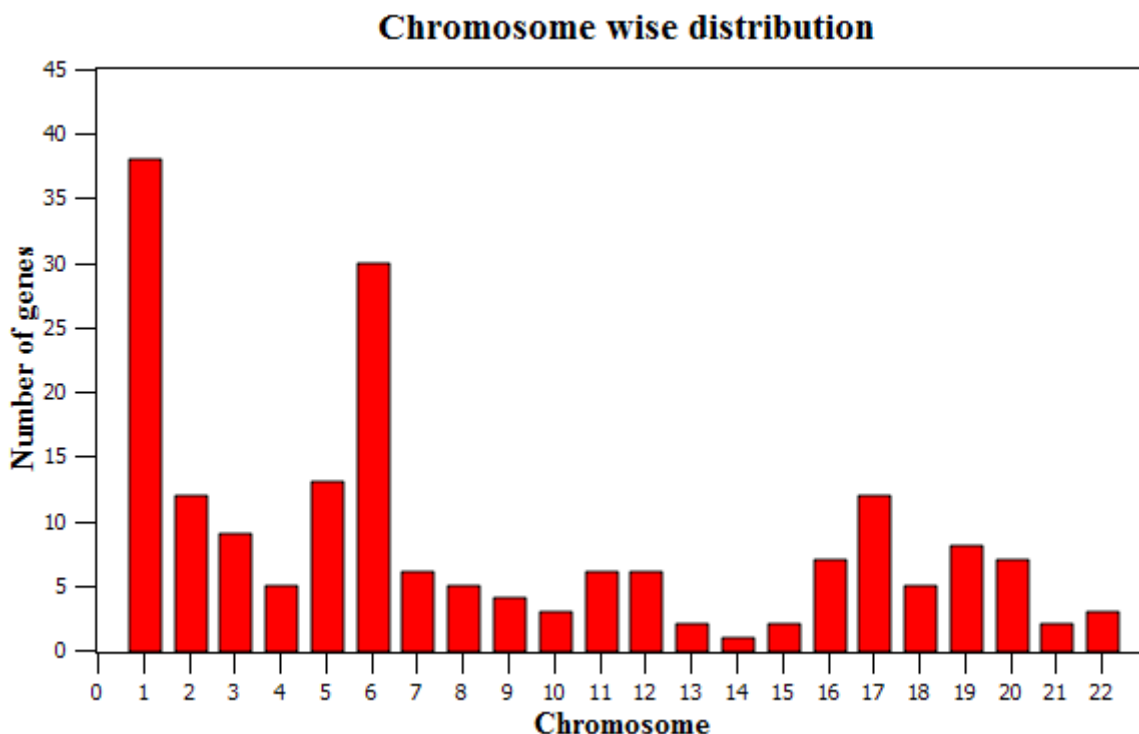


Fig 1. Chromosome-wise distribution of psoriasis susceptible genes

Objective 2: To develop solutions for controlling communicable and non-communicable diseases

i) Discovery of potential inhibitors of BMX non-receptor tyrosine kinase through e-pharmacophore based virtual screening

BMX is expressed in bone marrow, granulo/monocytic cells and arterial endothelium. The stimulated production of IL-6, an important mediator of autoimmunity, has been shown to be dependent on BMX in human fibroblast-like synoviocytes by the use of siRNA knockdown. IL-6 over expression is known to play critical role in progression of psoriasis (Saggini et al., 2014). BMX has been also shown to be overexpressed in human prostate cancer. This signifies discovery of potential BMX inhibitors would represent promising therapies for autoimmune disorders and decipher potential anti-cancer applications. Two E-pharmacophores were designed based on two crystal structure of BMX in complex with inhibitors (3SXR and 3SXS) available at Protein databank (Fig. 2). A dataset of 1678 ligands matched to two pharmacophores with fitness score >1.2 from an in-house repository generated from more than one million entries of ligandinfo metadatabase, were selected for three stage docking with BMX inhibitor binding pocket using virtual screening workflow in Maestro v9.8. The ligands were screened based on extra precision Glide scores (XP Gscore). The top ranked ligands were re-scored using Prime/MM-GBSA free energy calculations (ΔG). The binding affinity and binding orientations of five ligands compared favorably with dasatinib and PP2 (Fig. 3).

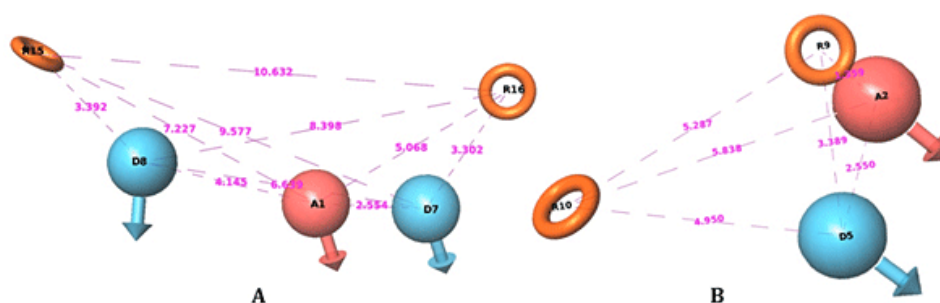


Fig. 2: E-pharmacophores A) BMX complex with dasatinib (3SXR) B) BMX complex with PP2 (3SXS)

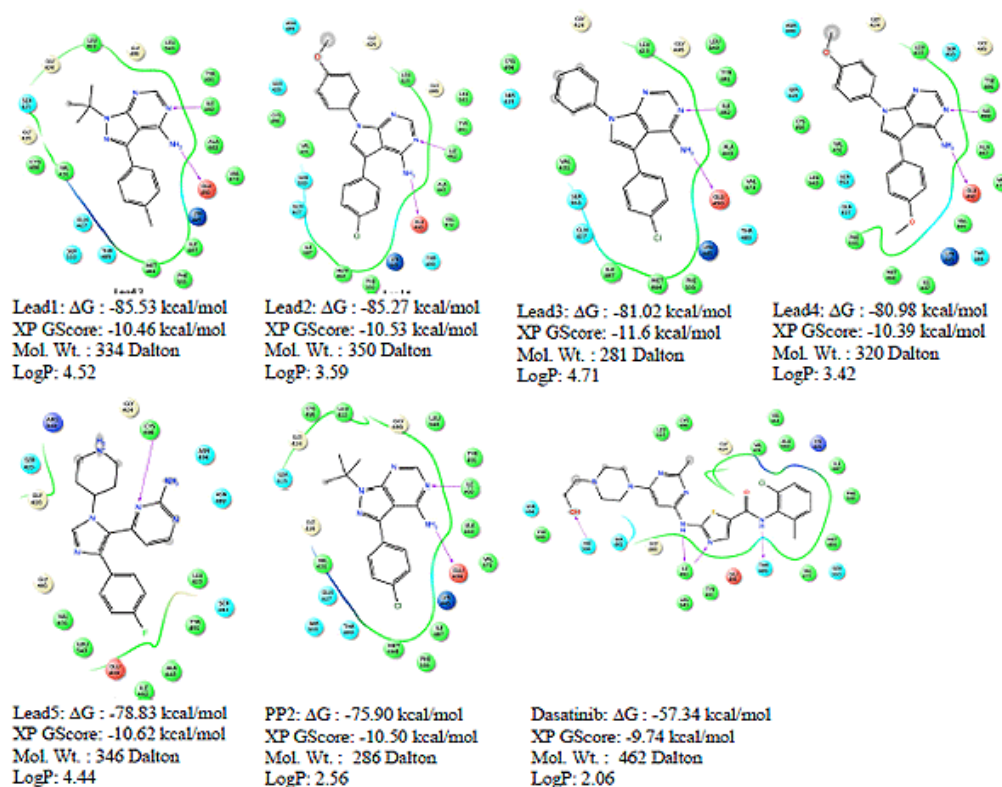


Fig. 3: Five proposed BMX inhibitors, Dasatinib and PP2.

ii) Probing Binding Mechanism of Interleukin-6 and Olokizumab: *In silico* Design of Potential Lead Antibodies for Autoimmune and Inflammatory Diseases

Biological therapy is an important strategy because of high efficacy and affinity with occurrence of immediate response against the target. Interleukin-6 (IL-6) a well-recognized, drug target for various autoimmune and inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis and cancer, was investigated *in silico* to design potential lead antibodies. In this study, crystal structure of IL-6 along with monoclonal antibody olokizumab explored to predict antigen-antibody (Ag-Ab) interacting residues using DiscoTope, Paratome, PyMOL and LIGPLOT. *In silico* mutations were introduced to selected residues of the paratope regions to design 899 theoretical macromolecules. Binding affinity of these theoretical macromolecules were evaluated through Ag-Ab docking (ZDOCK, ClusPro and Rossetta Dock server), binding free energy calculations using Poisson Boltzman with non-polar Surface Area (PBSA) method and interaction energy estimation in an implicit Distance-Dependent

Dielectrics model. Eight newly designed theoretical antibodies demonstrated better result in all assessments compared to olokizumab. The eight proposed antibodies showed decreased pI values and increased hydrophobicity compared to olokizumab, thus are expected to have enhanced half-life as well as better clearance rate from the body. Therefore, these newly designed macromolecules were proposed as potential lead antibodies to serve as a therapeutics option for IL-6 mediated diseases.

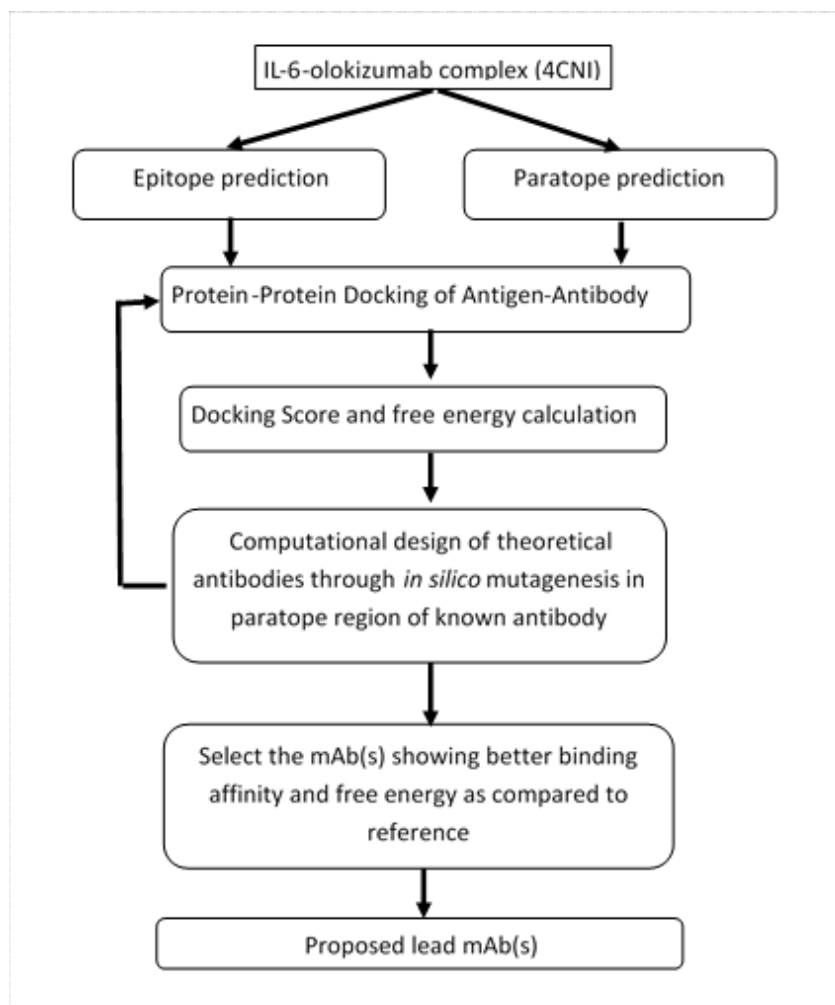


Fig.4: Flow of potential IL-6 antibody design

iii) Potential small molecule inhibitors of TNF-alpha, PDE4 and JAK2

TNF-alpha (tumor necrosis factor- α), PDE4b and JAK2 are well validated targets of many inflammatory and autoimmune diseases including psoriasis. Therefore, potential lead molecules for these three targets were identified through a structure based

virtual screening protocol (Fig. 5). Ten potential lead molecules were proposed for each of the three targets.

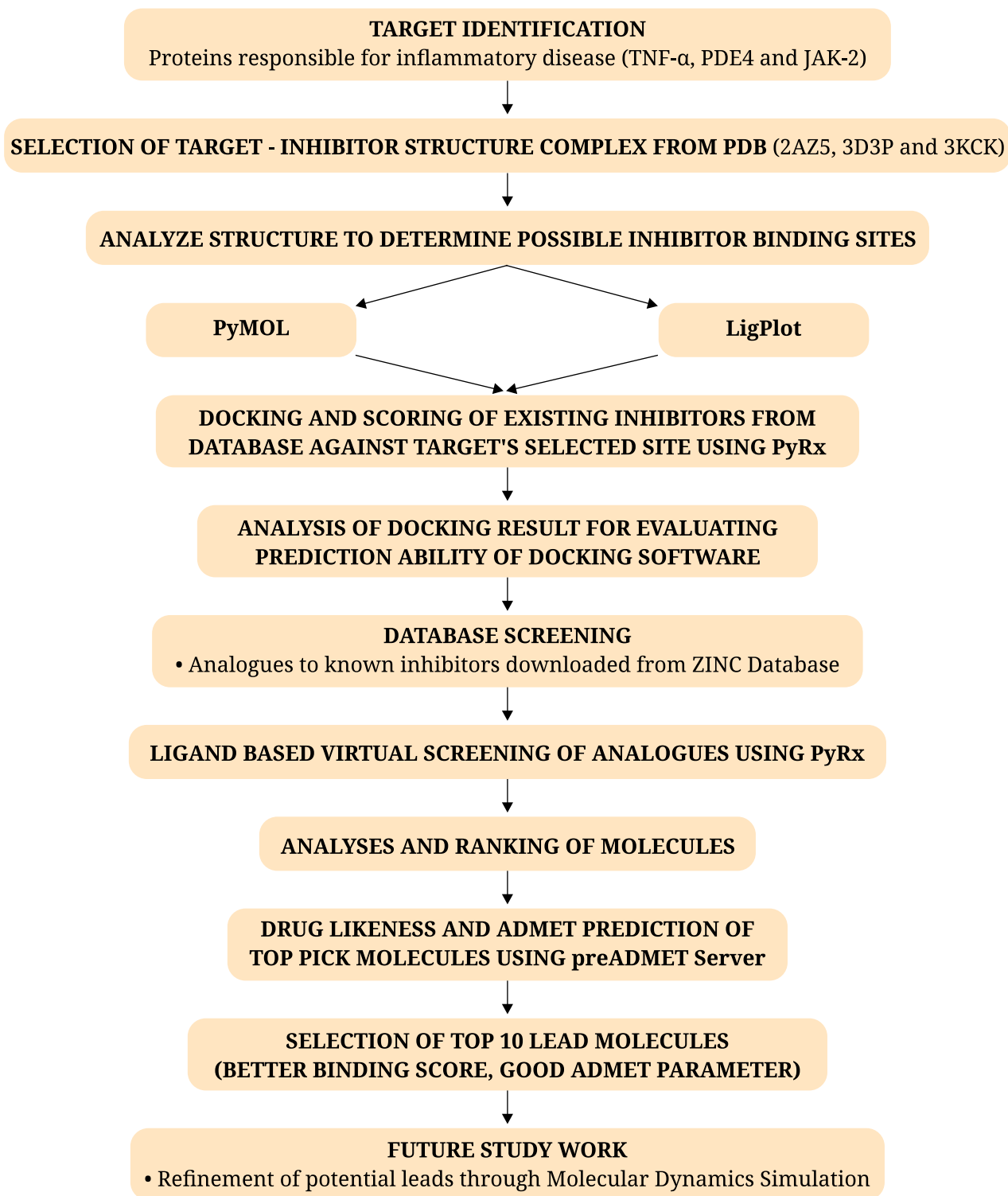


Fig. 5: Workflow for potential inhibitor identification

iv) Identification of putative drug targets of *R. mucilaginosa* and *R. dentocariosa*

R. mucilaginosa is a Gram-positive coccus that causes bacteremia, central nervous system infection, meningitis, peritonitis, osteomyelitis, cervical necrotizing fasciitis, endophthalmitis, and endocarditis. *R. dentocariosa*, originally isolated from carious lesions of human teeth, has been found to cause endocarditis, pneumonia, and infections of the peritoneum and lung. The severity of the disease caused by both pathogens also shifts attention towards developing targeted therapies. A systematic protocol including subtractive genomic analysis, pathway analysis and protein-protein interaction studies (Fig. 6) led to identify 64 proteins of *R. dentocariosa* and 45 proteins of *R. mucilaginosa*. The drug targets were prioritized through metabolic pathway analysis and protein-protein interaction studies to propose 26 and 11 proteins could act as potent therapeutic targets against *R. dentocariosa* and *R. mucilaginosa*, respectively.

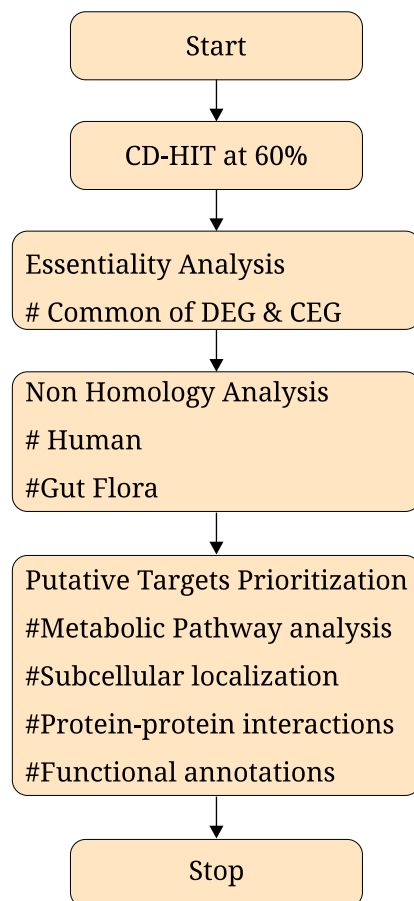


Fig. 6: Drug target identification workflow

v) Developing a software for drug target identification against infectious pathogens is under progress.

Drug target identification software for pathogenic diseases are under development. The software is being developed using Python 2.7 and Visual studio 2015. The software is mainly focused on subtractive genomic analysis with cross references to prioritization techniques such as metabolic pathway analysis, protein-protein interactions analysis, subcellular localization and functional annotations (Fig. 7).

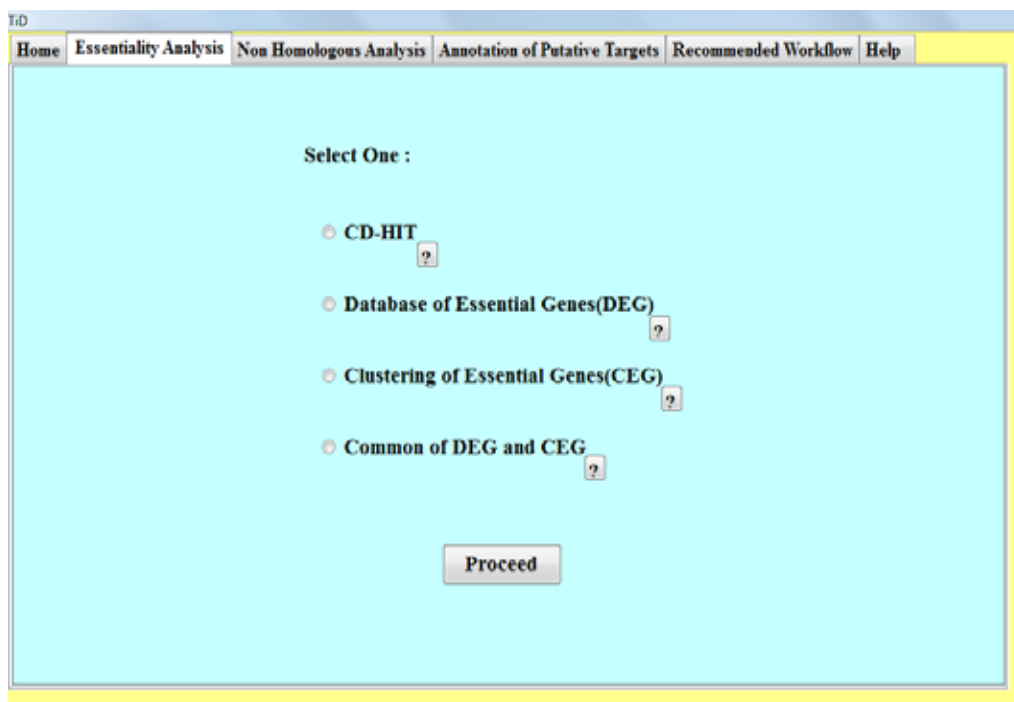


Fig. 7: Drug target identification software snapshot.

Objective 3: To develop a National Repository of clinical information/data, high-throughput data, genotype and phenotype

i) Psoriasis patient registry development

A psoriasis patient registry is proposed to develop with an objective to monitor Efficacy of systemic therapy in psoriasis, side effects related to systemic treatments, demographic distribution of the disease, comorbidities, linking genetic basis of the disease and finally as evidence based medicine at point of patient care.

Psoriasis patient registry form include data such as Name, Age, Gender, Religion, Community, Address, Phone number, Age of onset, Seasonal variation, Family History, Medical History, Duration, Affected area, Clinical diagnosis, Clinical notes, Hospital and doctor referred, Histopathological number, Histopathological findings, Site of biopsy, PASI score, Type of treatment, Date of treatment started, Side effects of treatment.

Last two years (2013, 2014) patient data collection completed.

ii) Tools to predict pollutants from patient samples is developed.

A Perl script is developed to predict possible pollutant contaminations in human blood sample. The tool was developed based on known RT values and spectra data of selected 63 pollutants. Combination of RT, Maxima and minima spectra led to predict probable presence of these 63 pollutants in a given sample with ~94% accuracy.

Objective 4: To promote applications of cutting-edge technologies in medical research

- i) A workshop on 'Bioinformatics tools for Biomedical Research' was organized by the centre during March 24-27, 2015 to promote cutting-edge technologies in Biomedical medical research. 21 PhDs, 3 MDs and 7 M.Sc. students were trained.
- ii) Six M.Sc. students completed six-month dissertation training in 2014-15.
 1. Ms. Monika Yadav, M.Sc. Biochemistry, Jamia Millia Islamia University, Delhi.
 2. Ms. Rashmi Verma, M.Sc. Biochemistry, Jamia Millia Islamia University, Delhi.
 3. Ms. Noor Saba Khan, M. Sc. Biotechnology, Invertis University, Bareilly.
 4. Ms. Afreen Khan, M. Sc. Biotechnology, Invertis University, Bareilly.
 5. Ms. Manisha Ray, Dept. of Bioinformatics, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.
 6. Ms. Prajna Parimita Kar, Dept. of Bioinformatics, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.
- i) Post M.Sc. Biomedical informatics trainee: 03 (continuing)
- ii) MD and PhD students were provided informatics support based on requirement.



ACADEMIC ACTIVITIES

GOLDEN JUBILEE YEAR CELEBRATIONS

NIP was established in 1965 hence we celebrated Golden Jubilee by organizing number of workshops, seminars and lectures.

INTERNATIONAL WORKSHOP

Organized Foundation workshop on 'Clinical and Laboratory Medicine' research for Member countries of South-Asian Forum for Health Research (SAFHeR) from 9th – 12th Feb. 2015.

The workshop was jointly organized by National Institute of Pathology (NIOP; ICMR), New Delhi and Moving Academy of Medicine and Biomedicine (MAMB), Pune from 9th-12th February, 2015 at National Institute of Pathology (ICMR), New Delhi.

This workshop had been organized to expose undergraduate MBBS students from SAFHeR countries to research methodologies. Two MBBS students and one faculty from each member country had been invited to participate in this workshop, beside 50 Indian Short summer studentship recipients. Students from Bhutan, Bangladesh, Nepal, Maldives and Sri Lanka have participated in the workshop.

The workshop included **Lecture series, Laboratory visits and an Interactive session between Expert Groups from India and International faculty**. A total of 14 Speakers were invited from India who shared their experience in various topics including study designs, biostatistics, commonly used statistical software, data management, medical ethics, GCP and GCLP, clinical protocol development, RCT, reviewing research papers, drafting and submission of research proposals to funding agencies and communication skills were covered. The laboratory demonstrations included various techniques in molecular biology such as DNA extraction, quality and quantitation PCR, Agarose gel electrophoresis, SDS-PAGE, 2-D PAGE, Western blotting, *In situ* hybridization, FACs etc. **Fifty students** (10 from other member Nations and 40 from medical colleges in different parts of India) and **four faculty members** (from Bangladesh, Nepal, Bhutan, Srilanka) participated in the workshop. Students were divided in groups and given the group-task to formulate a Research proposal

on various topics. There was an interactive session between between **Expert Groups from India** (comprising of four members- Dr. Karoon Agrawal, VMMC and SJH; Dr. N. K. Mehra, AIIMS; Dr. Shridhar Dwivedi, Jamia Hamdard and Dr. Bindu Dey, DBT) and **International faculty** on Medical research and Research oriented medical education in the SAFHeR.



NATIONAL WORKSHOPS

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Organized Hands on workshop on “In-situ Hybridization” from 2nd-4th April 2014 and from 12th – 13th January 2015

In-situ hybridization

In-situ hybridization including FISH is an important research tool which has made strong inroads into diagnostic pathology in recent years. It has an important diagnostic and prognostic value in oncology where it is used to detect cytogenetic and chromosomal abnormalities in various tumors. The workshop was intended for medical and biomedical faculty and students to become conversant with this technique and apply the knowledge in their respective fields. It was tailored in such a way that the participants would get hands- on experience about the old elaborate method of doing in-situ hybridization as well as the new advanced method of fluorescent in-situ hybridization (FISH). In addition theoretical concepts were provided through lectures and discussions to facilitate the learning process. The main objective of this course was to equip the participants with an understanding of concepts pertaining to in-situ hybridization and FISH and hands-on experience for its application in various fields. This workshop was attended by 24 participants including 11 medical and 4 biomedical scientists.



Organized workshop on Electron Microscope for Life sciences from 6th July – 7th July 2014.

Workshop was conducted on July 6th and 7th July as pre conference workshop for the Annual Meeting of Electron Microscopy society of India. It was conducted in collaboration with University of Delhi, EMSI and NIP. It was attended by 70 Delegates from all over the India and from abroad also. It covered all the aspects of Electron Microscopy in Life sciences.

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

The patron for workshop was Dr. VM Katoch, DG, ICMR and Mr. NK Ganguly, EX DG ICMR and was convened by Dr. S Saxena, Director, NIOP. The workshop was attended by 15 delegates from medical and non medical fields like pathologists, scientists, students from all over the India. The workshop provided Hands on training on Flow cytometry



Workshops Organized on “Tissue Microarray”

- **Tissue microarray** 18-19th December, 2014. The patron for the workshop was Dr VM Katoch Secretary, DHR and DG –ICMR and was convened by Dr Sunita Saxena, Director NIP. The workshop was attended by 16 delegates from all over India spread across north to south India and also North east India. Delegates included pathologists and biomedical scientists and they were given hands-on experience of making Tissue microarray blocks so that they can independently use this technology to answer their independent research questions.
- Participated in workshop organized on “Manual Construction of Tissue Microarray” at Dept. of Pathology, University of College of Medical Sciences, New Delhi on 12th April 2014

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

Bioinformatics tools for Biomedical Research was organized by the Biomedical Informatics Centre, National Institute of Pathology during March 24-27, 2015. The target audience for the workshop was medical and biomedical faculty, researchers and students with an objective to provide both demonstration and hands-on sessions on basics of Bioinformatics to advance tools and techniques essential for innovative biomedical research. A series of lectures, demonstrations and hand-on sessions were delivered by experts from academia (Jawaharlal Nehru University, New Delhi; Institute of Genomics and Integrative Biology, New Delhi; Indian Council of Medical Research, New Delhi; National Institute of Plant Genome Research, New Delhi; Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra) and

industries (Premas Life Sciences, New Delhi on Illumina and DNASTAR, USA) in topics such as Introductory Bioinformatics, Basics of machine learning, Linux OS overview, Database and tool development through Perl Programming, Comparative Genomics, Protein-Protein Interactions and Next Generation Sequencing data analysis. **Thirty** participants medical as well as non-medical background from different parts of the country were trained in the workshop.



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

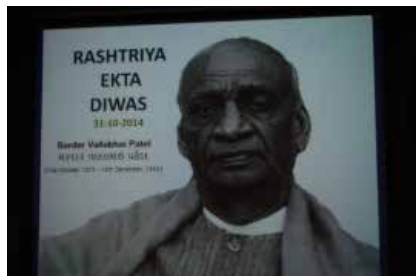
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Molecular cloning and expression of recombinant protein was conducted from March 18-20, 2015. Cloning of the gene of interest its expression and purification of recombinant protein is one of the most widely used molecular biology tool. High amount of purified desired recombinant protein can provide a wide range of utilities including its use in industrial application, pharmaceutical processes, and also in diagnostic or therapeutics. The purpose of this workshop was to provide both demonstration and hands-on experience to cloning and expression of recombinant protein. Main aim of this workshop was to encourage medical practitioners and scientists in relatively remoter parts of the country, to educate them in basics of research and inspire them to submit research proposals. About 17 students including medical and biomedical scientists attended this workshop. Few students as a follow up of this workshop have approached us for help in their experimental parts.

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



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



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



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

AWARDS/PATENTS

AWARDS, PATENTS OBTAINED/FILED

1. **Dr. Poonam Salotra** elected as Fellow of The World Academy of Sciences (FTWAS), 2014.
2. **Dr. Poonam Salotra** elected as Fellow of the National Academy of Medical Sciences, India (FNAMS), 2014.
3. **Dr. Sunita Saxena** awarded “Dr. PN Wahi” award of ICMR, 2014.
4. **Dr. LK Yerneni** and RM Chugh (2014). A method for processing of feeder cells suitable for adult stem cell proliferation. File Number 3115/DEL/2014, Filing date 30/10/2014.
5. **Dr. Nasreen Z. Ehtesham** was invited by visitor (President of India) of Central University of Kashmir as a member of Executive Council.

PATENTS

Indian patent filed (application no. 349/DEL/2014) for “Loop mediated isothermal amplification (LAMP) assay for a reliable and rapid diagnosis of *Leishmania* infection” (2014). Complete specification has been filed on 6th Feb, 2015. Inventors **Dr. Poonam Salotra**, **Dr. Sandeep Verma** and **Dr. Ruchi Singh**.

EXTRAMURAL RESEARCH PROJECTS

NEW PROJECTS

1. Investigation on Paromomycin resistance in *Leishmania donovani* using molecular and biochemical tools.

Dr. Poonam Salotra and Dr. Ruchi Singh, ICMR, 2014-17

2. Identification and characterization of artemisinin resistance associated genes in *Leishmania*.

Dr. Ruchi Singh and Dr. Poonam Salotra, ICMR, 2014-17

3. Evaluation of immune status and parasite load of Post Kala-azar Dermal Leishmaniasis (PKDL) in response to treatment with miltefosine and amphotericine-B.

Dr. Poonam Salotra and Dr. Ruchi Singh, ICMR approved

4. Virtual centre of Excellence (II) on Multidisciplinary approaches aimed at interventions against M.tb.

Dr. Nasreen Z. Ehtesham, DBT 2014-19

ONGOING PROJECTS

1. Study on micro RNA Signature associated with Breast cancer stem like cells (CSCs) and their role in drug response.

Dr. Sunita Saxena, Dr. SA Raju

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

Dr. Sunita Saxena, Dr. SA Raju

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

Dr. Sunita Saxena

4. Expression of aldo-ketoreductase: The effects of drug and tobacco exposure.

Dr. Sunita Saxena

5. Study of characterization of TMPRESS2: ERG and PCA3 as prostate cancer Biomarkers in Indian patients.

Dr. Sunita Saxena, Dr. Anju Bansal

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

Dr. Usha Agrawal

7. Proteomic analysis of Leishmania donovani membrane components involved in host parasite interaction. Post-doctoral Fellowship Project under supervision of **Dr. Poonam Salotra, approved by ICMR, 2014-2016**

8. Understanding the role of micronutrients in M.tb infection using guinea pig as a model.

Dr. Nasreen Z. Ehtesham, ICMR 2013-17

9. Functional Characterization of AipA(RJ1477), an invasion associated and secretion protein and its impact on virulence of Mycobacterium tuberculosis H37RV. Post Doctoral fellowship project under supervision of

Dr. Nasreen Z. Ehtesham, ICMR 2013-16

COMPLETED PROJECTS

1. Genome-wide analysis of genetic alterations in patients with esophageal cancer from north-east India using single nucleotide polymorphism array.

Dr. Sunita Saxena, Dr. Sujala Kapur

2. Epigenetic studies in esophageal cancer in high risk region of Northeast India.

Dr. Sunita Saxena, Dr. Sujala Kapur

3. Immunogenetic profile of nasopharyngeal cancer in a high prevalence region of northeast India.

Dr. Sujala Kapur, Dr. Sunita Saxena

4. Molecular mechanism of drug resistance in acute myeloid leukemia (AML): Role of ATP-binding cassette (ABC) Transporters.

Dr. Sunita Saxena

5. Development of new live-attenuated vaccine candidates for kala-azar.
Dr. Poonam Salotra, DBT 2011-2015.
6. Centre of Excellence (I) on Multidisciplinary approaches aimed at intervention against M.tb.
Dr. Nasreen Z. Ehtesham, ICMR 2009-14
7. Detection of Chlamydia trachomatis in synovial samples from patients with undifferentiated spondyloarthritis/reactive arthritis. ICMR-Senior Research Fellowship project under supervision of **Dr. Sangita Rastogi, 2010-14.**

PUBLICATIONS

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48. Siraj F, Ansari M, Sharma KC, Singh A. Chordoid meningioma: a diagnostic dilemma. *J Cancer Res Therap*, 11:663-665, 2015.
49. Jha A, Taneja J, Ramesh V, Singh A. Annular epidermolytic ichthyosis: a rare phenotypic variant of congenital ichthyosiform erythroderma. *Indian J Dermatol Venereol Leprol*, 81:194-97, 2015.
50. Gopal D, Puri P, Singh A, Ramesh V. Asymptomatic solitary cutaneous mastocytoma: a rare presentation. *Indian J Dermatol*, 59:634, 2014.

ACCEPTED PUBLICATIONS

1. Meena Lakhanpal, Laishram Chandreshwor Singh, Tashnin Rahman, Jagannath Sharma, M. Madhumangal Singh, Amal Chandra Kataki, Saurabh Verma, Santhi Latha Pandrangi, Y. Mohan Singh, Saima Wajid, Sujala Kapur, Sunita Saxena. Study of single nucleotide polymorphisms of Tumour necrosis factors and HSP genes in nasopharyngeal carcinoma in North-East India.” *Tumor Biology*.
2. Nitu Kumari, Pawan Vasudeva, Anup Kumar, Usha Agrawal. Adenocarcinoma of urinary bladder: A report of two patients. *Journal of Cancer Research and therapeutics*.
3. Sharma I, Singh A, Mishra AK, Singh LC, Ramesh V, Saxena S. Is CXCL10/CXCR3 axis a better indicator of leprosy type 1 reaction than inducible nitric oxide synthase? *Indi an J Med Res* 2015.
4. Mallya V, Siraj F, Sharma KC, Singh A. Giant cell glioblastoma with calcification and long-term survival. *Indian J Cancer* 2015.
5. Rastogi S, Yadav C, Das B. *In situ* localization of Chlamydia trachomatis and chlamydial heat shock protein 60 in endometrial curettage tissue of recurrent spontaneous aborters. *J Cytol Histol*, 2015.
6. Rasheedi S, Suragani M, Raviprasad P, Ghosh S, Suragani RN, Ramaiah KV, **Ehtesham** NZ. Functional characterization of PeIF5B as EIF5B homologue from *Pisum sativum*. *Biochimie*. (2015)

BOOKS

Monographs/ Books:

1. Recent Advancement in Cancer and its Prevention. S. Varma. Lambert Academic Publications, Germany, 2014.

BOOK CHAPTER

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

SCIENTIFIC ACTIVITIES

Dr. Sunita Saxena

1. 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.
2. Invited to attend joint Scientific Advisory committee (SAC) meeting for NIRT, Chennai and NJILOMD, Agra held on 10th and 11th January, 2014 at NJIL&OMD, at National JALMA Institute for Leprosy & Other Mycobacterial Disease, Agra.
3. Invited to chaired Institute Ethics Committee meeting held on 27th January, 2014 held at Safdarjang Hospital, New Delhi.
4. Invited to attend Doctoral Committee (DC) of Department of Medical Elementology & toxicology, Jamia Hamdard University, New Delhi held on 29th January, 2014.
5. Invited to attend and Chaired 2nd Session of “Medanta Hepatopathology CME” organized by Department of Digestive and Hepatobiliary Sciences, Medanta on 3rd February, 2014 in Gurgaon.
6. Invited to attend World Cancer Day held on 4th February, 2014 at Institute of Cytology & Preventive Oncology, NOIDA.
7. Invited to attend Post Summit Strategy Session in New Delhi on 6th February, 2014 organized by USA-India Chamber of Commerce, New Delhi.
8. Invited to attend Meeting of the ICMR condemnation Board held in ICMR on 10th February, 2014.
9. 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.
10. Invited to attend Ph.D. Viva-voce Examination of Ms. Rakshan Ihsan held on 24th February, 2014 held at Birla Institute of Technology & Science, Pilani.

11. Attended the meeting of Translational Research of ICMR held on 6th March, 2014 at ICMR, New Delhi.
12. Invited to attend as co-chairman of screening committee for screening the pilot projects of cancer biology at DBT held on 25th – 26th February, 2014 and 11th – 13th March, 2014 at National Institute of Immunology, New Delhi.
13. Invited to attend Curtain Raiser of the film “Scientifically Yours” on Indian Women Scientists held on 6th March, 2014 at Vigyan Prasara, Department of Science and Technology jointly with NISCAIR, CSIR, New Delhi.
14. Attended IAPM (Delhi Chapter) meeting held on 9th March, 2014 organized by Department of Pathology, Army Hospital (R&R), New Delhi.
15. Invited and facilitated in the workshop “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.
16. Invited to attend meeting of Translational Research to discuss phase II technologies held in ICMR, New Delhi on 10th March, 2014.
17. Invited to attend ICMR-AF workshop on chronic noncommunicable diseases, New Delhi organized under object research priorities for Indo-Finland Partnerships in chronic diseases held at ICMR, New Delhi during 11th – 13th March, 2014
18. Invited to attend Selection Committee and Review Committee meeting for review of ICMR Post Doctoral Fellowship held on 12th and 13th March, 2014 at ICMR, New Delhi.
19. Invited to attend and chair Institute Ethics Committee meeting held on 1st April, 2014 in Safdarjang Hospital, New Delhi.
20. Attended meeting of the Translational Research held on 4th April, 2014 in ICMR, New Delhi.
21. Invited to attend and deliver a talk on ‘Role of Tissue Microarray in Pathology’ in Workshop on “Manual tissue microarray construction” held at University College of Medical College, Delhi on 12th April, 2014.

22. Invited to attend Scientific Advisory Group (SAG) meeting of the Division of Basic Medical Sciences (BMS), ICMR held on 23rd April 2014 at ICMR Headquarters, New Delhi.
23. Participated and presented paper in the Human Genome 2014 meet held at Geneva, Switzerland during 27th – 30th April, 2014.
24. Nominated Examiner for Practical Examination of NBE held at K.S. Hegde Medical Academy, Deralakapte, Mangalore from 13th & 14th May, 2014.
25. Invited to attend meeting of the Translational Research of ICMR held on 22nd May, 2014 in ICMR Headquarters, New Delhi.
26. Invited to participate in the Selection Committee meeting to select Senior as well as Young Scientists for award of International Fellowships held on 2nd June, 2014 at ICMR, New Delhi.
27. Invited to attend Project Review Committee meeting of Oncology held at Indian Council of Medical Research, New Delhi on 3rd and 4th 12th June, 2014.
28. Invited to attend and chair the session of automation in molecular diagnostics in CME programme on the Armamentarium of Molecular Diagnosis in Lung diseases held at Vallabhbhai Patel Chest Institute, University of Delhi, Delhi on 11th July, 2014.
29. Attended the meeting of Subcommittee of Breast Cancer for discussions on the projects held at ICMR, New Delhi on 11th July, 2014.
30. Invited to attend meeting of the Screening Committee meeting for NASI Scopus Young Scientist Awards 2014 Medicine held at Stanford India Biodesign Centre, AIIMS, New Delhi on 17th July, 2014 and 8th September, 2014.
31. Invited to attend meeting of the Executive Council of the ICMR held on 21st July, 2014 in ICMR, New Delhi.
32. Invited to attend Scientific Advisory committee on Cancer Research (SACCR) held on 1st August, 2014 in ICMR, New Delhi.
33. Attended and chaired the Institute Ethics Committee meeting of the Safdarjang Hospital, New Delhi held on 7th August, 2014.

34. Invited to attend Research Advisory committee meeting of Rajiv Gandhi Cancer Institute and Research Centre, New Delhi held on 13th August, 2014.
35. Invited to attend doctoral Committee meeting of the Department of Medical Elementology & Toxicology, Jamia Hamdard University, New Delhi held on 14th August, 2014.
36. Attended the meeting of Indian Association of Pathologists and Microbiologists (Delhi Chapter) held on 23rd August, 2014 organized by Vardhman Mahavir Medical College & Safdarjang Hospita, New Delhi.
37. Invited to attend Task force project committee meeting held at ICMR, New Delhi on 28th August, 2014.
38. Invited to attend selection committee meeting for the selection of Emeritus Medical Scientist held on 4th September, 2014 at ICMR, New Delhi.
39. Invited to attend meeting of the Cancer Biology Task Force of the Department of Biotechnology, New Delhi held on 23-24 September, 2014 at ACTREC, Mumbai.
40. Invited to attend project completion PRSG meeting of the project “Development of PC based fully automatic batch analyser” held on 29th September, 2014 at CSIO, Chandigarh.
41. Invited to attend Indo-US workshop “Advanced Workshop on Multicolor Flow Cytometric Immunophenotyping” in collaboration with ISAC and BD Biosciences by Jamia Hamdard University during 27th -28th October, 2014 in New Delhi.
42. Invited to attend Scientific Advisory committee Meeting of Regional Medical Research Centre, Dibrugarh, held on 28th – 29th October, 2014.
43. Attended meeting of the ICMR condemnation Board held on 3rd November, 2014 at ICMR, New Delhi.
44. Attended Selection Committee meeting for finalizing the result for the post of Scientist ‘C’ at NIP held on 12th November, 2014 in ICMR, New Delhi.
45. Attended and chaired the Institute Ethics Committee meeting of the VMMC & Safdarjang Hospital held on 12th November, 2014 held at Safdarjang Hospital, New Delhi.

46. Invited to attend Joint Scientific Advisory committee (SAC) meeting for National Institute for Research Tuberculosis, Chennai and National JALMA Institute of Leprosy and Other Microbacterial Diseases, Agra held on 29th -30th November, 2014 at NJILOMD, Agra.
47. Attended Pre Scientific Advisory Committee meeting of Institute of Cytology and Preventive Oncology, NOIDA held on 11th December, 2014.
48. Invited to attend National Brain Research Centre's 11th Foundation Day Lecture held at India International Centre, New Delhi on 15th December, 2014.
49. Attended Scientific Advisory Committee meeting of National Institute for Research in Reproductive Health, Mumbai held on 17th -18th December, 2014 in Mumbai.
50. Attended Selection Committee meeting for award of ICMR Post doctoral Fellowship (PDF) held on 22nd December, 2014 in ICMR, New Delhi.
51. Attended Scientific Advisory Committee meeting of Institute of Cytology and Preventive Oncology, NOIDA held on 23rd December, 2014.
52. Attended Scientific Advisory Committee meeting of National Institute for Research in Environmental Health, Bhopal held on 25th December, 2014 in Bhopal.
53. Attended Scientific Advisory Committee meeting of Bhopal Memorial Hospital & Research Centre, Bhopal held on 25th December, 2014 in Bhopal.

Dr. Poonam Salotra

1. Attended ECD-PRC meetings at ICMR in July and Oct, 2014
2. Invited speaker at the 3rd International Conference on Clinical Microbiology and Microbial Genomics, held at Valencia, Spain in Sep, 2014.
3. Attended a lecture on "Imaging Drug Target Protein Using x-ray and Electron" by Dr. Xiaodan Li from Paul Scherrer Institute (PSI), Switzerland at NIP in July, 2014.
4. Attended NASI-Scopus Young Scientist Award Ceremony held at The Lalit New Delhi, in Sep, 2014.

5. Invited speaker at the 4th PKDL Consortium meeting held at Istanbul, Turkey in Dec, 2014.
6. Attended Project monitoring committee of Post Doctoral Fellowships at ICMR in Dec, 2014.
7. Participated in Workshop on India Innovation Growth Program 2015 at FICCI Jan, 2015.
8. Invited member at “Kala-azar Elimination Program” partners’ consultative meeting at WHO, Geneva, Switzerland in Feb, 2015. Chairperson of the session on “Case Detection and Surveillance”.
9. Attended the meeting on “*In Vitro* Diagnostics, World IVD Congress (India)” at New Delhi in Feb, 2015.
10. Attended National Science Day Symposium at University of Delhi, South Campus, New Delhi, Feb, 2015.
11. Invited participant at “Informal Expert Consultation on Kala-azar”, WHO-SEARO, New Delhi, March, 2015.
12. Attended series of meetings at DBT regarding Research Collaboration Agreement for studies on vaccine development, in May and Nov, 2014 and Feb, 2015.
13. Served as Co-ordinator for PhD program at National Institute of Pathology.
14. Continued as Co-ordinator of weekly Journal Club/ Seminars at National Institute of Pathology.
15. Reviewer for several projects submitted for funding to ICMR, DBT and DST.
16. Continued to serve as Associate Editor for the journal BMC Infectious Diseases.
17. Appointed reviewer of manuscripts submitted to various journals including PLoS Neglected Tropical Diseases, Antimicrob Agents & Chemotherapy, J of Antimicrobial Chemotherapy, PloS One, Journal of Clinical Microbiology, Experimental Parasitology.

Dr. Nasreen Z. Ehtesham

1. Invited to give lecture in Bioworld 2014 an International meeting “Protein structure and function” organized by IIT Delhi from Dec 4, 2014.
2. Delivered an invited lecture in the international conference on **Cellular and Molecular Mechanism of Disease process**” held in Kashmir from April 13-16, 2014.
3. Member of Institutional Animal ethical committee.
4. Faculty and examiner for Pre PhD course at NIOP for BITS, Pilani and Symbiosis, Pune.
5. Evaluated a thesis submitted at BITS, Pilani, Osmani University, Hyderabad and also was an External examiner for PhD thesis at Osmania University, Huderabad.
6. Reviewed manuscript for Plos One, 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 April 12, 2013.
9. Doctoral Committee member of Ph.D. students at IIT Delhi.

Dr. Sangita Rastogi

1. Awarded Travel Grant by DST for participation in 16th ICID at Cape Town, South Africa (2014).
2. Participated in 16th International Congress on Infectious Diseases held at Cape Town (South Africa) during 2nd – 4th April 2014 and presented poster titled, ‘*Is the role of Chlamydia trachomatis underestimated in reactive arthritis patients in India*’.
3. Attended Workshop on ‘*Flow cytometry data analysis*’ organized by Flow Cytometry Solutions Pvt. Ltd., New Delhi on 12th - 13th April 2014.
4. Attended a talk on ‘*Communicating science with public and policy makers*’ by Prof. D. Balasubramanian, Director of Research, LV Prasad Eye Institute, Hyderabad organized at INSA, New Delhi on 11th June 2014.

5. Invited to attend '**World IVD Congress India Focus Event**' at New Delhi on 5th - 6th February 2015.
6. Nominated for 'Fourth NIAS-DST Program for Women Scientists' on '**Science for Progress in India: Innovations in Technologies**' at National Institute of Advanced Studies (I.I.Sc.), Bengaluru during 23rd - 27th February 2015.
7. Attended seminar organized by *JOVE* at ICMR, New Delhi on 25th April 2014.
8. Faculty for Pre-Ph.D. course at NIP for BITS, Pilani and Symbiosis University, Pune.

Dr. AK Jain

1. Attended 14th Smt Pushpa Sriramachari Foundation Day Oration entitled "Decision to live or die : a cellular view" delivered by Prof. Chandrima Shaha on 7th May 2014.
2. External examiner for the practical examination of Computer Application and Bioinformatics of M.Sc. Biotechnology course of Invertis University, Bareilly on June 04, 2014.
3. Conducted pre-conference workshop on Electron Microscopy at National Institute of Pathology and University of Delhi on 6th and 7th July 2014.
4. Participated in International Conference of Electron Microscopy at Delhi University from July 8-10, 2014.
5. Attended lectures during Flow Cytometry workshop and Hands on training in Basic Flow Cytometry and its applications organized on 28 -29th, Aug, 2014 at National Institute of Pathology, New Delhi.
6. Delivered a lecture on Elemental analysis during XXX National Training Program on Electron Microscopy conducted by SAIF, AIIMS, New Delhi on Nov 22, 2014.
7. Attended a lecture on "Importance of Surfaces for a Successful Animal Cell Culture" on 14.11.2014 at National Institute of Pathology, New Delhi.
8. Attended a seminar "Bringing Nanoworld Together 2014" conducted by M/s Oxford Instruments on 27th to 28th November 2014 at Indian Institute of Technology, New Delhi.

9. External Expert for Specification and Tender Evaluation Committee for purchase of Scanning Electron Microscope and Ultramicrotome and condemnation of Old SEM for SAIF, AIIMS, New Delhi.
10. Attended the Screening Committee, the 36th Meeting of the Screening Committee for Registration of Public Funded Research Institutions/ universities, etc. on 16th December, 2014 at DSIR, Technology Bhavan , New Delhi.
11. Participated as external expert in 32nd Facility Management Committee Meeting (FMC) to monitor the performance and to assess the requirements of the Electron Microscope Facility (SAIF, New Delhi) on 6th January 2015.
12. Attended lectures of Hands on workshop on In situ hybridization at NIP on 12th & 13th January, 2015
13. Attended demonstration of Ion Proton Next Generation Sequencer on 16th Jan. 2015 in Central Instrumentation facility at National Institute of Pathology, New Delhi
14. Participated in organization and technical program of International South Asian Forum for Health Research (SAFHeR) Foundation workshop on Clinical and Laboratory Medicine Research from Feb 9 to 12, 2015 at National Institute of Pathology, New Delhi
15. Participated in a Hindi Seminar titled “Environmental and Occupational Health in the present Scenario” on 20th -21st February 2015 and delivered a talk entitled “Effect of plastic and chemicals used in plastics on reproductive system” at National Institute of Occupational Health, Ahmedabad.
16. Conducted National Workshop on Bioinformatics tools for Biomedical research from 24-27th March, 2015 at National Institute of Pathology, New Delhi.

Dr. Avninder Pal Singh

1. Participated as judge for the poster session in neuropathology at Annual IAPM Conference held at RML PGIMER, Delhi on March 1, 2015

Dr. Fouzia Siraz

1. Presented guest lecture titled- “Cancer Genetics” at SAFHeR 2015, Foundation workshop in Clinical and Laboratory Medicine Research, National Institute of Pathology, ICMR, 9-12 Feb 2015
2. Attended 30th Annual conference of Delhi Chapter IAPM, PGIMER & Dr R M L Hospital, 1st March 2015.

Posters presented

- Pancreatic tuberculosis mimicking pancreatic carcinoma in an immunocompetent host
 - CD10 positive stromal sarcoma of breast: A rare entity
 - Primary hydatid renal disease: A rare presentation
3. Attended PATHCON 2014: New generation diagnostics in anatomic pathology. G.B.Pant Hospital, 10th October, 2014

Dr. Poonam Gautam

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1. Invited talk entitled “Proteomic profiling of *Aspergillus fumigatus* for understanding the molecular mechanisms involved in pathogenesis” in ACBICON 2014 held from 10th-13th December 2014 at AIIMS, Jodhpur, India.
2. Attended 6th Proteomics Society, India meeting and Workshop on Protein Microarrays held from 7th-11th December, 2014 at IIT, Bombay.

STUDENTS ACTIVITIES

1. **Manish Bhuwan** presented in the international conference on **Cellular and Molecular Mechanism of Disease process**” held in Kashmir from April 13-16, 2014.
2. **Javeed Ahamad** presented poster in the international conference on **Cellular and Molecular Mechanism of Disease process**” held in Kashmir from April 13-16, 2014.
3. **Javeed Ahamad** presented poster Bioworld 2014 an International meeting “Protein structure and function” organized by IIT Delhi from Dec 4, 2014.

4. **Saurabh Pandey presented a poster** at International conference **BioWorld 2014** an International meeting “Protein structure and function” organized by IIT Delhi from Dec 4, 2014
5. **Md. Khubaib** attended International conference **BioWorld 2014: Protein structure and function** held on Dec. 2014 at Kusuma School of Biological Sciences, IIT Delhi.
6. **Saurabh Pandey and Md. Khubaib** submitted their Ph.D. thesis.
7. **Himanshu Kaushal** was awarded Dr. S. Sriramachari Young Scientist Award for the year 2014 held on 2nd May, 2014 at National Institute of Pathology (ICMR), New Delhi for the presentation entitled “Role of CD8⁺ T cells in protection against *Leishmania donovani* in healed Visceral Leishmaniasis”.
8. Kumar Avishek participated in the 17th Annual Conference on Vaccine Research, held from April 28-30, 2014, at the Bethesda, Maryland, USA and presented poster entitled “Pre-clinical study to assess the protective immunogenicity of centrin1 gene deleted live attenuated *Leishmania* vaccine candidate in human PBMCs”.
9. **Aditya** participated in a workshop on Theoretical and Practical Course “Molecular Biology of *Leishmania*” and presented a poster entitled “Transcriptome profiling reveals role of ABC mediated drug efflux and altered energy utilization in *Leishmania donovani* in experimental resistance to paromomycin”, held from 22nd to 24th October 2014 at ICGEB, Trieste, Italy.
10. **Deepak Kumar Deep** delivered oral presentation titled “Identification of mechanisms associated with miltefosine unresponsiveness in clinical isolates of *Leishmania donovani*.” in workshop on Theoretical and Practical course “Molecular biology of *Leishmania*”, held on 22th – 24th October 2014 at ICGEB, Trieste, Italy.
11. **Himanshu Kaushal** received two days training on 15th Indo-US workshop on Advance Multicolour Flow Cytometry” held on 27th-28th October, 2014 at BD- JH FACS Academy Jamia Hamdard, New Delhi.



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National Brain Research Centre (NBRC),
Near NSG Campus,
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Director,
Advance Center for Treatment,
Research & Education in Cancer,
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9. **Dr. Sudha Bhattacharya**
Professor & Dean,
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15. Dr. Ravi Mehrotra

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16. Dr. Vijay Kumar

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REFERRAL SERVICES

REFERRAL SERVICES

MOLECULAR BIOLOGY LABORATORY

A total of 130 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 Vanila Sharma, Mr Himanshu Kaushal, Mr Kumar Avishek, Mr Deepak Kumar Deep, Ms Aditya, Mr Uday, Ms Amandeep Kaur, Ms Kamlesh Sharma, Mr. Anish Saxena, Mr R.C. Chhetri

HISTOPATHOLOGY AND CYTOLOGY LABORATORY

The Diagnostic services are offered to the patients of Safdurjung Hospital for both tissue and FNAC samples. In addition we serve as a referral centre for pathology for samples from all over India. The spectrum of samples received encompass Surgery, Eye, ENT, Dermatology, Genito-Urinary and Neurosurgery. Ancillary tests such as immunohistochemistry, immunofluorescence, and frozen sections are also available with a large panel of diagnostic antibodies for differential diagnosis in Lymphomas, soft tissue tumours, round cell tumours, etc. Research is supported for scientists within the Institute as well as outside students from prestigious Institutes/Universities such as Delhi University, National Institute of Immunology, BHU, Varanasi and Jawaharlal Nehru University.

Scientific Staff: Dr. Sunita Saxena, Dr. Usha Agrawal, Dr. Avninder Singh, Dr. Anju Bansal, Dr. Fouzia Siraj, Dr. Shruti Sharma, Dr. Varsha Dalal, Dr. Manveen Kaur

DNB students: Dr. Mariya Khatoon Ansari, Dr. Ritu Jadhav, Dr. Reena Jain, Dr. Manju Bhamu, Dr. Anupama Jha, Dr. Rakesh, Dr. Reetika Menia, Dr. Manisha Choudhary, Dr. Sonam Jain, Dr. Kaushik Kar, Dr. Sadia Khanam, Dr. Chandrabhushan

Technical Staff: Mrs. Karuna, Mrs. Krishna, Mr. Jagdish Pant, Mr. Satyapal, Mr. Madan Lal, Mr. Raj Singh, Mr. Anil Verma, Mr. Shiv Bahadur, Mr. Shyam Sundar, Mr. Sanjay, Mr. Bala Dutt, Mrs. E. Shardha

MICROBIOLOGY LABORATORY

The Microbiology laboratory at NIP focuses on research in chlamydial infection pertaining to genital chlamydia and *Chlamydia*-induced reactive arthritis. In the year under report, synovial fluid and 48 urine specimens were further collected from Army hospital (R&R), New Delhi and Safdarjung hospital, New Delhi from patients with reactive arthritis/ undifferentiated spondyloarthropathy and recurrent aborters, respectively. Samples were processed for doing diagnostic and immunomolecular studies on chlamydial infection.

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

ANIMAL HOUSE FACILITY

The Animal House at NIP is a central facility registered with **CPCSEA** (*Committee for the Purpose of Control and Supervision of Experiments on Animals*), Ministry of Environment, Forests & Climate Change (GOI) which provides technical service to the Institute's scientists for small animal experimentation in **IAEC**-cleared projects as per the CPCSEA guidelines. During the reporting period, the registration of Animal House Facility was renewed (**new registration no.: 102/GO/ReBi/S/99/CPCSEA**) for the period, *w.e.f.* 28.04.2014 to 27.04.2017 by CPCSEA and two IAEC meetings were conducted at NIP for ethical clearance of research projects. Also, inspection of the facility was undertaken by the CPCSEA nominee on 17.10.2014.

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

INFLAMMATION BIOLOGY AND CELL SIGNALING LABORATORY (IBCL)

This group has been working on inflammation and pathogenesis and micronutrient-mediated immune modulation. It was shown that resistin additionally acted as a molecular chaperone during cellular stress, thereby establishing for the first time a link between inflammation, cellular stress, and protein folding. It was further shown that levels of resistin in human serum were directly linked to TB disease intensity with levels falling as a function of positive treatment outcome. While these data pointed to the presence of a new cellular axis of inflammation, infection and unfolded protein response (UPR) we could indeed show that *M. tuberculosis* MoxR protein, by virtue of its novel chaperone function, assisted folding of a pathogen virulence factor

RipA. Our collaborative project on comparative genomic and proteomic analyses identified molecular attributes of virulence and pathogenicity, and additionally generated molecular markers for TB diagnosis.

Staff: Dr. Nasreen Z. Ehtesham, Dr. Naresh Arora, Dr. Manish Bhuwan, Md. Khubaib, Saurabh Pandey, Javeed Ahmad, Simran Kaur Arora, Rishi Sharma, Javaid Ahmad Sheikh, Lallan Kumar

IMAGING LABORATORY

The imaging laboratory houses the Tissue microarray facility, Digital slide scanning station and Cytogenetics Workstation. The equipment is state-of-art and the scientists are well-trained in the operation of these workstations. The equipment is being used in both ongoing as well as new research projects. Three National workshops have also been organised in the past year to train scientists and students in these technologies.

Staff: Dr Usha Agrawal, Dr Avninder Singh, Dr Fouzia Siraj

BIOMEDICAL INFORMATICS CENTRE

Biomedical informatics Centre at National Institute of Pathology has initiated research and training facility for biomedical scientists, research scholars and students to promote and support informatics in medical research. The centre is furnished with Lenovo workstation with hexa-core Intel Xeon processor, 36 GB RAM; three state of art desktop machines equipped with academic licensed Bioinformatics software and 1 Gbps internet connectivity.

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

- Integrated catalogue on genome wide association studies and drug targets of psoriasis.
- *In silico* screening of TNF-alpha, PDE4 and JAK-2 inhibitors.
- Computational design of Interleukin – 6 antibodies.
- Putative drug target identification against zoonotic pathogens.
- Developed Perl scripts for predicting presence of toxic compound in patient samples.
- Bioinformatics centre web portal to in-house integrated catalogue and databases.

- Organized one National workshop to render Bioinformatics training to 30 Biomedical scientists/researchers.
- Six M.Sc. students were given training to complete dissertations.
- Developing NIP patient sample electronic record is undergoing.

Staff: Dr. Arun Kumar Jain, Dr. Dibyabhaba Pradhan, Dr. Shweta Aggarwal, Mr. Arnab Nayek, Mr. Prince Gautam

FLOWCYTOMETRY LABORATORY

In total 656 samples of blood, leukaemic patients, samples were acquired and analysed for surface antigen expression, intracellular cytokine response and apoptosis.

Staff: Dr Aruna Mittal, Dr. S. Verma, Mr. P.D. Sharma

CONFOCAL LASER SCANNING MICROSCOPY LABORATORY

Total of 69 images were acquired on confocal laser scanning microscope.

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

INFORMATION TECHNOLOGY/COMPUTER DIVISION

Computer Division of the Institute serves as the backbone for communication for all other divisions and department of the Institute through Local Area Networking and providing internet services. The Computer Division is equipped with the three servers for LAN, Antivirus and application programs along with latest computer systems, a laser printer, color inkjet printers, scanner and up to date software. The department helps the students in conducting weekly journal club meeting as well as other Data entry and formatting for their thesis. Through maintenance of Histopathology Software it helps in recording, storage and archival as well as of histopathology data. With the recent emphasis the Government on Digital India, Minimum Government Maximum Governance, e-governance etc, computer division has been entrusted with implementation of these policies and accordingly has been playing crucial and active role in this activity.

The department helps in the compilation and generation and printing of Annual Report, Highlights and other documents. The computer division also takes care of day to day assistance and maintenance of more than 100 computers in different divisions of the

Institute. Computer Division is also responsible for maintaining the Institute's Web Site, uploading latest information and updating the different web pages.

It is actively involved in financial accounting i.e generation of pay, paybills, schedules, pay slips, compilation of income tax, filing of quarterly and annual tax returns etc.

Staff : Dr. Arun Kumar Jain, Mr. Shiv Parkash, Ms. Seema Sharma

CENTRAL PROTEOMICS FACILITY

The facility has the set up for gel-based separation of proteins. The facility is equipped with IEF Cell, SDS-PAGE (small, medium and large format), ChemiDoc MP Image scanner and 2-D gel analysis software (PD Quest Advanced Software version 8.0), scanner for imaging for fluorescently labelled proteins, wet and semi-dry transfer apparatus. We are expanding the set up for liquid chromatography-based separation of proteins/peptides and procurement of high pressure liquid chromatography (HPLC). The facility is being used by various scientific groups in the Institute.

Staff: Dr. Poonam Gautam, Ms. Priyanka Tripathi

LIBRARY

During the reporting period, the library continued to cater to the needs of the Institute's scientific staff, administrative staff, DNB/ M.Sc./ Ph.D. students, project staff and trainees. The library further subscribed to **14** online journals through *Wiley* and **15** Indian journals. The facility continued to give services to its users including access to online journals and reprints through inter-library loan from Safdarjung hospital library, NIC, National Medical Library, INSDOC and others. Inter-library loan requests were received through email and photocopies were dispatched by post. Xerox facility was further continued for the benefit of scientists and students.

The Institute's library has more than **10,000** books, bound journals, CDs, thesis, annual reports, WHO and AFIP fascicles pertaining to pathology, cancer, computer science, immunology, infectious diseases, toxicology, statistics, electron microscopy, confocal microscopy, sub-branches of pathology and other specialized medical subjects; Hindi books were further added to the library collection. The library displayed recent scientific publications, annual reports, newsletters of other Institutes, newspaper clippings of scientific, technical and various government publications. It also provided other services like indexing and abstracting of books/ journals. Also, the publication of

NIP Newsletter was continued during the year and it was distributed to various ICMR Institutes and medical institutes.

NML-ERMED, JCCC@ ICMR (consortium between NML - ICMR - AIIMS), Cochrane, ICMR consortia and other online services were further provided to the scientific/ technical staff of NIP. JCCC and J-Gate custom content for consortia through *Informatics India Ltd.* was used for resource sharing with other ICMR libraries. The Local Area Network (LAN) facility (six nodes terminal internet connection) installed in the library for internet browsing and e-mail access was further continued. Online journals subscribed from Science Direct, Wiley, ICMR Consortia, NML-ERMED were accessed by scientists on their desktop. The facility thus allowed its users greater flexibility in meeting their needs.

Staff: Dr. Sangita Rastogi, Mrs. Anita Sharma, Mrs. Sangeeta Batra, Mr. Dharmender.
Email : ioplibrary@rediffmail.com



STAFF LIST

STAFF LIST

SL.NO.	SCIENTIFIC STAFF	DESIGNATION
1	Dr. Sunita Saxena, MD (Path)	Director
2	Dr. Poonam Salotra, Ph.D. (Biochem)	Scientist 'F'
3	Dr. AK Jain, Ph.D	Scientist 'F'
4	Dr. Nasreen Z Ehtesham, Ph.D	Scientist 'F'
5	Dr. Sangita Rastogi, Ph.D. (Zoology)	Scientist 'F'
6	Dr. Usha Agrawal, MD	Scientist 'E'
7	Dr. LK Yerneni, M.Sc., Ph.D.	Scientist 'E'
8	Dr. Anju Bansal, MBBS, MD (Path)	Scientist 'D'
9	Dr. Avninder Pal Singh, MBBS, MD (Path)	scientist 'D'
10	Dr. Ruchi Singh, Ph.D	Scientist 'D'
11	Dr. Saurabh Verma, M.Sc., Ph.D	Scientist 'D'
12	Dr. Appala Raju Bagadi, MD	Scientist 'C'
13	Dr. Fouzia Siraj, MD	Scientist 'C'
14	Dr. Shruti Sharma, DNB	Scientist 'C'
15	Dr. Neeraj Kumar, Ph.D (from Feb. 2015)	Scientist 'C'
16	Dr. Poonam Gautam, Ph.D	Scientist 'B'
17	Dr. Prakash K Sahoo (upto June 2015)	Scientist 'B'

SL.NO.	ADMINISTRATIVE STAFF	DESIGNATION
1	Mrs. R. Saratha	Administrative Officer
2	Mr. Raja Ram	Accounts Officer
3	Mr. Yogender Kumar	Section Officer (upto April 2015)
4	Mrs. Sunita Ahuja	Section Officer
5	Mr. VS Rawat	Section Officer
6	Mr. Jagdish Kain	Private Secretary
7	Mr. Dashrath G. Khambadkar	Assistant
8	Ms. Anita Sharma	Assistant (Library)
9	Mrs. Rekha Rani	Personal Assistant
10	Mr. Subhash Babu	Assistant
11	Mr. Mangey Ram	Assistant
12	Mrs E. Sharda	Personal Assistant
13	Mrs. Sushma Ralhan	Assistant
14	Mrs. Sharmila Kamra	Assistant
15	Mr. Ajay Joshi	U.D.C.
16	Mr. Sonia Khattar	U.D.C.
17	Mr. Rajesh Kumar	U.D.C.
18	Mr. Brijender Singh	U.D.C
19	Mrs. Archana	U.D.C
20	Ms. Jyoti	U.D.C

SL.NO.	TECHNICAL STAFF	DESIGNATION
1	Dr. L.C. Singh	Technical Officer 'A'
2	Mrs. Madhu Badhwar	Technical Officer 'A'
3	Mr. Shiv Prakash	Technical Officer "A"
4	Mrs. Seema Sharma	Technical Officer 'A'
5	Mrs. Anita Bhatia	Technical Officer 'A'
6	Mr. Chandi Prasad	Technical Officer 'A'
7	Mrs. Karuna	Technical Assistant
8	Mrs. Krishna	Technical Assistant
9	Mr. Kuldeep Kumar Sharma	Technical Assistant
10	Mrs. Valsamma Mathew	Technical 'C'
11	Mr. Jagdish Pant	Technician 'C'
12	Mr.P.D. Sharma	Technical Assistant
13	Mr. Suresh Bhimrao Kamble	Technician 'C'
14	Mr. Pushp Raj	Technician 'C'
15	Mr. Satyapal Singh	Technician 'C'
16	Mrs. Sangeeta Batra	Jr. Librarian
17	Mr. Ishwar Singh	Technician 'B'
18	Mr. Madan Lal	Technician 'C'
19	Mr. Shiv Bahadur	Technician 'C'
20	Mr. Manwar Singh	Driver
21	Mr. Puran Singh	Driver
22	Mrs. Santosh Deora	Receptionist- cum-Tele. Operator
23	Sh. Bijendra Kumar	Technician 'B'
24	Sh. Kamal Dev	Technician 'B'
25	Mr. Anil Kumar Verma	Technician 'B'
26	Mr. Ajit Singh Lehra	Technician 'B'
27	Mr. Shyam Sunder	Technician 'B'
28	Mr. Daya Sagar	Technician 'B'
29	Mr. Sajid Hussain	Multi Tasking Staff (TECHNICAL)
30	Mr. Bala Dutt	Multi Tasking Staff (TECHNICAL)
31	Mr. Jagdish Ram	Multi Tasking Staff (TECHNICAL)
32	Mr. Dharmendar Singh	Multi Tasking Staff (TECHNICAL)
33	Mr. Ram Chander Das	Multi Tasking Staff (TECHNICAL)

SL.NO.	TECHNICAL STAFF	DESIGNATION
34	Mr. Puran Chand	Multi Tasking Staff (TECHNICAL)
35	Mr. Rajendra Kumar	Multi Tasking Staff (TECHNICAL)
36	Mr. Manoj Kumar	Multi Tasking Staff (TECHNICAL)
37	Mr. Anish Kumar Saxena	Multi Tasking Staff (TECHNICAL)
38	Mr. Raj Singh	Multi Tasking Staff (TECHNICAL)
39	Mr. Chandrika Prasad	Multi Tasking Staff (TECHNICAL)
40	Mr. Sanjay Dutt Upreti	Multi Tasking Staff (TECHNICAL)
44	Mrs. Charanjit Kaur	Multi Tasking Staff (TECHNICAL)

