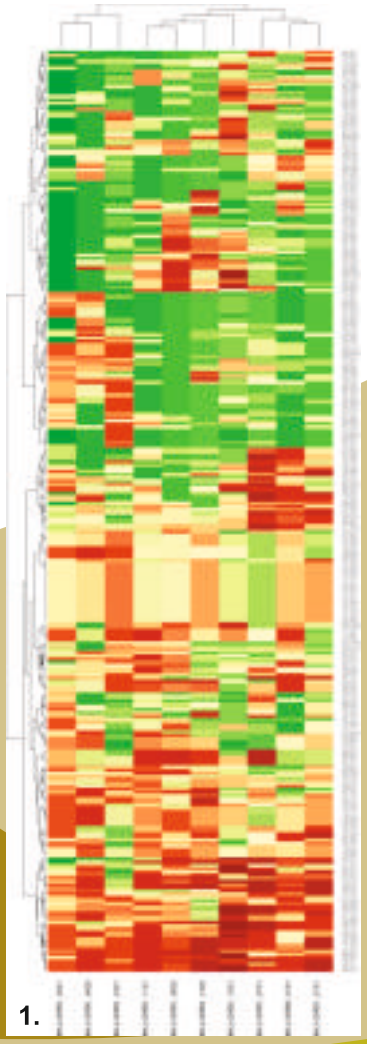

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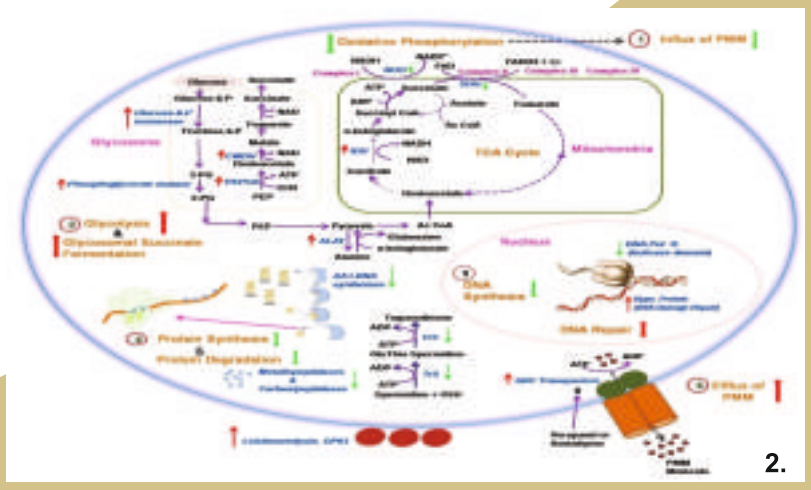

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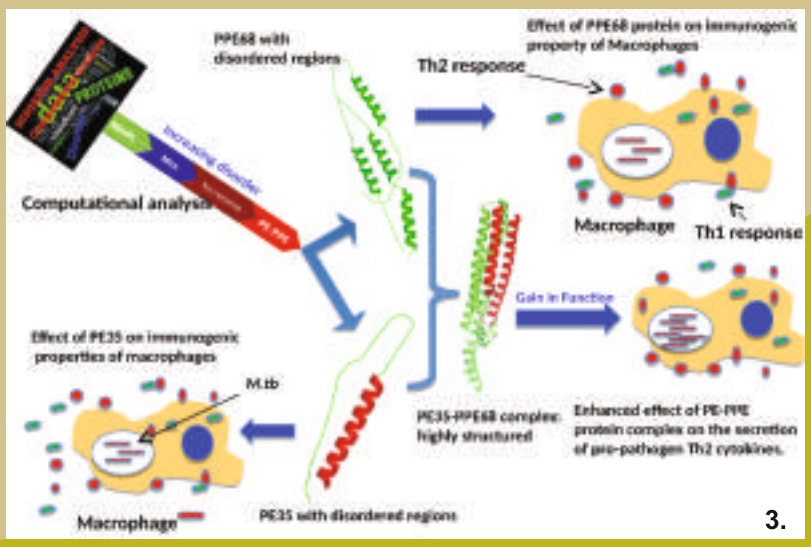

3.

ANNUAL REPORT

2016 - 2017

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

PO Box 4909, Safdarjang Hospital Campus, New Delhi 110 029

Website : www.instpath.gov.in Email : info@instpath.gov.in

1: Clustered heat map of human candidate proteins associated with late onset complex diseases and their binding affinity threshold with common HLA class II alleles.

2: Predicted adaptations in *L. donovani* in Paromomycin (PMM) resistance

3: Proposed model for gain in immunogenicity through protein-protein interaction and disorder-to-order transition of PE-PPE protein pairs.

ANNUAL REPORT

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CONTENTS

Executive Summary

It is my proud privilege to present the Annual Report of our Institute for the year 2016-17. The National Institute of Pathology has continued its stride towards making significant scientific contributions in the various thrust areas of the institute during the year 2016-2017, including tumour biology, infectious diseases (leishmaniasis, tuberculosis, leprosy & chlamydiasis), stem cell biology and environmental toxicology. Scientists made seminal contributions in basic & translational research by identifying markers of diagnostic and prognostic importance against various cancers, developing novel simplified and reliable diagnostic assays, development of a vaccine for visceral leishmaniasis and carrying forward the technology for epidermal expansion for auto transplantation in burns patients.

Under translational research we have developed a LAMP assay for detection of *Leishmania* in clinical samples, for which the technology transfer to industry is under consideration. Pre-clinical studies are being pursued to develop live attenuated vaccine against kala-azar. An improved technology to grow cultured epithelial autografts (CEA) for burns patients has been established and work is ongoing for its clinical trials. New genetic markers for diagnosis of *M. tuberculosis* are being taken up for validation.

The tumour biology group is working in cancers of breast, genitourinary system, gall bladder, brain and haematological malignancies to identify markers of diagnostic and prognostic importance and novel drug targets. Whole exome and transcriptome profiles of these tumours in the Indian population are being characterised. Proteome studies in urinary bladder cancer have identified proteins of interest present in urine and are being validated for their application as a screening marker in patients on follow-up. Immunoproteomic studies are underway for detection of autoantibodies having the potential to identify multiple proteins eliciting humoral response in gall bladder cancer patients at early stages. studies on hormone sensitive and hormone refractory prostate cancer have identified chromosomal regions and global transcriptional changes that are important for tumour progression and aggressiveness. Studies on molecular regulation of mTOR signalng in acute lymphocytic leukaemia patients revealed its correlation with clinical response

The technology for growing epidermis for application in burns patients had been earlier standardized and efforts are ongoing for cGMP construction to facilitate clinical trials. During this year the studies on Identification of growth stimulating proteins expressed by the optimal feeder cells led to the first ever demonstration of fibroblast cell proteins as exclusive growth promoters of human epidermal keratinocytes. Work was also initiated to develop technology to grow non-xenogeneic CEA using human dermal fibroblasts as feeders.

Towards understanding the molecular mechanisms of resistance to paromomycin and artemisininn in *Leishmania donovani*, identification of genes/pathways associated with drug resistance was carried out. Various adaptations responsible for paromomycin resistance were predicted. Validation of the LAMP assay was completed at both RMRI, Patna and IMS, BHU, Varanasi. Direct Blood Lysis (DBL) approach was attempted, in order to eliminate the DNA isolation step.

Molecular antimicrobial resistance screening methods are needed to improve the diagnosis of nosocomial and community acquired multi drug resistant bacterial infections. Development of LAMP assays was initiated for rapid surveillance of carbapanem resistance gram negative bacteria.

Studies have been ongoing to decipher pathogenesis of tuberculosis and developing diagnostic assays. Signature sequences unique to *M.tuberculosis* were identified and are being characterized to understand their role in pathogenesis and their vaccine potential. Studies on immunomodulatory role and antigenicity of PE/PPE genes indicated that these protein complexes influence the host immune system towards favouring pathogen survival. the role of micronutrients in Mtb infection using guinea pig model was investigated.

Work has been ongoing to understand the immunopathogenic mechanisms involved in *Chlamydia trachomatis*-infected tubal ectopic pregnancy (EP). In the current year, the role of matrix metalloproteinases (MMPs) in the pathogenesis of EP in infected women was investigated. The mRNA expression of MMP-2 and MMP-14 was found to be increased in *C. trachomatis*-infected women undergoing salpingectomy (SALP) for EP in comparison to the controls.

Biomedical Informatics Centre of ICMR (Phase II) at the Institute has been carrying out research activities in line with the primary objectives outlined by the taskforce. The Centre is constantly striving to promote and support informatics in biomedical research at the institute and by training researchers at National level through workshops. BIC has developed a comprehensive database of genes and genetic markers associated with psoriasis, dbGAP, an integrated knowledgebase representing a gateway to psoriasis associated genomic data.

Actively involved in the MRHRU program of DHR, NIP created the infrastructure for carrying out diagnostic studies and management of diseases prevalent in and around Bhunga, Hoshiarpur Dist., Punjab. The construction of the building was completed last year and installation of major equipment was undertaken in the current year. Projects on Dengue surveillance and Nutritional profile in the area were initiated.

In order to disseminate knowledge to budding medical and biomedical researchers, three National workshops were organized on topics of wide interest, including on Next generation Sequencing data analysis. Eminent biologist Dr. Rajesh Gokhale delivered 16th Pushpa Sriramachari oration entitled “Demystifying the vitiligo Conundrum” on the foundation day at NIP. On this occasion Young scientist award was given to Mr Javeed Ahmed.

Some of our scientist & research scholars were honoured for their achievements by National & International scientific bodies. The year was highly productive as evidenced by a large number of published research papers, majority of which were in reputed journals with high impact value. An Indian patent was granted for A CULTURE SYSTEM FOR THE GROWTH OF STEM CELLS.

I am deeply appreciative of the efforts put in by the scientists, students, technical & administrative staff for the growth of the Institute. I would like to acknowledge the constant support & encouragement provided by Dr. S. Swaminathan, DG ICMR & Secretary DHR.

Poonam Salotra
PhD, FNA, FNASc, FTWAS, FAMS

RESEARCH ACTIVITIES

TUMOUR BIOLOGY

TUMOR BIOLOGY

1. Differential Protein Profile for Identification of Markers in Recurrent Urothelial Cancer

Scientific staff : Dr Usha Agrawal and Dr Nitu Kumari
Collaborators : Dr Anup Kumar, Dr Pawan Vasudeva
Funding Agency : ICMR

Identification of urinary biomarker may decrease the frequency of cystoscopy and predict recurrence of urothelial bladder tumor using non-invasive samples. In the present study, we have identified differentially deregulated proteins in urothelial bladder cancer compared to adjacent normal mucosa using iTRAQ-LC-MS/MS. The identified deregulated proteins (SYNDECAN1, MMP1, Tenascin C, VEGFA, PRDX1 and PRDX2) were further verified by immunohistochemistry on formalin-fixed paraffin embedded tissue of bladder tumor and adjacent normal mucosa (n=119) and common upregulated proteins were validated on urine sample of non-maglinant patients and bladder cancer patients by ELISA (n=150). Estimation of ELISA showed significant elevation of urinary PRDX1, PRDX2 and VEGF in urine of bladder cancer and recurrent bladder cancer patients ($p<0.001$). Median concentration of urinary PRDX1 (29.4 ng/ml) and urinary PRDX2 (21.94 ng/ml) were used for Kaplan Meier survival analysis and result showed that the concentration of urinary PRDX1 was not associated with survival whereas urinary PRDX2 was associated with recurrence and poorer survival of bladder cancer patients though it was not statistically significant. Urinary VEGF concentration of higher than 1954.58pg/ml was significantly associated with recurrence of patient or poorer survival and p-value was <0.039.

2. Genome-wide analysis of genetic alterations and gene expression profiles in hormone sensitive and hormone refractory prostate cancer

Scientific staff	:	Dr. Anju Bansal, Ms Prachi Gupta, Mr. Rohit Singh Rawat, Mr. Satish Kumar
Collaboration with	:	Dr. Anup Kumar, Department of Urology, Safdarjung Hospital, New Delhi
Duration	:	2015-2018
Funding Agency	:	ICMR

Aims, Objectives and Background

Prostate cancer is the second most common cancer in men with 1.1 million cases worldwide and is the fifth leading cause of death from cancer in men. In India, the incidence and mortality rate shows an increasing trend since last few decades.The genetic changes underlying the development and progression of prostate cancer are poorly understood. Comparative genomic hybridization (CGH) for amplification and loss of DNA sites allows screening of the whole genome for sequence copy number alterations. Array CGH analysis can substantially narrow down the regions of interest relevant in clinical tumor specimens. Next generation sequencing technology permits the investigation of an entire cancer genome and transcriptome with unprecedented resolution and throughput. RNA-seq enables comprehensive analysis of gene expression simultaneously revealing multiple aspects of the transcriptome. The aim of this study is to identify those chromosome regions and global transcriptional changes that are important for the development of prostate cancer and to identify genetic markers of tumor progression and aggressiveness.

Work done during the year

Array-CGH was performed for twenty four prostate tissue samples comprising of twenty tumor samples (10 from each group) and four benign tissue samples, selected as cases and controls respectively. Hybridization and analysis were performed using

Agilent Sureprint CGH+SNP 4x180K (Agilent Technologies, Inc., Santa Clara, CA, USA) following the manufacturer's instructions. The arrays were scanned at 2-µm resolution using an Agilent microarray scanner and analyzed using Feature Extraction v10.10 and Agilent Cytogenomics CGH + SNP software version 4.0 software (Agilent Technologies). Chromosomal aberrations in 24 samples (20 malignant and 4 benign) with respect to Agilent Male_dbSNP141 reference genome (hg19) were determined using aberration detection method-2 (ADM-2) algorithm. Comparison of chromosomal aberrations between malignant and benign tissue samples was carried out. Array- CGH data showed more number of gains and less number of losses. 22cytogenic locations (15 gains and 7 losses) were observed to be representing CNAs associated with prostate cancer. Analysis of averaged frequencies of copy number gains and losses revealed that CNAs were detected across the entire genome. Regions of gains detected in cases of Group 1 were 1q31.3, 2p21, 7p21.1, 7q36.3, 10q24.31-q24.32, 14q23.1, 16p11.2-p11.1, 19q11-q12 while region of loss detected in group 1 was 17q11.2. Similarly, Regions of gains detected in cases of Group 2 were 2p11.2, 4p16.1, 22q11.21, yq11.21, yq11.221 and losses in group 2 were 6p25.3, 10q11.22, 15q11.1-q11.2, 16q23.3-q24.3, 17p13.3-p11.2.

Four prostatic adenocarcinoma samples were processed for RNA-Seq analysis. These 4 samples had 2 samples in each subgroup.High-throughput Illumina2500 sequencing was completed, the data generated as Fastq text file.Differential gene expression analysis was performed.Quality metric for the RNA-Seq reads were evaluated using FastQC. The sources of bias like GC content, rRNA depletion, errors produced during sequencing, adaptors removal and trimming was performed to generate improved quality raw data that can be used for creating de-novo assembly and for mapping against the hg19 reference genome using TopHat2. Abundance of each transcript (FPKM) was computed using Cufflink. Differentially expressed genes with respect to 4 control samples(logFC> 1 or logFC< -1; FDR <0.05) were identified using Cuffdiff. Gene ontology annotation and pathway enrichment were carried out using Cluster profiler bioconductor package implemented in R.The four RNA-Seq processed samples were evaluated to follow necessary QC quality metrics required for RNA-Seq analysis. Total 1480 genes were found to be up-regulated when control was compared with Group1 and 2295 up-regulated genes when control was

compared with Group2. Similarly, 1596 genes were found to be down-regulated when control was compared with Group1 and 2089 down-regulated genes when control was compared with Group2. Functional enrichment analysis of dysregulated genes showed their involvement in vital biological processes (Figure1-4).

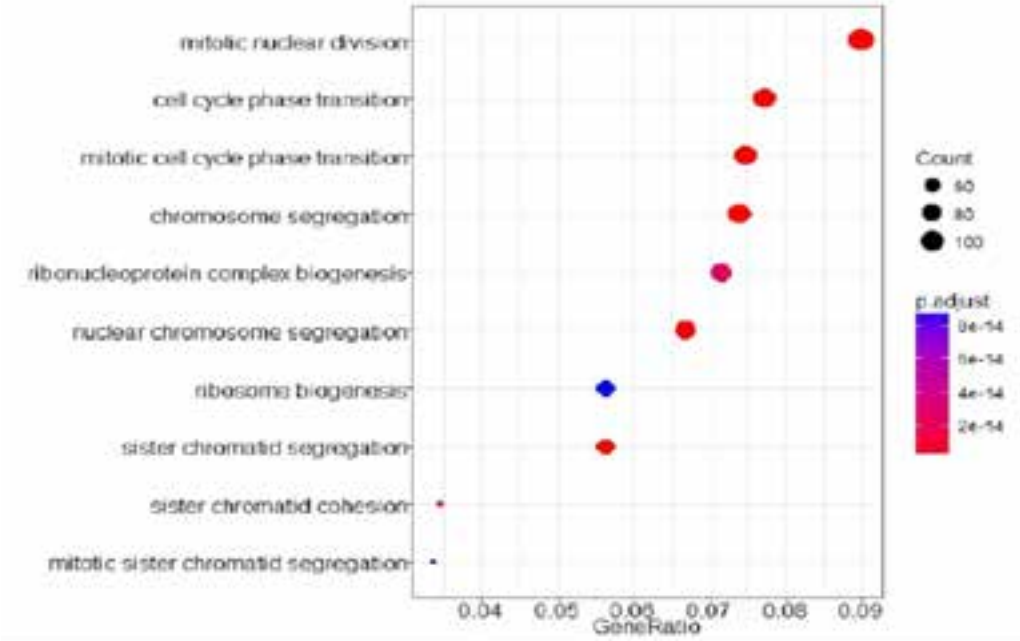


Fig.1: Biological processes up-regulated in group 1.

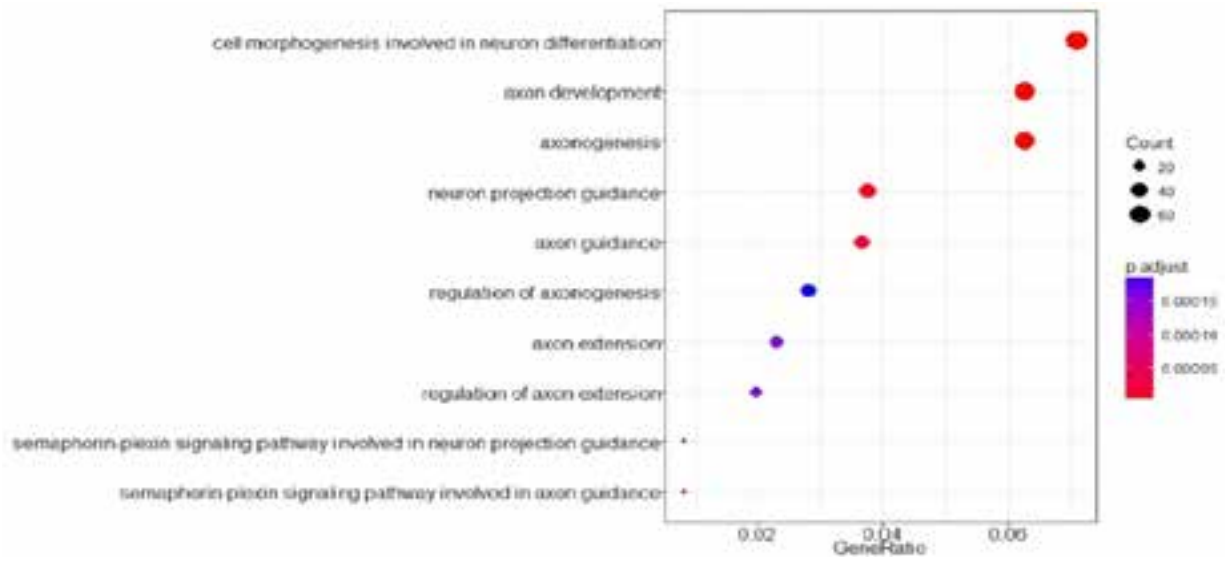


Fig. 2: Biological processes down-regulated in group 1.

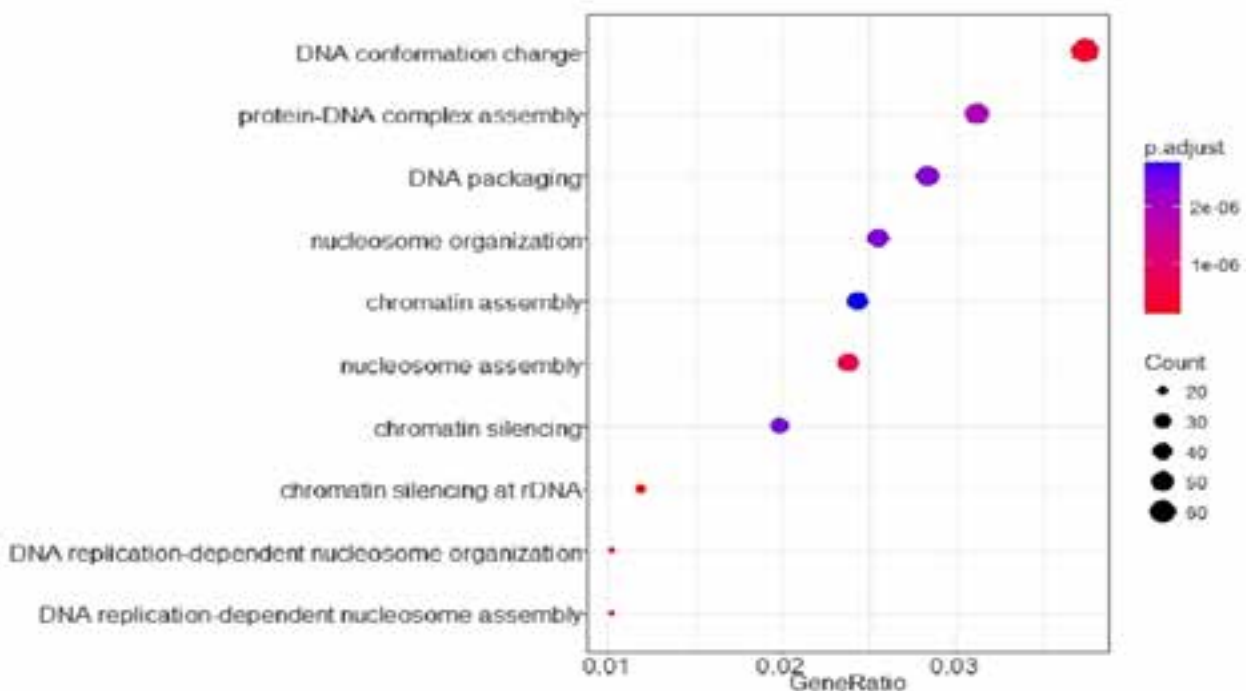


Fig. 3: Biological processes up-regulated in group 2.

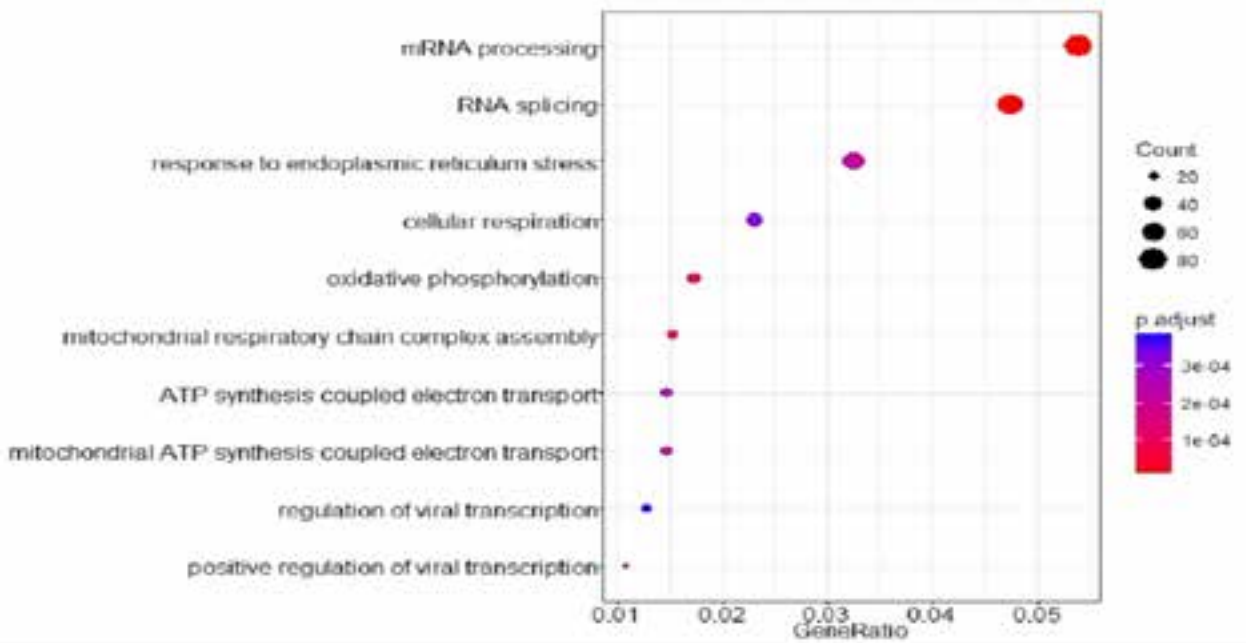


Fig. 4: Biological processes down-regulated in group 2.

3. Identification and Determination of potent inhibitors against cyclooxygenases receptors using computer aided drug design approach.

Scientific Staff : Dr Saurabh Verma and Dr. Ankita Sahu

Collaborator : Dr D. Pradhan, Dr AK Jain (NIOP)
Dr SA Raju Bagadi (NIOP)

Techincal Staff : Mr. P.D. Sharma

Year : 2016-2018

Funding Agency : ICMR

Background

Cyclooxygenase enzyme (COXs) consists of two isoforms, i.e., cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), catalyze the conversion of arachidonic acid to PGH2 in the first two steps. COX-1 isoform has been suggested as a constitutively expressed enzyme, which is responsible for maintaining normal physiological function. On the other hand, the second COX-2 isoform is inducible and the expression is stimulated in the inflammation process. The protein is found to be over expressed in the multiple types of human cancers including melanoma, Ewing sarcoma, ovarian, glioblastoma, prostate, etc.

Structurally, human COX-1 and COX-2 show as homodimerization stabilized by hydrophobic interactions, and hydrogen and electrolytic bridges. They contain single peptides differ in length and glycosylation patterns. Numerous reviews explain the structure and function of the isoforms of cyclooxygenase enzyme and their relationship. Both COX isoenzymes activate signaling reaction which involve electron transfer (reduction/oxidation or redox) reactions. This is an intrinsic, highly active peroxidase (POX) activity. Both activities are different, physically as well as functionally. There are 21 PDB structures of human cyclooxygenase -2 are available in http://www.rcsb.org/.

AIM

The present study deals with the identification and characterization of potent inhibitors against cyclooxygenase enzyme using bioinformatics approach.

Objectives

- To analyze the COX structures for docking and virtual screening and to find out protein-ligand interaction for identifying the new drugs in protein model.
- To find out the new active drug on the basis of *in-silico* and *in vitro* method

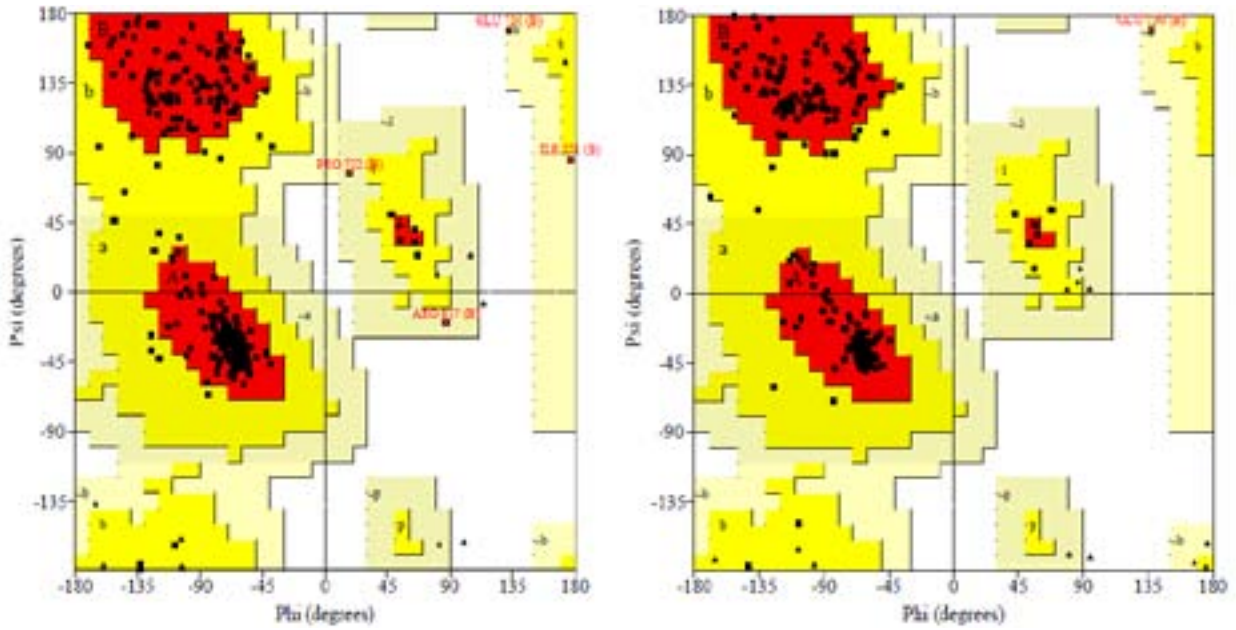
Work Done

An independent literature survey was performed in PubMed. Among the 9 resolved crystal structures of human COX 2, 06 were cocrystallized with inhibitors and were considered in the present study. Multiple crystal structures for human cyclooxygenase- 2 in the Protein Data Bank (PDB) were made for structure based virtual screening and multiple docking.

Protein preparation

5IKR structure of human cyclooxygenase 2 bound with ligands were retrieved from the Protein Data Bank (PDB) and prepared using Protein Preparation Wizard in Schrodinger Maestro v9.8. Optimized protein structures were minimized using optimized potential for liquid simulations (OPLS-2003) force field, by converging heavy atoms to RMSD of 0.3 Å. Bond orders and formal charges were added for hetero groups and hydrogen atoms to all the atoms in the system. Protein molecule was optimized at neutral pH.

Compared 3D model by ramachandran plot give the information regarding between without energy minimized model and with energy minimized shown in fig 1. The present findings show the proposed model is stereochemically stable and the minimized model more accurate and flexible than without energy minimized model. This model used further for docking study.



Before Energy Minimization **After Energy Minimization**

Fig. 1: Ramachandran Plot: A Ramachandran plot output without ligand of 5IKR structure based on without energy minimization and with energy minimization, modified from Schrodinger Propreparation. The areas marked in red are most favored region, additionally allowed (yellow), generously (fawn) and disallowed (white) regions for amino acid

Ligand Preparation

1207 ligands were downloaded in .smi format from Zinc15 database. These ligands were prepared by ligprep using Schrödinger suite.

Docking studies

The six cocrystal ligands prepared through standard Maestro v9.8 ligand preparation protocol were docked into the 5IKR binding site. In this study, rigid receptor docking (RRD) was followed to calculate the scores and binding affinity of the interactions between 5IKR and ligands. A receptor grid was generated with the default parameters of van der Waals scaling factor 1.00 and charge cutoff of 0.25 around the binding site residues of COX-2 (5IKR) structure using Glide v6.3. It provides the better ranking accuracy for virtual screening. Three level docking method of RRD from lower stringency to higher

stringency with high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) were performed to filter the ligands. Qsite refinement was done with Glide SP docking to generate multiple poses and partial atomic charges were calculated for each pose of the bound leads in 5IKR lead complexes. Using QikProp v4.0, compounds having reactive functional groups and high-energy ionization states or compounds disobeying the Lipinski's rule of five were slewed out from the generated conformations. Virtual screening of docking site was analyzed using Schrodinger and crosscheck the result by AutoDock vina represented in table 1.

Virtual screening using Schrodinger

We used PyMoL plugin that allows carrying out molecular docking, virtual screening and binding site analysis to screen our in-house database. Zinc database, chembank and chemspider are searched for getting approved drug/compound list on the basis of similarity search criteria. Output results retrieve 1207, 162 and 597 compounds on the basis of relative similar target drugs. Schrodinger suite used which allows carrying out molecular docking, virtual screening and binding site analysis by sitemap to screen our in-house database. 1039, 145 and 263 ligands present in a directory were prepared. This format contains information about atomic charges, atom-type definitions and, for ligands, topological information (rotatable bonds). After binding site definition and receptor and ligand preparation, docking runs were performed against the in-house database. Out of 1447 compounds, 11 were showed better binding affinity (9 from Zinc database and 2 from chembank) compared to approve drug of 5IKR. The binding affinity of these compounds lies in the range of -10.738 to -7.955 shown in table 1. On the basis of binding affinity, Schrodinger showed the better binding score than AutoDock Vina. PyRx is performed as an alternative source to perform virtual screening. Schrodinger is commercial software and works quite well with accurate results.

Docking and Virtual screening using AutoDock Vina

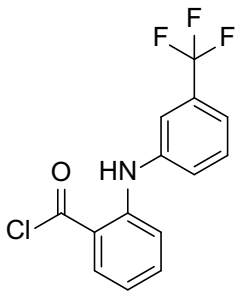
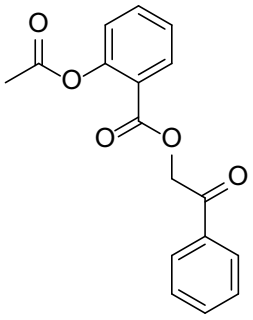
Docking of the target protein with selected ligand was also carried out using the AutoDock vina in PyRx platform. Docking was performed to obtain a population of possible

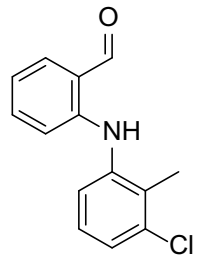
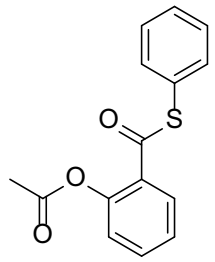
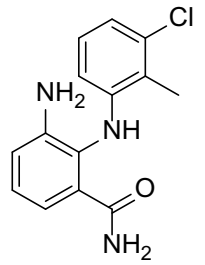
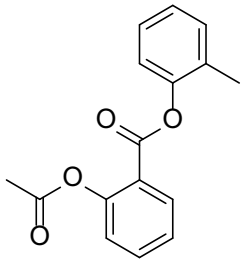
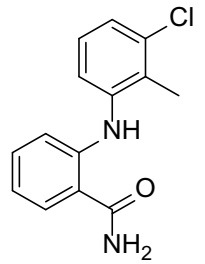
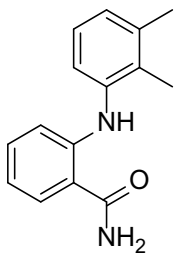
conformations and orientations for the ligand at the binding site. Using AutoDock vina, the target protein and ligands docking was performed using a grid generation. The best conformation was chosen with the lowest docked energy or binding affinity pose, after the docking search was completed shown in table 1.

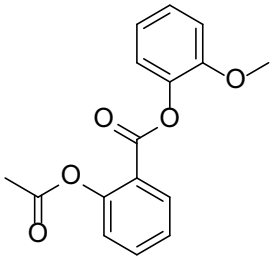
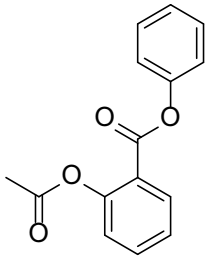
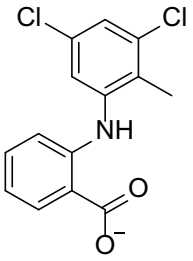
Prime/MM-GBSA

Additionally, for calculating Delta G value (DG) by the Prime approach the docked complexes from each docking method were subjected to molecular mechanics/generalized Born surface area (MM-GBSA) analysis. The binding energy calculated through MM-GBSA OPLS-2005 were much accurate than the XP GScore. MMGBSA continuum model was used to carry out the simulations by using Prime v3.6. For better representation of the solvent accessible surface area, Gaussian surface area model was employed instead of vdW surface area model. XP and MMGBSA results were shown in table 1:

Table: 1 Comparison of Binding affinities using Schrodinger and AutoDock Vina

S. No.	Title	Structure	XP Score (Schrodinger)	XP Score (PyRx)	MMGBSA dG Bind Score
1.	ZINC000039428234		-10.738	-8.4	-63.204
2.	ZINC000000244236		-10.378	-8.3	-63.321

3.	Compound A		-10.238	-8.1	-64.081
4.	ZINC000196009905		-10.211	-7.1	-64.968
5.	ZINC000130010902		-10.160	-6.2	-63.957
6.	ZINC000039914985		-10.059	-7.5	-66.457
7.	Compound B		-10.028	-7.9	-64.042
8.	ZINC000048442590		-10.007	-7.6	-62.951

9.	ZINC000000000348		-9.748	-8.1	-68.009
10.	ZINC000000351310		-9.736	-8.3	-63.343
11.	ZINC000039127665		-7.955	-6.2	-63.859

For analysis, the docking results were used for visualization. The best ligands selected based on the binding affinity. 11 ligands were found with high binding affinity. The higher affinity value was found in drug ZINC000039428234 (-10.738 kcal/mol), it indicates the good-quality results for cyclooxygenase enzyme.

AMET Properties

Cyclooxygenase enzyme plays the unique biological functions in cancer. Drug discovery is a complex and expensive endeavour that usually requires major steps such as disease selection, target hypothesis, leads compound identification (screening), lead optimization, pre-clinical trial, clinical trial and pharmacogenomic optimization. The best 11 compounds were selected which shows the high binding affinity, using Molinspiration calculator to test the bioavailability characteristics such as Adsorption, Distribution, Metabolism, Elimination (ADME). All compounds follow the lipinski's rule of five as well as bioactivity

score for finding the more suitable result including GPCR ligand, ion channel modulator, kinase inhibitor, protease inhibitor and Enzyme inhibitor as shown in Table 2.

Table 2: Molinspiration table showing molecular and bioactivity properties

S. No.	Compound Name	miLogP	TPSA (Å2)	natoms	MW	nON	nOHNH	violations	nrotb	volume	GPCR Ligand	ICM	KI	Nuclear receptor ligand	Protease Inhibitor	Enzyme inhibitor
1.	ZINC000039428234	5.21	29.10	20	299.68	2	1	1	4	231.76	-0.29	-0.21	0.01	-0.23	-0.46	-0.26
2.	ZINC000000244236	2.84	69.68	22	298.29	5	0	0	7	263.74	-0.37	-0.52	-0.66	-0.21	-0.43	-0.28
3.	Compound A	4.70	29.10	17	245.71	2	1	0	3	216.94	-0.52	-0.27	-0.35	-0.36	-0.96	-0.38
4.	ZINC000196009905	3.35	43.38	19	272.32	3	0	0	5	237.09	-0.26	-0.49	-0.74	-0.37	-0.24	-0.19
5.	ZINC000130010902	3.17	81.14	19	275.74	4	5	0	3	239.51	-0.27	-0.25	0.09	-0.43	-0.42	-0.03
6.	ZINC000039914985	3.79	52.61	20	270.28	4	0	0	5	244.51	-0.39	-0.24	-0.43	-0.07	-0.40	-0.20
7.	Compound B	3.73	55.12	18	260.72	3	3	0	3	228.22	-0.35	-0.31	-0.08	-0.48	-0.54	-0.21
8.	ZINC000048442590	3.50	55.12	18	240.31	3	3	0	3	231.25	-0.31	-0.31	0.05	-0.50	-0.38	-0.13
9.	ZINC000000000348	3.00	61.84	21	286.28	5	0	0	6	253.50	-0.33	-0.21	-0.37	-0.14	-0.35	-0.17
10.	ZINC000000351310	3.39	52.61	19	256.26	4	0	0	5	227.95	-0.40	-0.17	-0.49	-0.14	-0.40	-0.17
11.	ZINC000039127665	5.63	49.33	19	296.15	3	2	1	3	238.49	-0.24	-0.17	-0.21	-0.10	-0.61	-0.17

miLogP: LogP (partition coefficient); MW: molecular weight; nON: hydrogen bond acceptor; nOHNH: hydrogen bond donor; TPSA: Topological Polar Surface Area; natoms: number of atoms; nviolations: number of violations; nrotb: number of rotatable bonds; ICM:Ion Channel Modulator; KI: Kinase Inhibitor; PI: protease inhibitor;EI: Enzyme Inhibitor.

Conclusion

The approaches to develop therapeutics for inflammatory involve drugs, such as human COX-2 ligands, that specifically activate cyclooxygenase-2 enzyme in the relevant cell population. The present work deals with the COX-2 enzyme about the structural characterization using in silico approach. We have decrease the search space of active compounds using 'similar target search' criterion against Zinc database, chembank and chemspider to prepare in house database of 1207, 162 and 597 compounds. The screening experiment successfully identifies 11 compounds that have high binding affinity from zinc database and chembank compared to native compound. All the identified compounds follow Lipinski's rule of five. So, these drugs and their probable derivatives

may represent another important contribution to the arsenal of breast cancer therapy. ZINC000039428234 show the highest XP score among all 11 compounds. This study will further shed a light on them to use as possible alternatives for the prophylactic treatment of breast cancer.

Future Action

In vitro study

Screened out the top 11 best compunds which will provide useful information inhibitors. These best hits not only provided novel lead compounds but also suggested that their binding models were favorable to find a new path to designing novel COX inhibitors. We will try to find out IC_{50} value for anticancer activity by using breast cancer cell lines by MTT assay at different concentrations using microtitre plate assays ELISA. IC_{50} values will be determined through a series of experiments. On the basis of *in silico* and *in vitro* we can find out the best new drug that will help for breast cancer therapy.

4. Sub-Cellular Tissue Proteomics of Gallbladder Carcinoma for Understanding Molecular Pathogenesis and Identification of Signature Proteins for Diagnostic and Therapeutic Applications

Scientific staff : Dr. Poonam Gautam, Dr. Usha Agrawal, Mr. Javed Akhtar, Ms. Ratna Priya

Collaborator : Dr. Puja Sakhuja, Dr. Anil Agrawal

Duration :

Funding Agency :

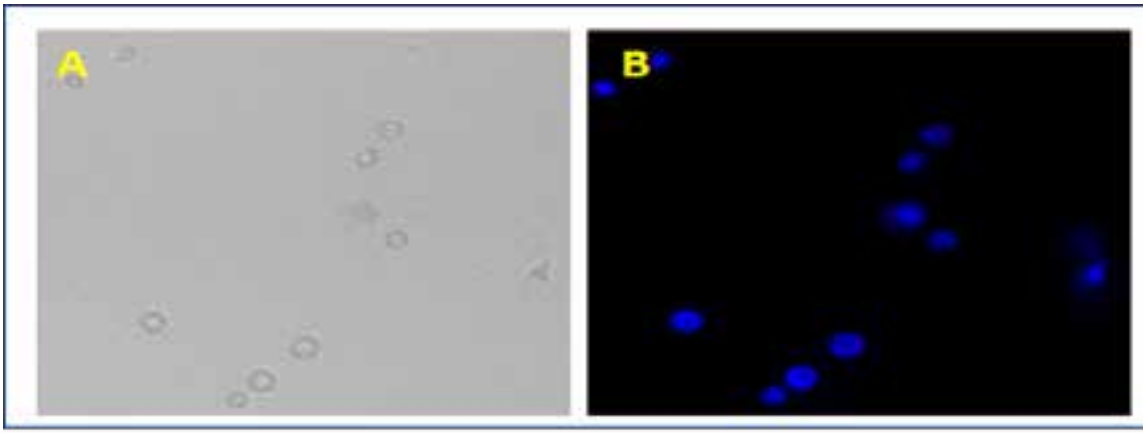
Background:

Gallbladder carcinoma (GBC) is the fifth most common malignancy of the gastrointestinal tract. Five-year survival rates for advanced stages are drastically low (≤ 5%) in

comparison to early. Survival of patients is directly related to the stage of the disease. The poor prognosis of GBC is thought to be associated with the diagnosis at advanced stage, due to the anatomic position of the gallbladder, or the vagueness and non-specificity of symptoms. Proteomic approaches to study altered expression of genes and proteins in different stages of GBC would further help to understand molecular changes in a holistic way that may lead to better management of the disease. Tissue subcellular fractionation is an ideal tool to enrich and analyze different cellular compartments. This is an effective method for increasing the detection coverage of the proteomic studies allowing analysis of proteins based on its subcellular compartmental distribution and how the distribution of a protein may differ between different cellular compartments.

Work done during the year

In the present study, we have optimized the isolation of different subcellular fractions-nuclear, mitochondrial, microsomal and cytosolic from mouse liver tissue based on differential centrifugation method. These fractions were further characterized by fluorescent microscopy and Western blot analysis. Analysis of nuclear fraction by fluorescent microscopy indicated high purity of these fractions (Figure 1).



Nuclear Fraction
bright light

Nuclear Fraction
DAPI-stained

Fig. 1: Images of nuclei acquired using light and fluorescent microscopy. Nuclear fraction was isolated from the mouse liver tissue by subcellular fractionation and stained with DAPI. The microscope (Axio Imager Z2, Carl Zeiss, Germany) at 20× magnification was used for visualizing stained nuclei. No intact cells were observed suggesting efficient lysis of cells.

SDS-PAGE analysis shows protein profile of different fractions (Figure 2). Western blot analysis showed significant enrichment of sub cellular fraction-specific marker proteins i.e. lamin A/C (LMNA) for nuclear fraction, cytochrome c oxidase subunit 4 isoform 1 (COX4I1) for mitochondrial fraction, calnexin (CALN) for microsomal fraction and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for cytosolic fraction (Figure 3). Thus, we have optimized the method for tissue subcellular fractionation and will be applying the same to gallbladder tissue samples (controls and cases) to study differentially expressed proteins using high throughput quantitative proteomic analysis in different stages of gallbladder carcinoma. We are in process of collecting the clinical samples. The study will lead to an in depth understanding of molecular pathogenesis in GBC and identification of signature proteins useful for diagnostic and therapeutic applications.

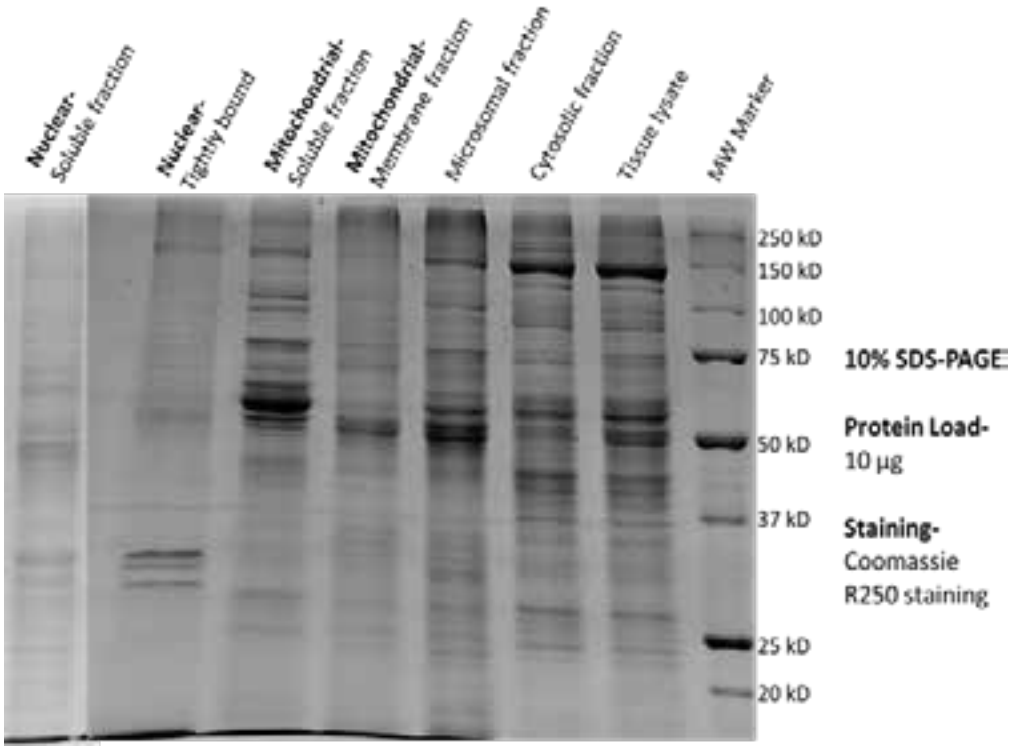


Fig. 2: SDS-PAGE analysis showing protein profiling of different subcellular fractions mouse liver tissue.

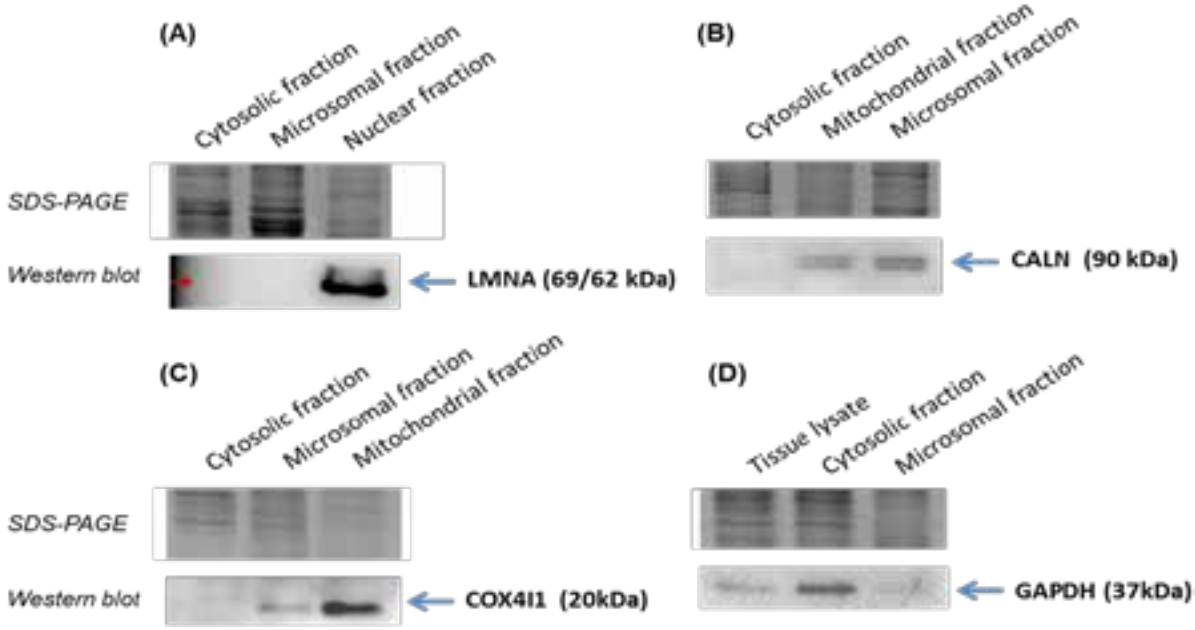


Fig. 3: Western blot analysis showing enrichment of marker proteins specific to subcellular fractions (A) Lamin A/C, marker protein for nuclear fraction, (B) cytochrome c oxidase subunit 4 isoform 1 (COX4I1) for mitochondrial fraction, (C) calnexin (CALN) for microsomal fraction and (D) glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for cytosolic fraction. Western blot analysis showed a significant enrichment of specific marker protein in the respective fraction. A total of 10 µg protein was resolved by 12% SDS-PAGE and then electrotransferred onto PVDF membrane. The blot was incubated with primary antibody and then seconodary antibody conjugated with HRP. The blot was developed using Clarity Western enhanced chemiluminescence (ECL) substrate (Bio Rad) and image acquired using Chemidoc MP image scanner (Bio-Rad).

5. Autoantibody Response And Identification Of Tumor-Associated Antigens In Gall Bladder Carcinoma - Immunoproteomics Approach

Scientific staff : Dr. Poonam Gautam, Mr. Javed Akhtar, Ms. Ratna Priya
Collaborator : Dr. Puja Sakhuja, Dr. Anil Agrawal
Duration : June 2016 - June 2019
Funding Agency : DST-SERB

Background

Early diagnosis for GBC is important for better treatment leading to increased survival of these patients, however, only in 10% of cases, GBC is diagnosed at early stage. Currently available blood tests and serum markers are not specific and sensitive enough to reliably detect GBC at early stages. We propose to apply Serological proteome analysis (SERPA), an immunoproteomics approach based on a classical proteomics workflow, for detection of autoantibodies having the potential to identify multiple proteins eliciting humoral response in cancer patients at early stages.

Work done during the year

Isolation and characterization of microsomal fraction from mouse liver tissue

We have optimized isolation of microsomal fraction and its characterization by Western blot analysis as described in previous project (Figure 2 and 3). We have also optimized the separation of microsomal proteins using 2-D gel electrophoresis, which showed separation of over 200 proteins. 2-D gel image were acquired using Chemidoc MP image scanner (Bio-Rad) and was analyzed using PD Quest Software version 8.0.1 (Bio-Rad).

Further, plasma membrane proteins will be isolated. This method would be then applied to tissue samples from early stages of gallbladder carcinoma cases as proposed in the

study. We are in the process of clinical sample collection. A total of 50 samples (23 GBC cases and 27 controls) have been collected. After the required number of samples is collected for all the groups, we will perform the experiments with clinical samples.

6. Molecular regulation of mTOR signaling in acute lymphoblastic leukemia

Scientific staff : Dr. Sunita Saxena, Dr. Fouzia Siraj, Asheema Khanna
Duration : 2015-2018
Funding Agency : DST

Aims, Objectives & Background

Despite major improvements in understanding of the molecular genetics of acute lymphoblastic leukemia (ALL), the mechanisms that lead to the abnormal proliferation and survival of T and B lymphoblasts remain largely unknown. Current ALL treatment protocols use combinations of multiple cytotoxic chemotherapeutics with overlapping toxicity and the potential for long term sequelae, especially in the most intensively treated patients. Therefore, treatment of leukemia remains a challenge for clinicians. New agents with activity against ALL are needed, and targeted biologic agents have the potential to add efficacy without additional toxicity in patients with ALL. Major efforts have been made to develop new compounds targeting signaling pathways implicated in ALL cell proliferation and survival. One such pathway is represented by the mammalian target of rapamycin (mTOR).

There is not much data available on mTOR expression in relation to leukemia etiology. The objective of the study was to study the expression of mTOR gene in acute lymphoblastic leukemia (ALL) samples using real time PCR and to identify subset of patients having high expression of mTOR and its association with response to chemotherapy.

Earlier mTOR expression was analyzed in ALL patients and correlated with clinical response

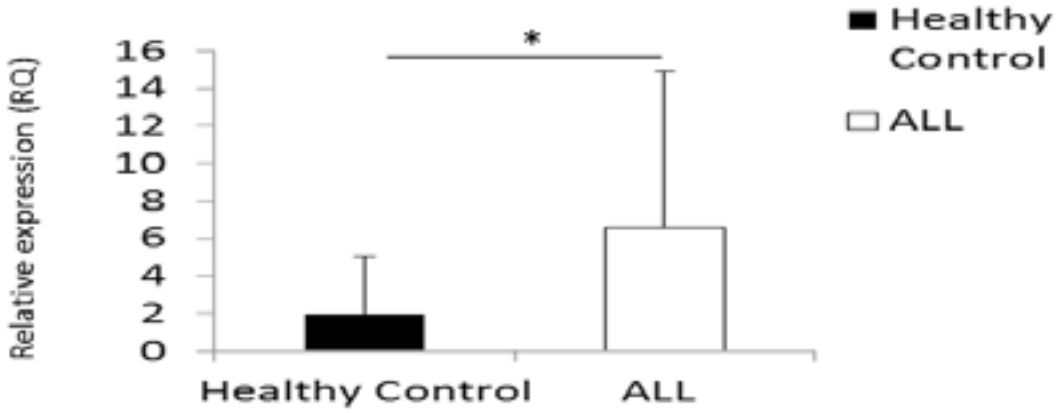


Fig 1: Relative expression of mTOR gene in ALL samples compared to normal PBMC cells

Correlation with Induction chemotherapy

Response to chemotherapy was determined at the end of completion of induction chemotherapy. Expression of mTOR was found to be significantly up regulated in non-responder patients of ALL as compared to responders.

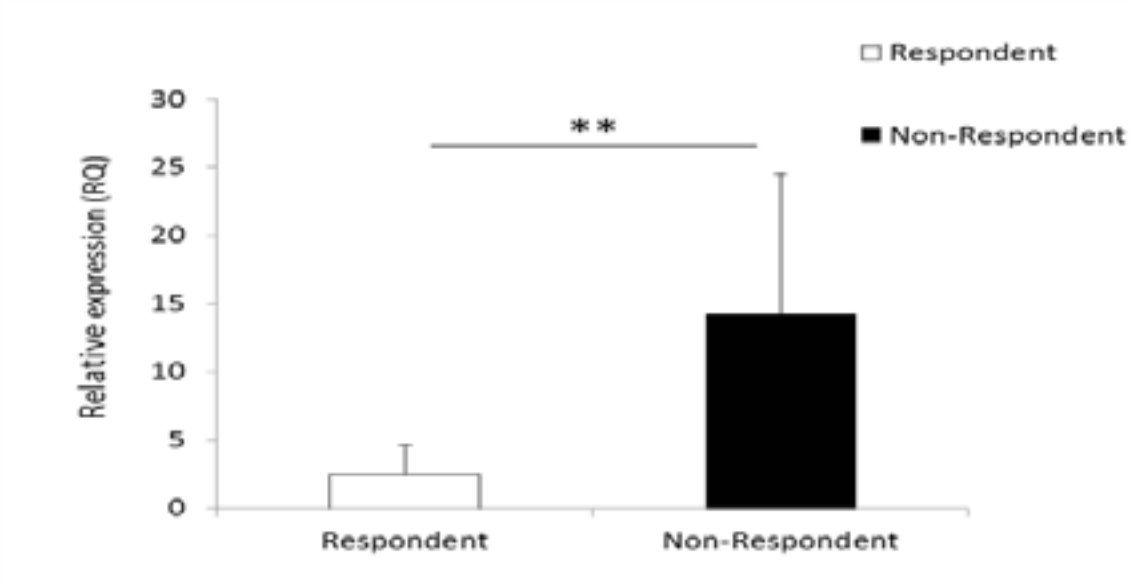


Fig 2: Expression of mTOR gene in ALL samples responded and non-responded to induction chemotherapy

Work done during the previous year (2016-17)

To determine the regulation of mTOR, we first analyzed the expression of mTOR in ALL cell lines i.e. MOLT 4 and JURKAT. (Figure 1)

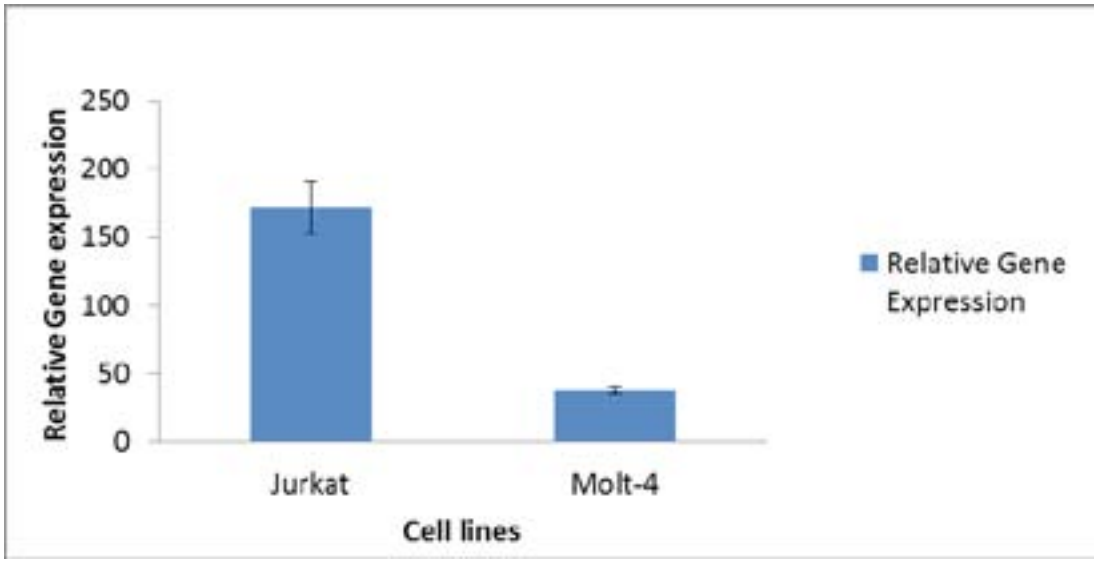


Fig. 1: Expression of mTOR in ALL cell lines

Cell Proliferation assay using trypan blue

Cell proliferation assay was done to calculate IC_{50} of rapamycin (mTOR inhibitor) in ALL cell lines. (Figure 2 and Figure 3)

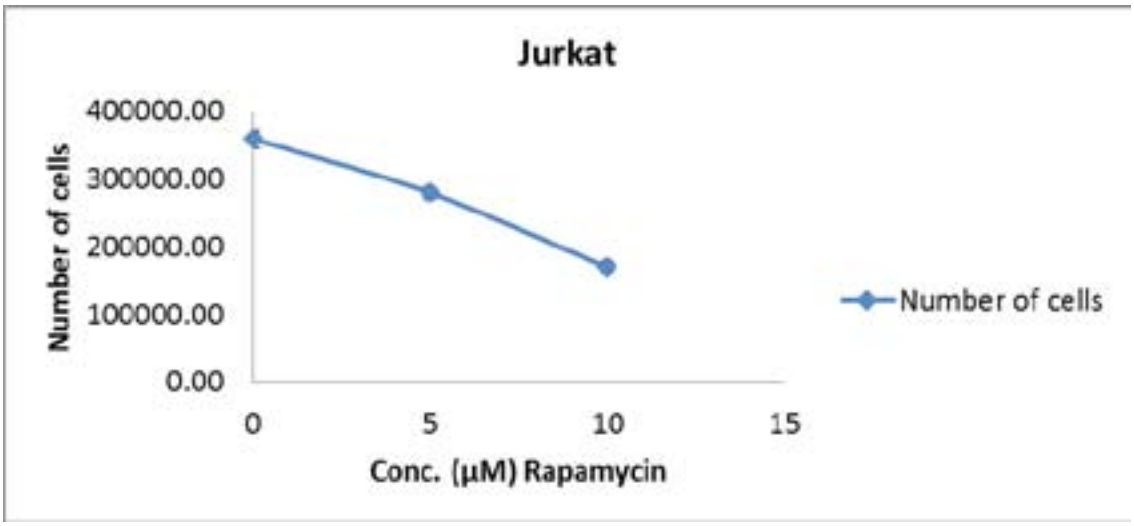


Fig. 2: Effect of rapamycin on cell proliferation of jurkat cells.

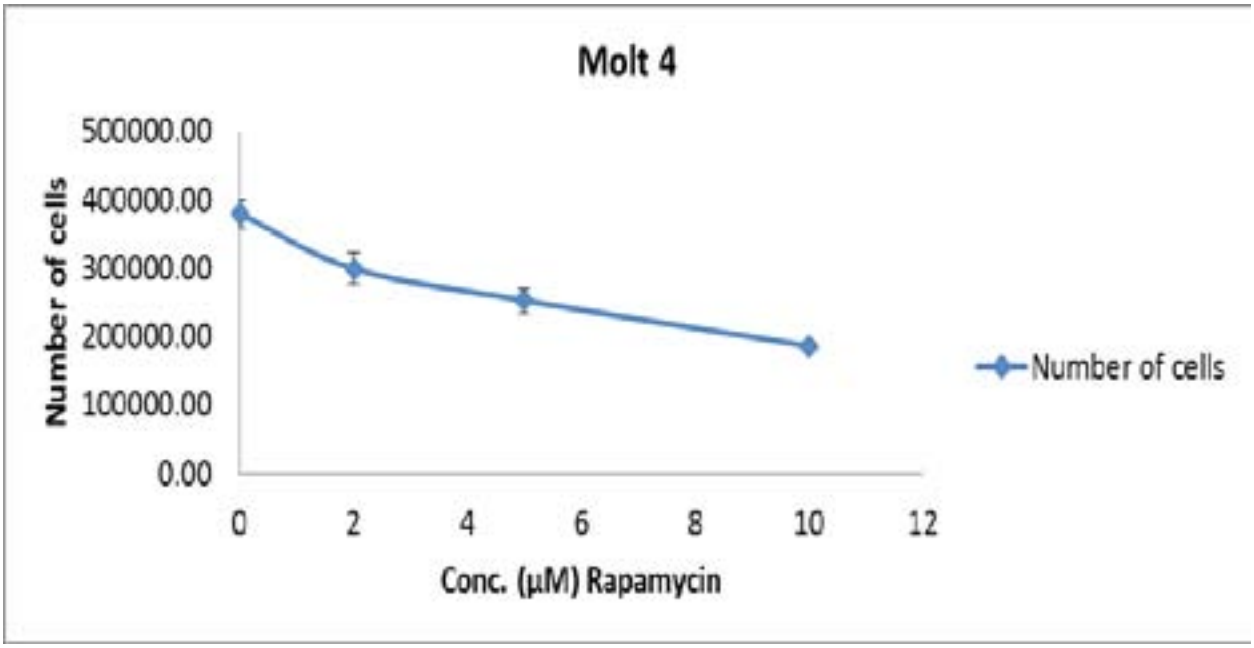


Fig. 3: Effect of rapamycin on cell proliferation of Molt 4 cells.

IC_{50}value of rapamycin was fund to be 10µM in both JURKAT and MOLT-4 cell line. This concentration of rapamycin is used in further experiments.

mTOR was inhibited using rapamycin in Jurkat cell line and its effect was analyzed on cell cycle and cell apoptosis.There was increase in G0/G1 population in rapamycin treated cells. Also, it was found that the treatment with rapamycin tilted the survival axis to a pro-apoptotic state.

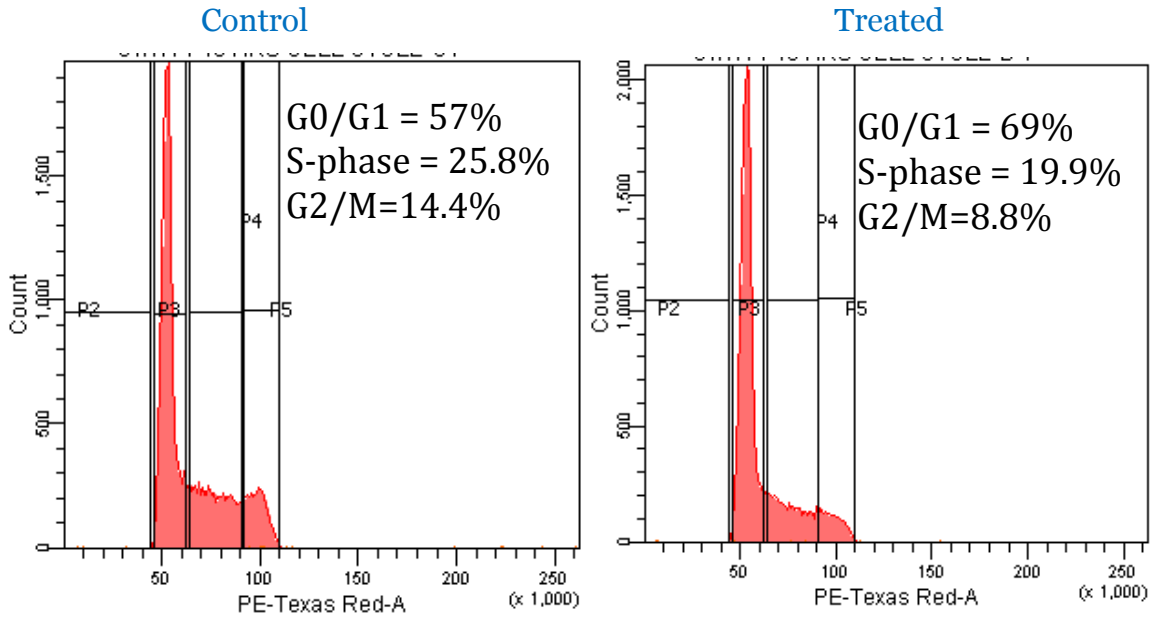


Fig. 4: Effect of inhibition of mTOR using rapamycin on cell cycle

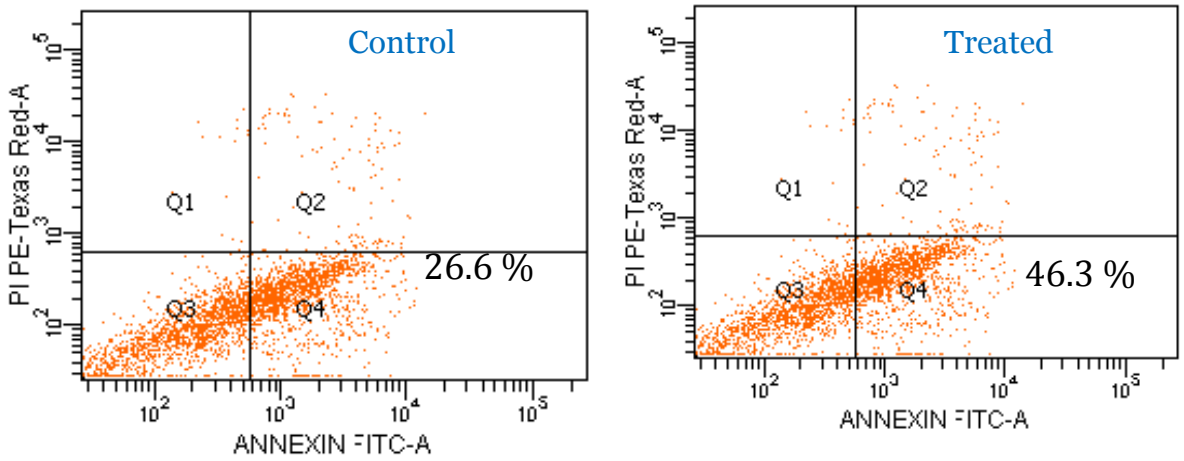


Fig. 5: Effect of inhibition of mTOR using rapamycin on apoptosis

Future Work to be done

Effect of chemotherapeutic agents in conjunction with rapamycin on cell cycle and apoptosis will be evaluated

INFECTIOUS DISEASES

LEISHMANIASIS

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

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

Duration : 2015 – 2018

Funding Agency : ICMR-Extramural

Aims, Objectives & Background

Paromomycin (PMM) is a new treatment option for VL control in India as a monotherapy and in combination therapy. Although its use is still restricted in the field, unraveling the molecular mechanism of resistance towards PMM is the key to preserve the drug efficacy. We have previously shown ≥ 2 fold modulated expression of 267 (approx. 2.9%) genes in PMM resistant parasites exploiting microarray technology. During the period under study, detailed bioinformatics analysis using BLAST2GO and AmiGO databases for functional classification of genes were carried out. KEGG pathway analysis tool was utilized to identify the pathways in which these genes were involved. Further, modulated gene expression of selected genes was validated by real time qPCR.

Work done during the year

Functional classification and pathway analysis of genes showing modulated expression in PMM-R parasites:

Both up-regulated and down-regulated genes were analysed using BLAST2GO, AmiGO databases and KEGG pathway analysis tool to identify the pathways involved in PMM resistance. The percentage of differentially modulated genes according to GO function categories is shown in **Fig.1.**

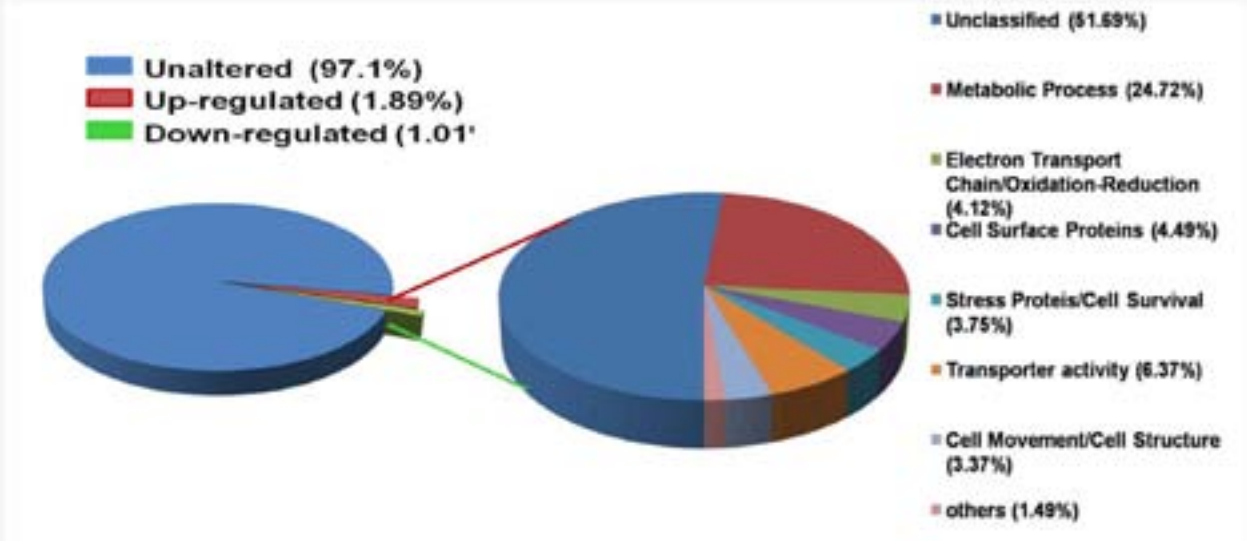


Fig. 1. Distribution of genes differentially modulated during PMM resistance in *Leishmania donovani* according to gene ontology (GO) functional categories.

Overall distribution of genes shows total number of unique genes up-regulated or down-regulated is 2.9%. Unclassified proteins include hypothetical proteins (proteins with unknown function and not tested experimentally) and proteins with no GO categories (unclassified) that have been experimentally characterized.

According to functional description and interacting partner of specific genes at protein level (String 9.01 database), a total of 129 genes were classified into eight different categories as discussed below.

A. Glycolysis, Substrate level phosphorylation & oxidative phosphorylation:

Up-regulation of 2 enzymes of glycolytic pathway, glucose-6-phosphate isomerase and phosphoglycerate mutase enzymes was observed. Among the enzymes that take part in TCA cycle, isocitrate dehydrogenase was up-regulated (1.5 fold), while succinate dehydrogenase was down-regulated (2.17 fold). Enzymes of glycosomal succinate fermentation viz. glycosomal phosphoenolpyruvate carboxykinas and cytosolic malate dehydrogenase were up-regulated. A down regulation in NADH-ubiquinone oxidoreductase (Complex system I) was observed.

B. Lipid Metabolism:

Lipase involved in lipid metabolic process was up-regulated. Another gene coding for choline/ethanolamine phosphotransferase, involved in synthesis of phosphatidylcholine, was up-regulated. A down-regulation in lathosterol oxidase like protein involved in biosynthesis of sterol was observed. 3-ketoacyl-CoA thiolase that catalyzes β-oxidation of long chain fatty acid showed down-regulation in expression.

C. Down-regulation of genes involved in DNA synthesis & translation machinery:

Genes responsible for DNA replication, DNA polymerase θ, H1 histone like protein and endonuclease/exonuclease activity were down-regulated. Genes involved in translational machinery like tryptophenyl t-RNA synthetase, 5.8 S ribosomal RNA and 28 S ribosomal RNA were also down-regulated. On the other hand a gene encoding a hypothetical protein (LinJ.26.1540), interacting with other proteins responsible for DNA damage repair, was up-regulated. Up-regulation of exoribonuclease and RNA binding protein was also observed in PMM resistance.

D. Modulation in Protein/Amino acid metabolic process:

Genes encoding phosphoglycan beta 1,3 galactosyltransferase and ubiquitin-conjugating enzyme protein were up-regulated. We also observed up-regulation of SNF7 like proteins which are involved in protein sorting and interact with proteins responsible for vesicular protein trafficking. A number of peptidases (carboxypeptidase and metallopeptidase) involved in protein degradation were down-regulated.

E. Modulated expression of transporters:

ATP Binding Cassette transporters (ABC transporters) ABC1 and ABCA7 showed higher mRNA expression in PMM-R parasites. Interestingly an ABC transporter, ABCB3 was down regulated. Nucleoside transporter1 and vacuolar proton translocating ATPase showed down-regulated expression in PMM resistant isolates. Folate/Biopterin transporter, glucose transporter, P-type ATPase showed up-regulated expression.

F. Cellular components / cell movement/ Cell Signalling:

Expression of genes coding for cellular movement like paraflegeller rod component, and dynein like protein were up-regulated, while genes for coronin and centrin were down-regulated in PMM-R isolates. Ca^{2+} dependent signalling, which eventually contributes to microtubule formation during cell division, was up-regulated. Protein phosphatase 2c like protein, serine/threonine phosphatse and a protein kinases were up-regulated in PMM-R.

G. Proteins involved in intracellular survival:

Isoprenyl cysteine alpha-carbonyl methylesterase (ecotin putative), inhibitor of serine peptidase, that helps parasites to avoid killing by host macrophages (Faria et al., 2011) was up-regulated. Up-regulation was also observed for tryparedoxin-like protein and Leishmanolysin, GP63, having protein tyrosine phosphatase activator activity. Peroxidoxin (peroxiredoin), trypanothione synthetase and pteridine reductase 1 (PTR1) were down-regulated.

H. Cell surface proteins:

Genes encoding cell surface proteins, such as GPI anchored proteins, amastin like proteins and ppg3 related protein, exhibited up-regulation in PMM-R isolates.

Validation of modulated gene expression with real time PCR:

A total of 15 (8 up- and 7 down-regulated) genes indicative to play role in PMM resistance were validated for their expression by real time q-PCR in three PMM sensitive and PMM resistant parasites. Fold change in gene expression of all the three PMM-R/PMM-S obtained by q-PCR was compared with the fold difference in gene expression obtained by microarray experiments and was plotted in a graph (Fig.2). Results obtained by q-PCR were in agreement with the transcriptomic data derived by microarray experiments for all the selected genes in all the parasites.

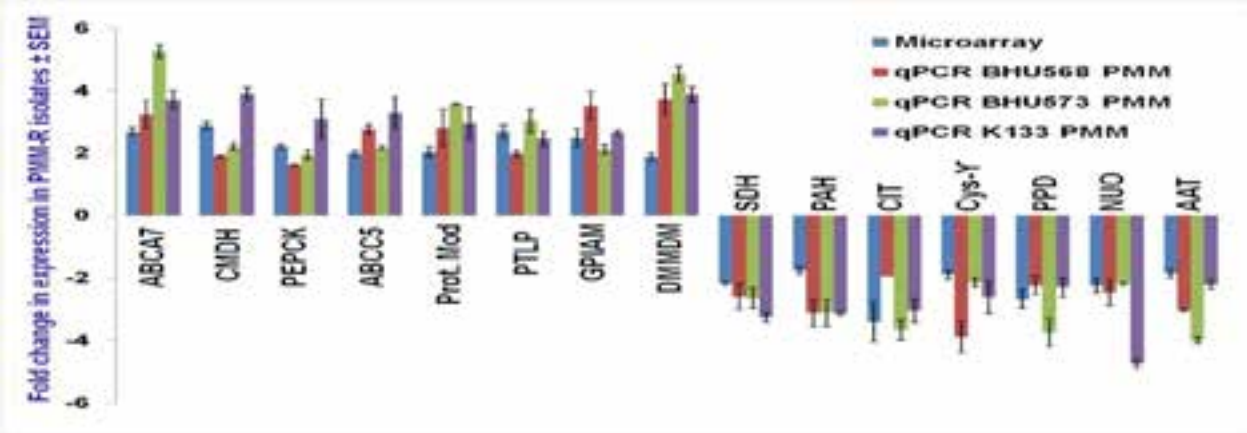


Fig. 2. Validation of modulated gene expression by quantitative real time PCR (qRT-PCR). Fold change in gene expression of three PMM-R parasites (K133 PMM, 573 PMM and 568 PMM) with respect to PMM-S parasites (K133 WT, 573 WT and 568 WT) ± SEM are represented here. The qRT-PCR data were normalized using two endogenous control, glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and cystathionine β-synthase (CBS).

Detailed pathway analysis of 129 modulated genes indicated probable adaptations in drug resistant lines which included a) reduced oxidative phosphorylation; b) increased glycosomal succinate fermentation and substrate level phosphorylation; c) dependency on lipids and amino acids for energy generation; d) reduced DNA synthesis and increased DNA damage repair and e) decreased protein synthesis and degradation. Based on results obtained, we propose that the mechanism of PMM resistance is multifactorial and involves various processes as depicted in Fig. 3. The study provides the comprehensive insight into alteration of parasite behaviour associated with PMM tolerance in *L. donovani* that will help to design strategies to increase lifespan of this important antileishmanial drug.

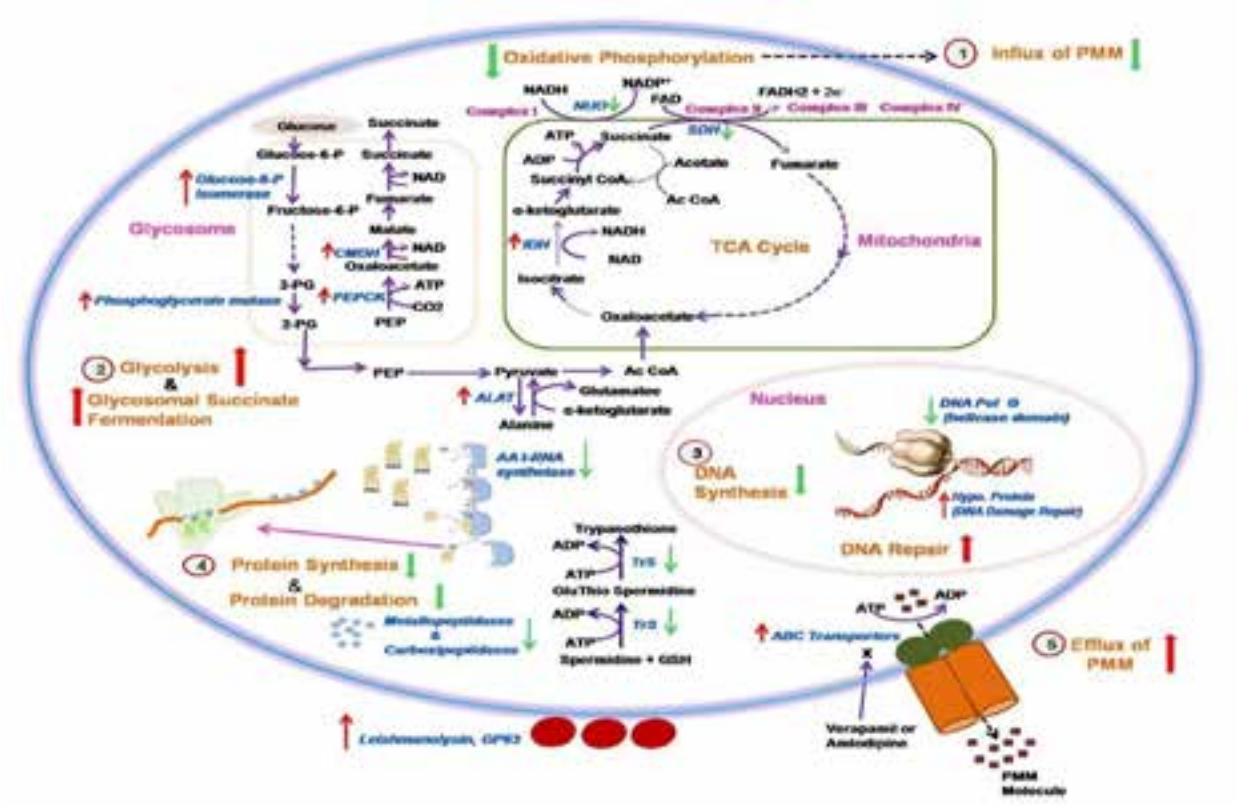


Fig. 3. Predicted adaptations in *L.donovani* in PMM resistance. Genes altered in PMM-R parasites are represented here. Genes marked with up and down arrow represent respectively the up-regulated genes and the down-regulated genes in PMM-R parasites. 1,2,3,4 and 5 are probable adaptations in PMM-R parasites.1. Down-regulation of oxidative phosphorylation leads to reduced influx of PMM drug, 2. Enzyme for glycolysis and glycosomal succinate fermentation were up-regulated indicating increased substrate level phosphorylation, 3. Down-regulated DNA polymerase θ suggesting reduced DNA synthesis. Hypothetical protein responsible for DNA damage repair were up-regulated 4.Tryptophenyl-t-RNA synthatase and several ribosomal proteins were down-regulated suggesting reduced protein synthesis, Down-regulation of metallo-and carboxipeptidases indicated reduced protein degradation, 5. Up-regulation of ABC transporters and reversion of resistant phenotype in presence of modulators, verapamil or amlodipine suggested probable role of ABC transporters in selection of PMM resistance. Abbreviations used are as follows: 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; AA t-RNA Synthetase, Aminoacyl t-RNA Synthetase; Ac CoA, Acetyl Coenzyme A; ALAT, Alanine aminotransferase; CMDH, Cytosolic malate dehydrogenase; DNA pol. θ, DNA Polymerase θ; Fructose-6-P, Fructose-6-phospahet; Glucose-6-P, Glucose-6-phosphate; GSH, Glutathione; Hypo. Protein, Hypothetical protein; IDH, Isocitrate dehydrogenase; NUO, NADH ubiquinone oxidoreductase; PEP, Phosphoenol pyruvate; PEPCK, Phosphoenol pyruvate carboxykinase; SDH, Succinate dehydrogenase; TrS, Trypanothione synthetase.

Future Work

Modulation in generation of Th1/Th2 cytokines, reactive oxygen and nitrogen species in PMM-S/PMM-R infected macrophages will be analysed.

2. Identification of genes associated with artemisinin resistance in *Leishmania donovani*

Scientific Staff : Dr. Ruchi Singh, Dr. Poonam Salotra, Ms. Aditya Ms Sushmita Ghosh

Duration : 2015 – 2018

Funding Agency : ICMR-Extramural

Aims, Objectives & Background

Artemisinin, a sesquiterpene endoperoxide isolated from *Artemisia annua*, is potent antimalarial compound that has demonstrated effectiveness in experimental models of leishmaniasis. Studies to unravel the possible mechanisms of action and resistance towards this drug are necessary. Previously we reported, inherent *in vitro* sensitivity of different field isolates of *L. donovani* towards artesunate (ART) and generation of experimental ART resistant lines to study difference in parasite behavior upon selection of ART resistance. In continuation to that, during the period under study, we evaluated comparative tolerance of wild type (K133 WT) and ART resistant parasites (K133 AS-R) towards nitrosative and oxidative stress and determined modulation in nitric oxide (NO) and reactive oxygen species (ROS) production in K133 WT vs K133 AS-R infected macrophages. Microarray based gene expression analysis for identification of differentially expressed genes in K133 WT Vs K133 AS-R parasites was also carried out.

Work done during the year

Tolerance of K133 WT and K133 AS-R towards nitrosative and oxidative stress:

Responses of the K133 WT and K133 AS-R strains towards nitrosative and oxidative stress was compared. K133 AS-R promastigotes were significantly more tolerant to SNAP (NO donor) than K133 WT promastigotes. The resistant parasites were >9 fold tolerant to NO stress (Fig. 1A). Both K133 WT and K133 AS-R parasites were similarly susceptible towards H_2O_2 (mimicking oxidative stress) (Fig. 1B).

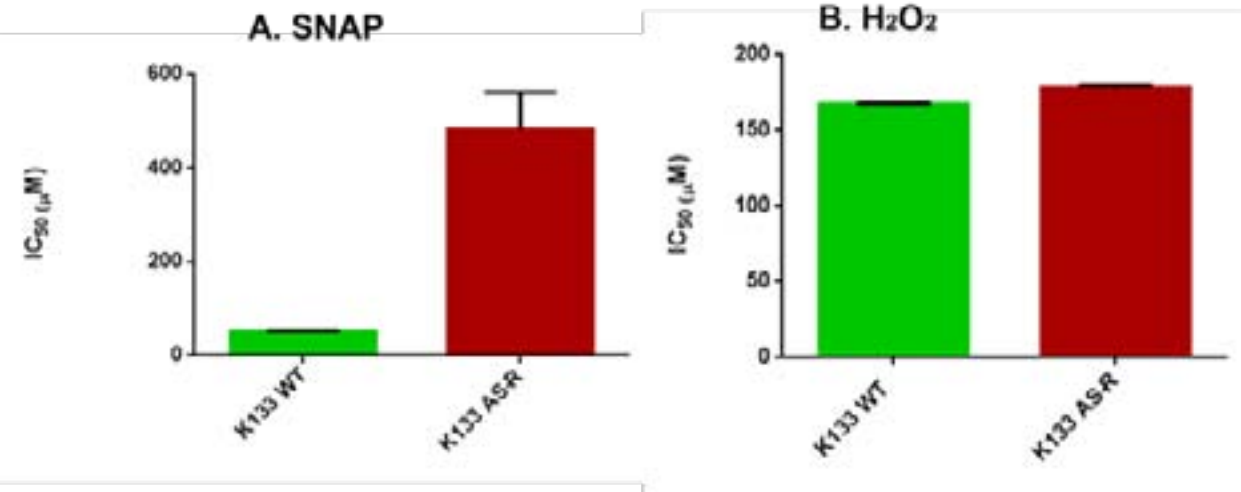


Fig. 1: Susceptibility of K133 WT and K133 AS-R isolates towards A. SNAP and B. H_2O_2. IC_{50} (±SD) values represented are the mean of two independent experiments performed in quadruplicate.

Measurement of NO production by host macrophages upon infection with K133 WT/K133 AS-R isolates:

NO levels were evaluated in cell culture supernatant of mice PECs infected with K133 WT or K133 AS-R isolates, colorimetrically by Griess reaction. Resistant parasite significantly ($p<0.0001$) modulated NO production upon infection to mice macrophages and induced reduced NO compared to wild type parasites.

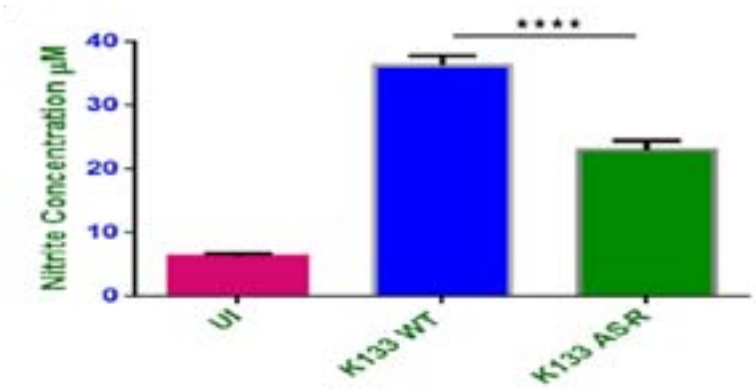


Fig.2. Assessment of levels of NO produced by K133 WT and K133 AS-R infected mice PECs. NO concentration represented is the mean of three independent experiment triplicates (**** p≤0.0001).

Measurement of ART induced oxidative stress response

We observed no significant difference in levels of ROS produced by K133 WT and K133 AS-R infected mice PECs. In presence of artemisinin, macrophages infected by resistant parasite produced significantly less (p<0.0001) ROS compared to K133 WT infected macrophages (Fig.3).

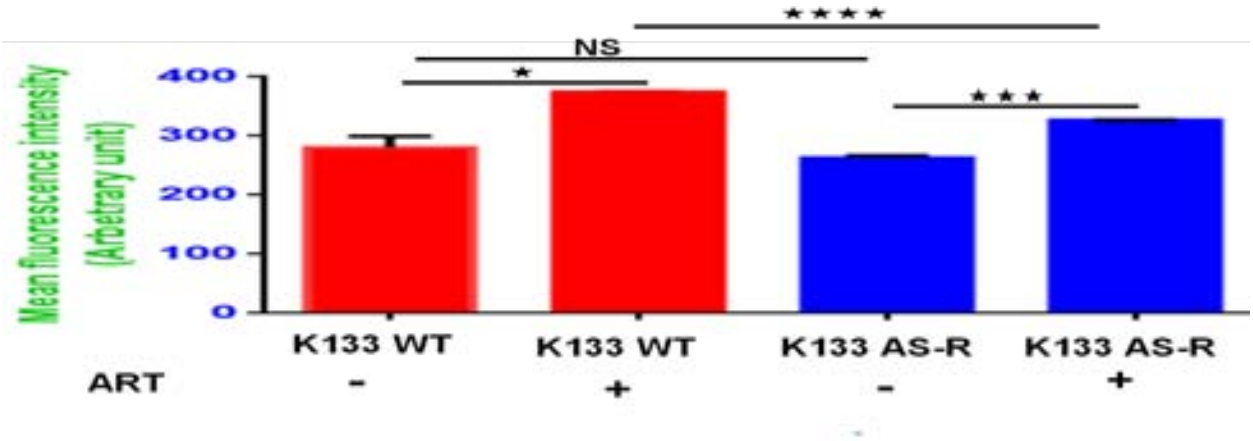


Fig. 3. Accumulation of ROS in mice PECs infected with K133 WT or K133 AS-R parasites with or without ART exposure (20µM). Data represents mean ± SD of three independent experiments, each in triplicate.(*p≤0.5,***p≤0.001,****p≤0.0001).

Transcriptome analysis of K133 WT and K133 AS-R *Leishmania* parasites:

To investigate the global mRNA expression profiles of K133 WT and K133 AS-R *L. donovani*, one colour microarray based gene expression profiling was carried out using 60mer oligonucleotide microarray slide [8X15K format] representing genome of *L. infantum* and *L. major*. Genes with expression ratio greater than 2.0 between K133 WT and K133 AS-R parasites were considered as differentially regulated. Comparative transcriptome profiling of K133 WT and K133 AS-R using single colour DNA microarray technology revealed that 208 genes (approx. 2.26%) were differentially modulated based on 2 fold cut off in PMM-R parasites (Fig.4).

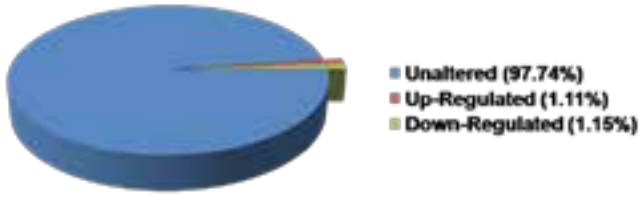


Fig. 4. Overall distribution of genes shows total number of unique genes up-regulated (1.11) or down-regulated (1.15) is 2.26%.

Of these 102 genes showed up-regulated expression while 106 genes showed down-regulated expression in AS-R isolates. The distribution of overall up-regulated and down-regulated gene expression based on fold changes is shown in Table 1. Most of the differentially regulated genes exhibited 2-3 fold modulation in expression while a few genes showed >4 fold modulation.

Table 1: Categorization based on fold change in up-regulated and down-regulated gene expression in artemisinin resistant *L. donovani* parasite:

Fold Changes	AS-R, Up-regulated	AS-R, Down regulated
>2.0-3.0	70	85
>3.0 – 4.0	20	16
>4.0	12	5
Total genes	102	106
Percent of modulated genes*	**1.11%**	**1.15%**

*The percent modulated genes calculated from the total 9,170 genes obtained in QC after filtering.

The plot log_2 transformed expression ratio of K133 AS-R (red line) compared to K133 WT (green line) as a function of the chromosomal location of microarray probes is shown in Fig. 5.

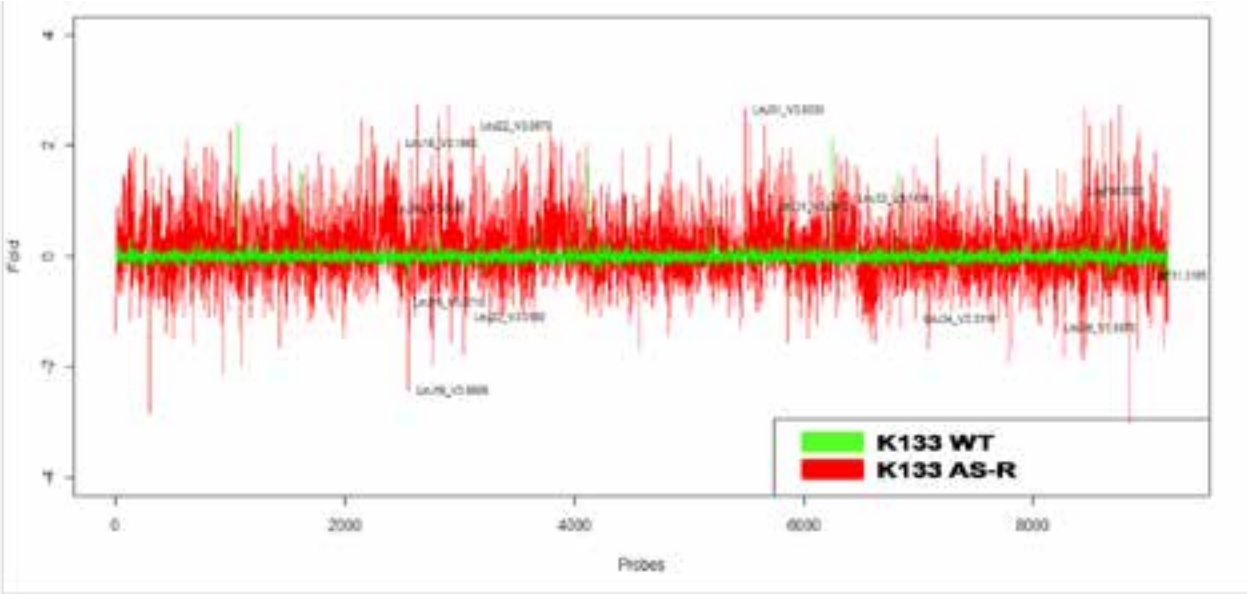


Fig. 5: Comparative transcriptional responses following ART adaptation in *L. donovani.* Overlap of log_2 transformed K133 AS-R and K133 WT expression ratio plotted as a function of chromosomal location of probes representing the full genome microarray. The plot represents the average values of three independent hybridizations for each isolate.

Chromosome map showing comparative gene expression in K133 AS-R Vs K133 WT parasites:

To analyse gene expression level on genomic scale, chromosome map was generated using Custom R program (Fig. 6). Up regulated genes are indicated by red lines whereas down regulated genes are indicated by green lines. Further analysis of chromosome map identified that higher number of up regulated genes were located on chromosomes 18, 25, 31 and 33. There were no down regulated genes on chromosome 25. Higher numbers of down regulated genes were located on chromosome 33 and 36 in K133 AS-R isolates.

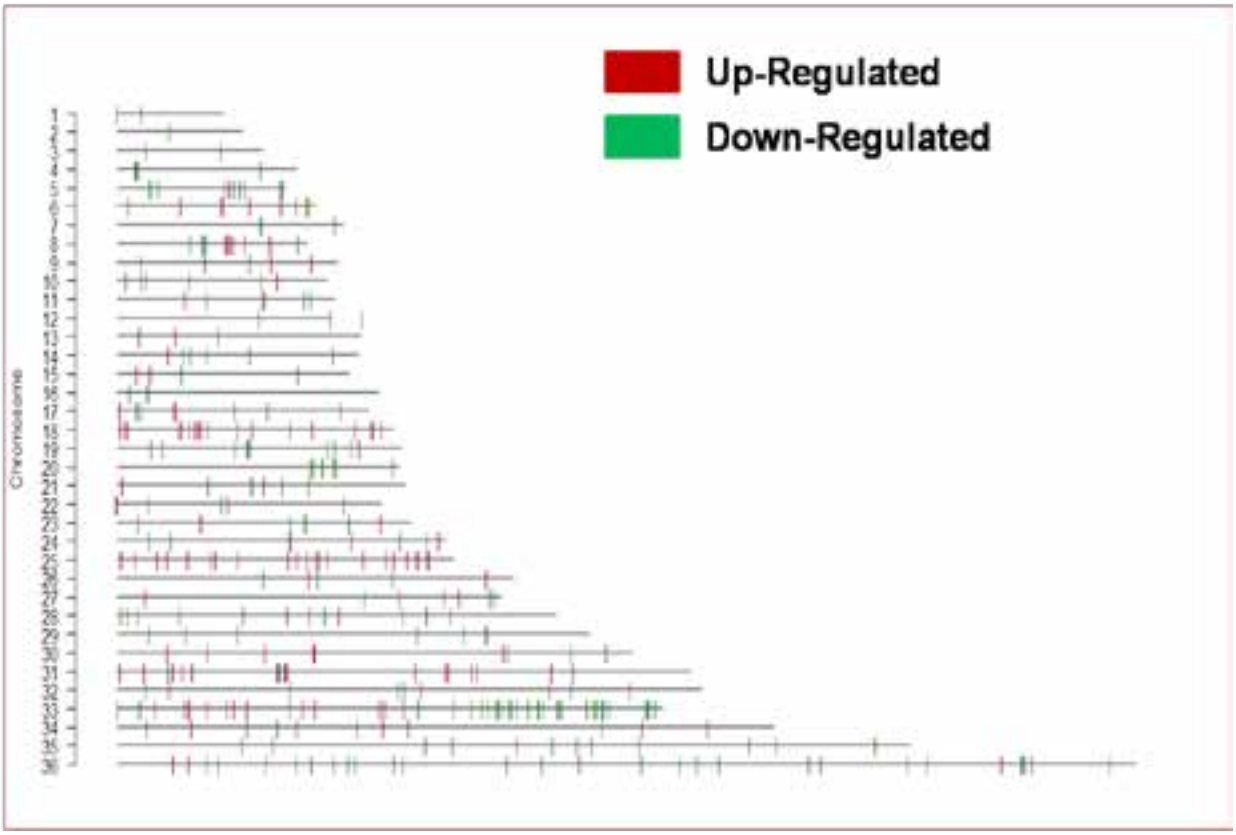


Fig. 6: Differential gene expression of K133 WT and K133 AS-R parasite on chromosomal map. Chromosome map for expression data was generated using Custom R program. Red lines indicate up regulated genes in PMM-R parasite, whereas green lines indicate down regulated genes.

Future Work

Both up-regulated and down-regulated genes in ART resistant parasites will be analysed by using BLAST2GO, AmiGO databases for functional classification of genes showing modulated expression. KEGG pathway analysis tool will be utilized to identify the pathways in which these genes were involved in. Validation of modulated gene expression in K133 AS-R parasites by real time q-PCR.

3. Identification of *Leishmania donovani* membrane proteins interacting with macrophages by 2-Dimensinal gel Electrophoresis and Mass Spectrometry

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

Duration : 2016-17

Funding Agency : ICMR

Aims, Objectives & Background

Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania donovani* parasite. VL is endemic in India and with no anti-leishmanial vaccine available, chemotherapy remains the mainstay of VL control strategy. Disappointingly few drugs are available in clinical practice and their efficacy is limited due to the toxicity and increasing multiple drug resistance. Membrane proteins play a pivotal role in host-pathogen interactions and in regulatory pathways. The process of host cell invasion requires contact with the host cell plasma membrane and these interacting proteins were targeted in many infections. Identification of such virulence factors responsible for parasite establishment may provide leads for unraveling new drug targets. Very few proteomic studies have been carried out with *Leishmania* parasite.

Work done

In the present study, invasion proteins of *Leishmania donovani* were identified and studied. *L. donovani* membrane proteins were isolated and Cy5 dye labelled. They were then allowed to interact with the intact host cells (THP-1 macrophages). The interacting proteins were identified using 2-dimensional gel electrophoresis followed by mass spectrometry analysis. Activated C kinase, Peroxidoxin, Small Myristoylated Protein 1 (SMP-1) and Cytochrome C oxidase were identified as interacting membrane proteins of *L. donovani*(Figure 1). We also found that beta -Actin, Filamin A interacting protein 1 (FILIP 1) and alpha2 macroglobulin proteins of macrophages are involved in interaction with parasite and may have roles in the parasitic invasion process.

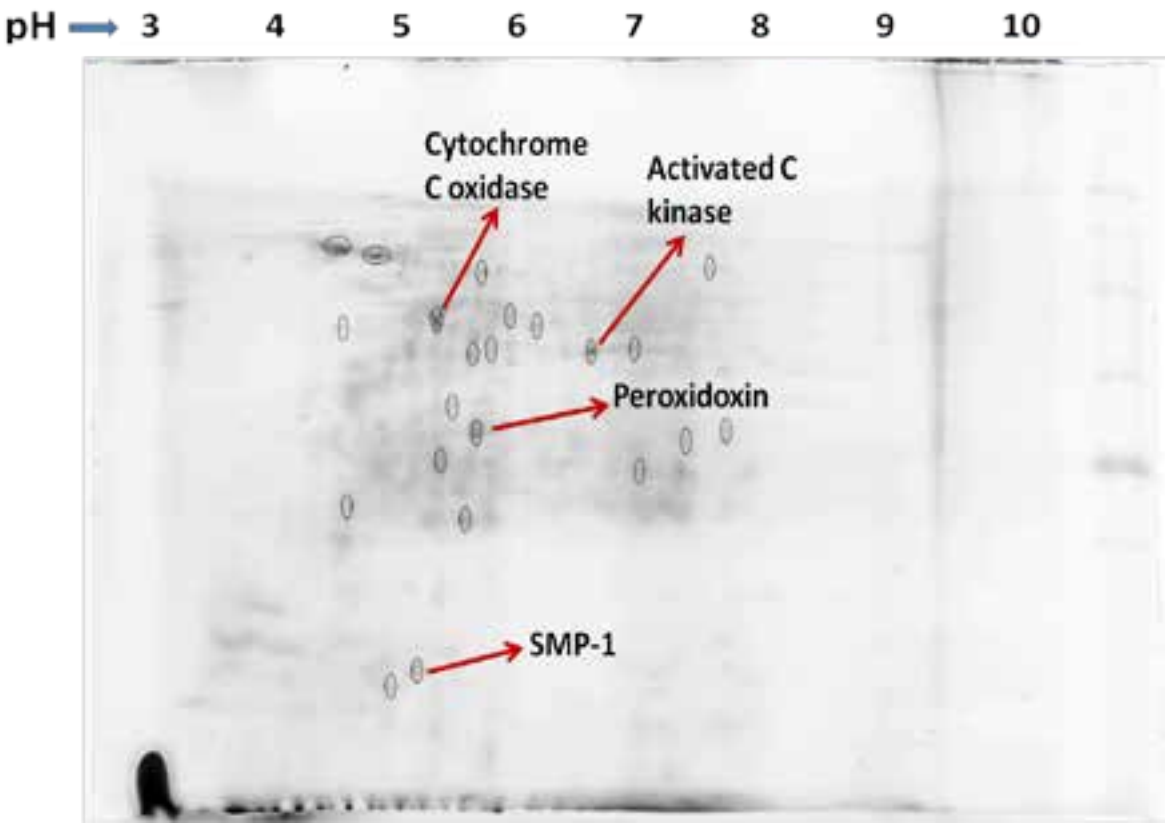


Fig. 1. Typhoon scanned gel for identification of interacting parasite membrane proteins (circled, n=19). Proteins indicated were identified by mass spectrometry.

Functional characterization to establish the role of protein in host-parasite interaction was attempted for Activated C kinase protein. The interaction was investigated using Withaferin A, an inhibitor of Activated C kinase protein. In the presence of Withaferin A, infection of macrophages (THP-1) with *L. donovani* parasites was inhibited markedly (Fig 2) indicating the significant role of Activated C kinase protein in parasite interaction with macrophages.

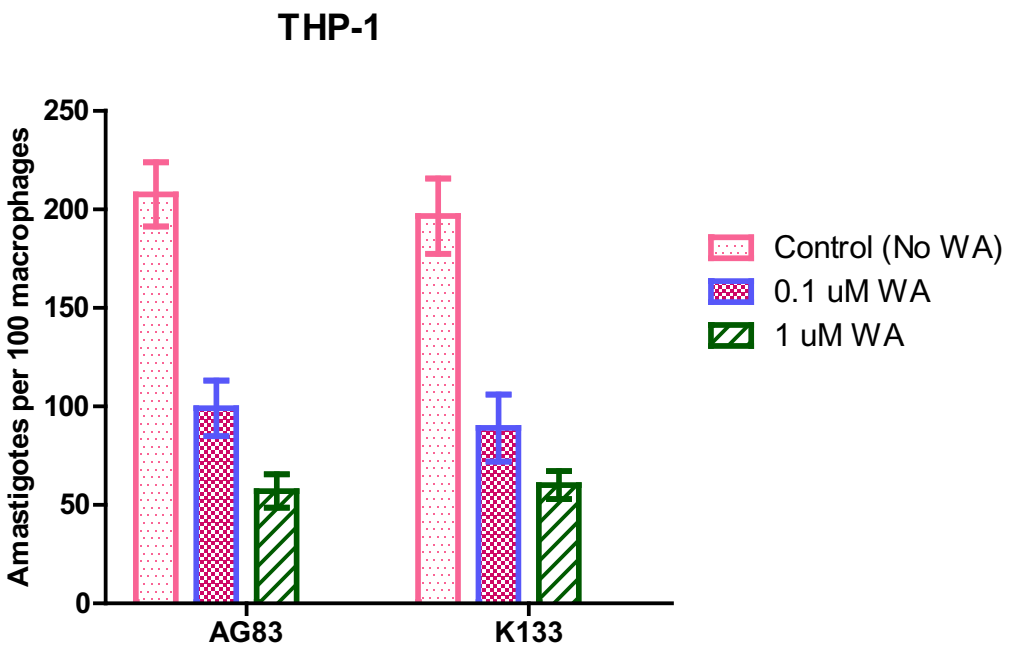


Fig. 2. THP-1 macrophage infection with *L. donovani* AG83 and K133 parasites in the absence and presence of Withaferin A (WA), an inhibitor of Activated C kinase protein. Error bars show standard deviation.

4. Application of LAMP for rapid and sensitive detection of *Leishmania* using direct lysis of clinical samples

Scientific Staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Dr. Sandeep Verma, Ms. Keerti Kaumudee Dixit

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

Duration : 2016- 2019

Funding Agency : ICMR-Intramural

Aims, Objectives & Background

LAMP technique for specific amplification of target DNA under isothermal condition is a simplified method for parasite detection without the need of sophisticated instruments. Previously we have applied SYBR Green I based LAMP assay based on 6 primers targeting

kDNA region of *Leishmania* as a diagnostic tool for VL and PKDL. The assay was applied on confirmed VL and PKDL samples at NIOP and validation was done by performing the assay on confirmed VL cases in endemic region (RMRI, Patna). We have now further validated the assay at Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi. Furthermore in an attempt to enhance the potential of LAMP assay in field applicability, Direct Blood Lysis (DBL) approach has been tried on VL and PKDL samples. The DBL-LAMP approach eliminates the step of DNA isolation which has improved the assay making it both time and cost efficient.

Work done during the year

1. Validation study conducted at Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi

Previously, we have done the validation of LAMP assay by performing it on confirmed VL cases at RMRI, Patna which yielded a sensitivity of 98% and specificity of 97.14%. This year validation of the LAMP assay was carried out at Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi. Standard operating protocols were prepared and provided at the validation centre. The research scholars at IMS, BHU centres were trained to perform the LAMP assay for diagnosis of VL. Only rK39 strip positive and LD (Leishman-donovan) bodies in splenic aspirate confirmed by microscopy cases were included in the validation study. The LAMP assay gave sensitivity and specificity of 98.44% and 96.22% respectively. The obtained sensitivity and specificity of LAMP assay in diagnosis of VL at both validation centres corroborated with our reported sensitivity and specificity of 96.9% and 100% respectively.

2. Sensitivity and specificity of LAMP assay in diagnosis of VL.

VL patients originating from Bihar and reporting to the Department of Medicine, Safdarjung Hospital, New Delhi were included in this study at pre-treatment stage. The patients presenting with characteristic symptoms of VL (fever, hepatosplenomegaly, anemia and leucopenia) were included in this study.

The LAMP assay gave a sensitivity of 96.9% with column extracted blood DNA and 92.42% with direct blood lysed DNA. The assay was negative for all the control samples which were inclusive of Malaria, Typhoid and healthy controls. (Fig. 1-A)

3. Sensitivity and specificity of LAMP assay in diagnosis of PKDL

Patients reporting to Department of Dermatology, Safdarjung Hospital, New Delhi, diagnosed as PKDL based on clinico-histopathological observations and rk39 strip test & Q-PCR positive confirmed cases were included in the study at pre-treatment stage. PKDL column extracted slit DNA and blood DNA were subjected to LAMP assay which provided a sensitivity of 83.78% and 81.08% respectively. Furthermore, application of LAMP assay by using direct blood lysed DNA gave a sensitivity of 78%. The assay was negative for all other disease and healthy control samples giving it a specificity of 100% (Fig. 1-B).

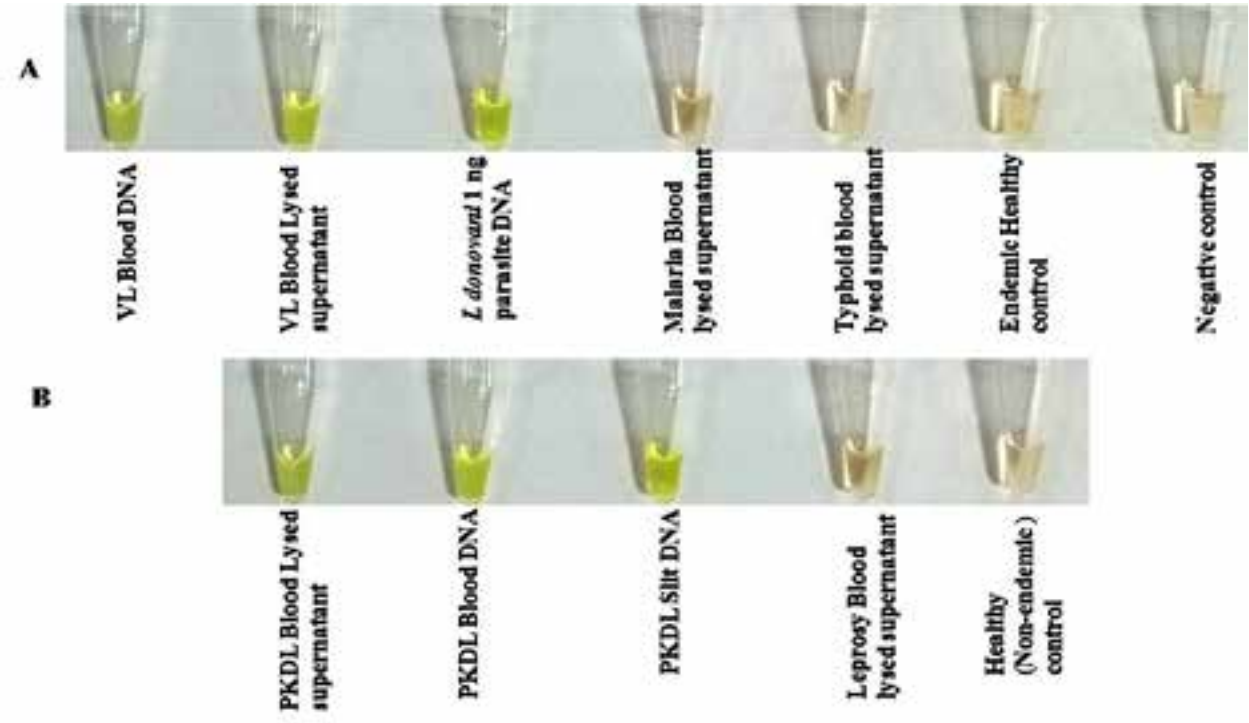


Fig. 1: Sensitivity and Specificity of LAMP assay in diagnosis of VL and PKDL. A- LAMP assay with direct blood lysed samples of VL, Malaria, Typhoid, Endemic healthy control. **B**- LAMP assay with slit, blood DNA and direct blood lysed sample of PKDL, Leprosy and Non-endemic healthy controls. 5 µL of lysed supernatant was used for amplification. 2µL of blood and slit DNA was used for putting up LAMP assay. *L. donovani* (1ng/µL) genomic DNA was used as positive control for the assay.

Outcome

The LAMP assay was found to be highly sensitive and specific for diagnosis of VL and PKDL. Validation of the assay, carried out at IMS, BHU, Varanasi further strengthened its utility as a potential diagnostic tool. Further efficient DNA amplification using the crude DNA derived by direct lysis of clinical sample (DBL-LAMP assay) for diagnosis of VL and PKDL reduces both cost and turnaround time advocating its applicability in field conditions.

Future Work

The LAMP assay has the potential application in mass surveillance of *Leishmania* infected cases in endemic areas. Detection of PKDL cases becomes critical for sustenance of target of VL elimination of less than 1 case per 10,000 population at block level under KA elimination programme in Indian sub continent. The application of LAMP assay need to be further explored by employing the assay using FTA cards for sample collection making it least invasive. Moreover, LAMP assay also has a probable implementation in mass screening of asymptomatics which needs to be assessed.

5. Development of LAMP assay for quick surveillance of carbapenem-resistant gram negative bacteria in clinical samples

Scientific staff : Dr. Ruchi Singh, Dr. Poonam Salotra
Collaborator : Dr. Rajni Gaind, VMMC and Safdarjung Hospital
Year in which project started : 2017
Proposed year of completion : 2019
Funding Agency : Intramural

Aims, Objectives and Background

Nosocomial and community acquired infections caused by multidrug resistant Gram-negative bacteria (GNB), represent a growing problem worldwide. Treatment is increasingly

complicated by the escalating incidence of antimicrobial resistance. Carbapenem-resistant gram negative bacteria including *Acinetobacter baumannii* (CRAb) and Eneterobacteriaceae (CRE) are pathogen associated with high morbidity and mortality as well as longer hospital stays. In most clinical settings, the detection of antimicrobial resistance is done by conventional disk diffusion susceptibility assays that remain the 'gold standard' are labor-intensive and time consuming and often the results are received too late to help the patients.

Molecular antimicrobial resistance screening methods can improve the diagnosis of resistant infection as they have quick turnaround time. Recently, field applicable portable Isothermal amplification with fluorescence based detection system have been developed and used for detection of various infectious agents. CRAb isolates in Asia mostly possess $bla_{OXA-23,}$ while bla_{OXA-48} is commonly identified in the CRE *Escherichia coli* and *Klebsiella pneumoniae*. bla_{NDM1} has been detected in both CRAb and CRE isolates. We propose to develop a method for detecting these target genes directly from clinical specimens using LAMP assay. We also propose to establish the utility of the assay as a surveillance tool in a hospital.

Work done during the year

The set of primers targeting blaOXA-23 and blaNDM-1 were designed using Primer Explorer V4 software (Net Laboratory, Kanagawa, Japan) based on the reported genome sequences. DNA extracted from blood culture sent to Microbiology Department for antimicrobial sensitivity test was used to for amplification of genes imparting carbapenem resistance, i.e. blaOXA-23 and blaNDM-1 by LAMP and PCR based assays. The assay was tested in 14 samples along with verified isolates positive for OXA-23 and NDM1 gene and negative control *E.coli* ATCC 25922. Both the LAMP assay and PCR assay detected the blaOXA-23 in 35% of the isolates whereas blaNDM-1 was detected in 14% isolates.

CHLAMYDIASIS

1. Study on matrix metalloproteinases and their inhibitors in women with *Chlamydia trachomatis*-associated ectopic pregnancy

Scientific Staff : Dr. Sangita Rastogi, Ms Shipra Pant

In collaboration with : Dr. Sunita Malik, Dr. Ruchi Hooda, VMMC & SJ Hospital, New Delhi

Duration : 2017-20

Aims, Objectives and Background:

Ectopic pregnancy following scarring of the fallopian tubes is a serious sequelae of sexually transmitted infections. More than 95% of all ectopic pregnancies are located in the fallopian tube. *Chlamydia trachomatis* infection is considered to be the most important etiologic agent for ectopic pregnancy that causes considerable maternal morbidity/ mortality. Past chlamydial pelvic inflammatory disease is also associated with an increased risk of ectopic pregnancy. Moreover, women with ectopic pregnancy are at an increased risk of repeat ectopic pregnancy and infertility. Diagnosis is difficult due to lack of biomarkers and cellular mechanisms imply modification of the extracellular matrix during ectopic implantation. Studies in fallopian tube organ cultures infected with *C. trachomatis* and animal models suggest that regulation of matrix metalloproteinases (MMPs) plays a central role in the pathogenesis of tubal/ murine oviduct damage caused by *Chlamydia,* however, there are limited studies available in humans. The present study aims to investigate whether the presence of chlamydial heat shock protein (chsp) 60 can be used together with MMPs/ tissue inhibitors of matrix metalloproteinases (TIMPs)/ activins/ inhibins as marker of ectopic pregnancy for an early diagnosis/ management of an unruptured tubal pregnancy in *C. trachomatis*-positive women.

Work done during the year:

Enrollment of patients and collection of clinical samples:

Women undergoing salpingectomy (SALP) during laparotomy for ruptured/ unruptured tubal ectopic pregnancy comprised the study group (Group I) while age-matched women undergoing mini-laparotomy for tubal ligation (Group II) served as controls. The patients were enrolled under the guidance of the collaborating gynecologist from Department of Obstetrics & Gynecology, Safdarjung hospital. Those with tubo-ovarian mass, history of tuberculosis and ectopic pregnancy undergoing medical management were excluded from the study. Various parameters including gestational age and intra-operative findings such as location of ectopic pregnancy in the fallopian tube/ status of the contralateral tube/ adhesions/ clinical presentation of *Chlamydia* (Fitz Curtis syndrome) were noted in each patient. The fallopian tube sample obtained at the time of SALP/ tubal ligation was subjected to histopathological examination for confirmation while the remaining tissue was cryopreserved in liquid nitrogen and stored at -80^{0}C for the nucleic acid amplification tests. Non-heparinized blood was collected to investigate the presence of serological markers of *C. trachomatis*. Samples were also screened for other co-infections.

Detection of infection and quantitative analysis of MMPs:

Infection with *C. trachomatis*, genital mycoplasmas and herpes simplex virus (HSV) was detected in 3/10 (single infection), 2/10 and 1/10 patients (Group I), respectively; while co-infection with *Ureaplasma spp.* and HSV was detected in 1/10 patients (Group I). 1/5 patient was found to be infected with HSV in Group II. The expression of MMP-2 and MMP-14 genes was studied in the tissue of enrolled subjects by q-PCR and analysis of Ct values showed that the mRNA expression of MMP-2/ MMP-14 was increased among the infected Group I patients in comparison to the controls. In Group I patients, the expression of MMP-2/ MMP-14 was high in *C. trachomatis*-positive women undergoing SALP for ectopic pregnancy *versus Chlamydia*-negative women undergoing SALP for ectopic pregnancy. Further studies are underway to study the expression of MMPs to establish whether they play a role in the pathogenesis of tubal ectopic pregnancy in infected women.

TUBERCULOSIS

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

Scientific Staff : Dr. Nasreen Z. Ehtesham, Manjunath P., Javeed Ahmad, Dr. Javaid A Sheikh, Simran Kaur Arora

In Collaboration with : Dr. Seyed E. Hasnain IIT Delhi and Jamia Hamdard

Duration : 2012-2017

Funding : Intramural

Aims, Objectives & Background

Globally there were an estimated 1.5 million deaths from Tuberculosis (TB). TB ranks alongside HIV as a leading cause of death worldwide (World tuberculosis report, 2016).

Comparative computational analyses of different species of mycobacterium have shown that the size of genome decreases with gain in pathogenesis (Rahman SA et al, 2014) highlighting the importance of genes which are present only in pathogenic *Mycobacterium tuberculosis*. Despite intense research on *M.tb* pathogenesis, detailed molecular mechanisms of the distinct mycobacterial virulence factors remain incompletely understood. In order to understand mechanism of pathogenesis, the functions of numerous *M.tb* gene products which are absent in other non-pathogenic mycobacterial species gains special importance. Based on the computational analysis of 13 mycobacterial species two hypothetical proteins, termed as 'signature proteins' SP1 and SP2 were identified. One of the signature protein (SP2) displays iron binding activity and protection of DNA from hydroxyl radical damage. Moreover, bioinformatic analysis revealed the presence of Methyltransferase and DNA binding leucine zipper motif. Secondary structure prediction showed the presence of highly ordered structure with very less disorder content around. This was also reported

to be an essential protein for the survival of *M.tb.* To further understand the role in virulence, *M.smegmatis* was transformed with SP2.

These two signature proteins SP1 and SP2 were functionally and immunologically characterized in order to understand their role in *M.tb* biology. Both signature proteins were found to induce pro-inflammatory immune response in immunized mice and showed high IgG reactivity in sera of immunized mice and also in human TB patients. One of these signature protein induced the maturation of dendritic cells and enhanced effective memory response in immunized mice.

Work done during the year

Generation of recombinant *M. smegmatis*

Complete ORF of SP2 gene was successfully cloned in pST-Ki and confirmed by restriction digestion as shown in (**Fig. 1A**). pST-Ki-SP2 construct was electroporated into *M. smegmatis* and transformed colonies (Ms_SP2) were confirmed by colony PCR (**Fig. 1B**)

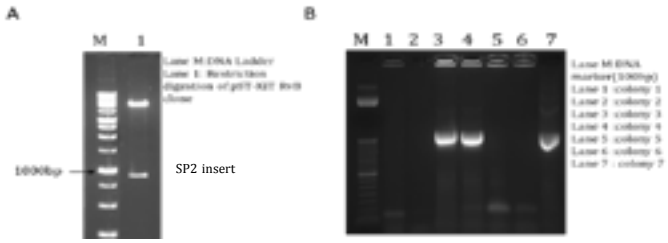


Fig 1A: Clone confirmation of pST-Ki_SP2 through restriction digestion. **Fig 1B**: Clone confirmation of pST-Ki_SP2 by colony PCR after Electroporation in to *M. Smegmatis*

Analysis of SP2 Expression in *Mycobacterium smegmatis*

Transformed colonies were grown in 7H9 media supplemented with ADC, tween80 and cultures were grown for 48 hrs. *M. smegmatis* (Ms_Vec) transformed with vector alone (pST-Ki) were also grown in similar conditions as control. After 48 hrs cultures were pelleted by centrifugation and protein expression analysis done by western blotting using antibody against SP2 developed in rabbit (Fig. 2).

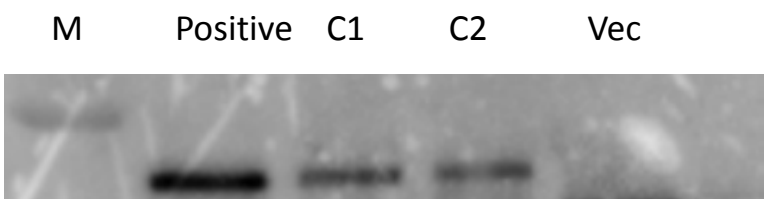
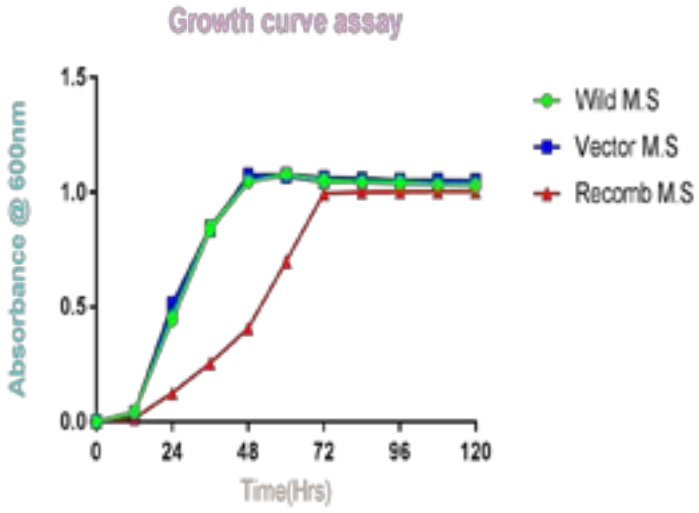


Fig. 2: Western blot showing the expression of SP2_*M. smegmatis*

Growth characteristics of Ms_SP2, Ms_Vec and wild type *M. smegmatis*

To analyse the effect of SP2 gene of *M. smeg,* Ms_SP2, Ms_Vec and M.s were grown in similar condition. Growth analysis was compared at different time points through absorbance at 600nm (Table 1). After 72 hrs growth curve was plotted and compared with Ms_Vec and wild type *M. smeg.* Very interestingly it was observed that the expression of SP2 in *M. smeg* drastically decreases the growth of *M. smeg* as compared to vector transformed *M. smeg* (Ms_Vec*)* and wild type (Fig. 3).

Table 1 Absorbance readings at different time points



Time	Wild	Ms_Vec	Ms_SP2
0	0.00	0.00	0.00
6	0.012	0.011	0.008
12	0.146	0.145	0.016
24	0.456	0.516	0.126
30	0.623	0.697	0.198
36	0.849	0.898	0.256
42	1.026	1.106	0.315
48	1.046	1.108	0.407
60	1.079	1.068	0.698
66	1.046	1.042	0.803
72	1.045	1.025	0.996

Fig. 3: Growth curve analysis of Ms_SP2 (red), Ms_wild (green) and Ms_Vec (blue)

Morphological analysis of Ms_SP2, Ms_Vec and wild type *M. smeg* :

Once the OD600 reaches 0.5 samples from each (Ms_SP2 and Ms_Vec) were screened by Ziehl Neelsen staining and compared with wild type *M. smeg*. It was observed that length of Ms_SP2 is much larger as compared to Ms_Vec and wild type *M. smeg* and also the colony morphology is rough compared to Ms_Vec and wild type. *M. smeg.* (Fig.4)

Fig. 4: Morphological analysis: (A) Ms_SP2, (B) Ms_Vec (C) Wild type *M. smegmatis.* Ms_SP2 shows long filamentous morphology compared to others.

Comparative protein profiling of *M. smegmatis* and Ms_SP2:

As the growth retardation and morphological changes in Ms_SP2 were observed, protein profile comparison by 2D Electrophoresis was performed. 2D Electrophoresis showed altered gene expression levels along with some new genes being expressed (Fig 5).

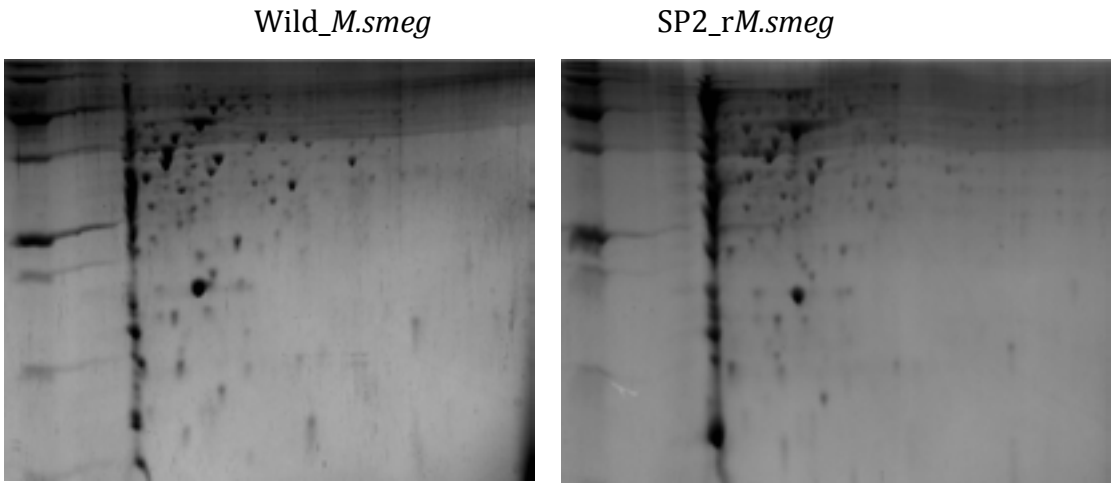


Fig. 5: 2D gel Electrophoresis of wild and Ms_SP2 showing differential gene expression levels.

Intracellular survival of recombinant *M. smegmatis* in macrophage

RAW264.7 cell line were infected with Ms_SP2 and Wild type *M.smeg* expressing GFP to check whether the altered gene expression levels play a role in intra cellular survival. It was observed that the uptake of Ms_SP2 was low compared to wild type *M.smeg*. However survival of Ms_SP2 in macrophage were for longer duration, ultimately leading to the necrosis of macrophages. (Fig.6)

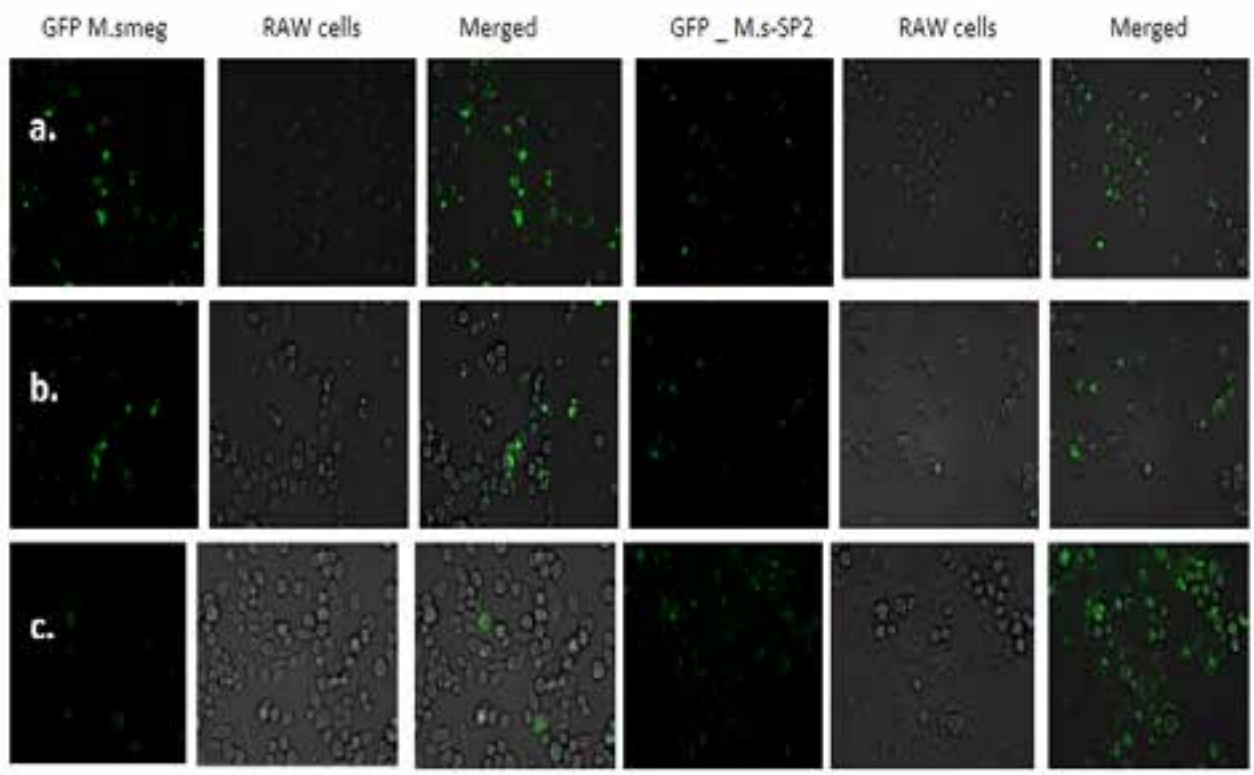


Fig. 6: a. RAW cells uptake of bacilli after 4 hours of incubation with SP2 and wild type *M.smegmatis*. b. After 24 hours of post infection of RAW cells with wild and SP2_*M.smegmatis*. c. After 48 hours of post infection of RAW cells with wild and SP2_*M.smegmatis*.

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

Scientific Staff : Dr. Nasreen Z. Ehtesham, Dr. Mohd Shariq, Dr. Manish Bhuwan
Duration : 2014-2019
Funding Agency : ICMR - RA

Aims, Objectives and Background

M.tuberculosis usually employs arsenal of proteins called invasins to infect phagocytic cells. RipA (inv1, invasin1) a cell associated and secreted protein that interacts with resuscitation promoting factors RpfB and RpfE and synergizes transglycosylase activity.

Previous studies have demonstrated that RipA interacts with a chaperone MoxR1 and is secreted through a TAT secretion pathway. This secreted RipA might gain entry into the host cells through its association with mammalian cell entry protein Mce2B to exhibit virulence (Bhuwan M et al. 2016). In order to understand its functions in virulence after entering into the host cells experiments were designed.

Work done during the year

C-terminal GFP-tagged RipA was transient transfected into HEK293T cells and analyzed using immunofluorescence microscopy. GFP vector was used as a negative control. After transfection, GFP-tagged RipA localized in the cytoplasm as a punctuated foci, indicating that it may be localized in specific organelle in the cytoplasm. DAPI stain was used to mark the nucleus (Figure 1).

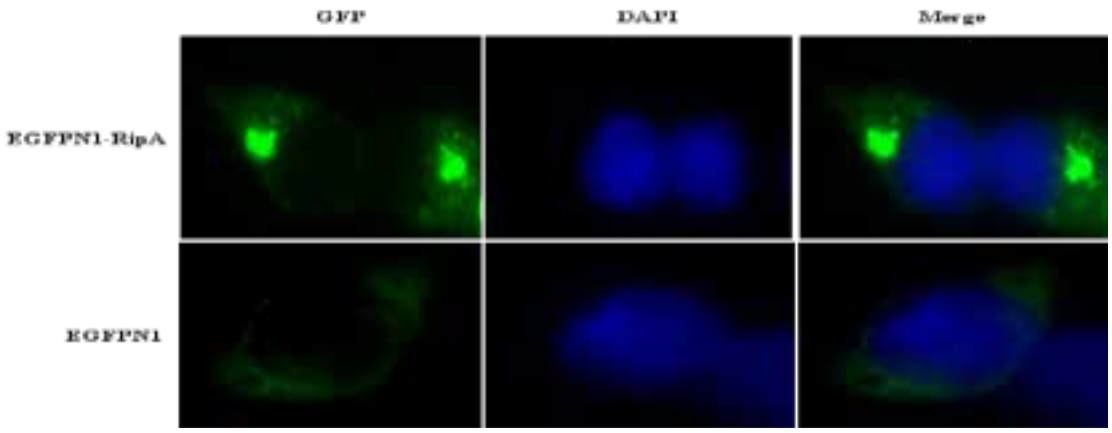


Fig. 1: Immunofluorescence microscopic images showing localization of GFP-tagged RipA. GFP-tagged RipA localized in the cytoplasm of transiently transfected HEK293T as punctuated foci. DAPI was used to stain the nucleus. Localization of GFP alone was used as a negative control.

Furthermore, RipA protein sequence was analyzed for the presence of a possible organelle targeting sequence. All the used tools predicted presence of a mitochondria targeting sequence and possible mitochondrial localization with high probability (>90%). To confirm this HEK293T cells were transiently transfected with GFP-tagged RipA. Mitochondrial positions in the cells were probed using Mitotracker deep red dye. Nuclei positions were marked using nuclear stain DAPI (Figure 2).

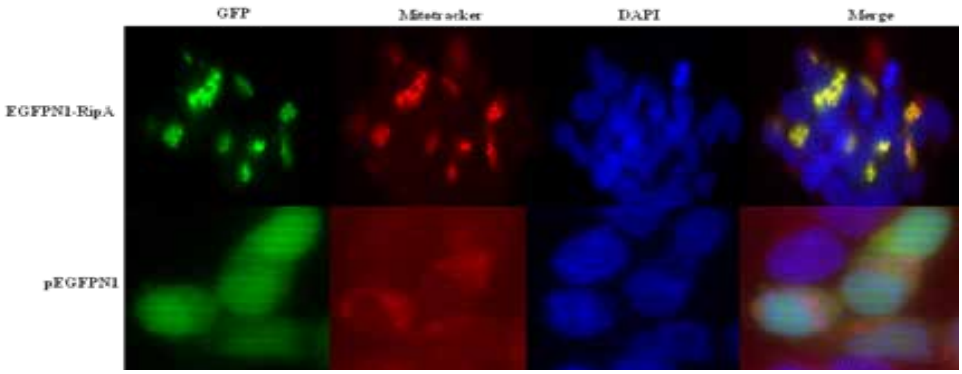


Fig. 2: GFP-tagged RipA co-localized to mitochondria. HEK293T cells were transiently transfected with GFP-tagged RipA or GFP alone as a control. Mitochondrial positions were probed using Mitotracker deep red dye. Nucleus was stained using nuclei staining dye DAPI.

Co-localization of RipA to mitochondria was further confirmed by i) transiently transfecting HEK293T cells either with pCDNA-RipA or pCDNA and probing it with anti RipA antibody

ii) GFP-tagged RipA was transiently transfected to into HEK293T. Mitochondrial positions in the cells were probed using Mitotracker deep red dye. Nuclei positions were marked using nuclear stain DAPI (Figure 3).

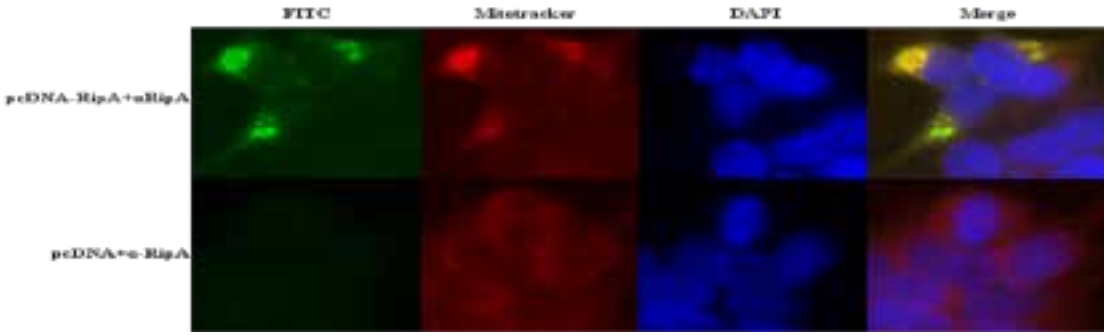


Fig. 3: Immunofluorescence microscopic images demonstrating co-localization of endogenous RipA to mitochondria. HEK293T cells were transiently transfected using pc-DNA-RipA without tag or pc-DNA vector alone. RipA positions were probed using polyclonal anti-RipA antibody. Mitochondrial positions were marked using mitochondrial staining dye mitotracker deep red. Nuclei positions were marked using nucleus staining dye DAPI. FITC labelled secondary antibody was used for signal detection.

Transient transfection with either GFP tagged RipA or untagged RipA probed with anti RipA antibody confirmed the localization of RipA to mitochondria.

3. Immunomodulatory role and antigenicity of Co-operonic PE-PPE genes belonging to the *Region of difference* of the *Mycobacterium tuberculosis* genome.

Scientific Staff : Dr. Nasreen Z. Ehtesham, Dr. Mohd Khubaib, Javeed Ahmad
In collaboration with : Prof. Seyed E. Hasnain, IIT, Delhi, Dr. Saroj, AIIMS
Duration : 2015-2017

Aims, Objectives and Background

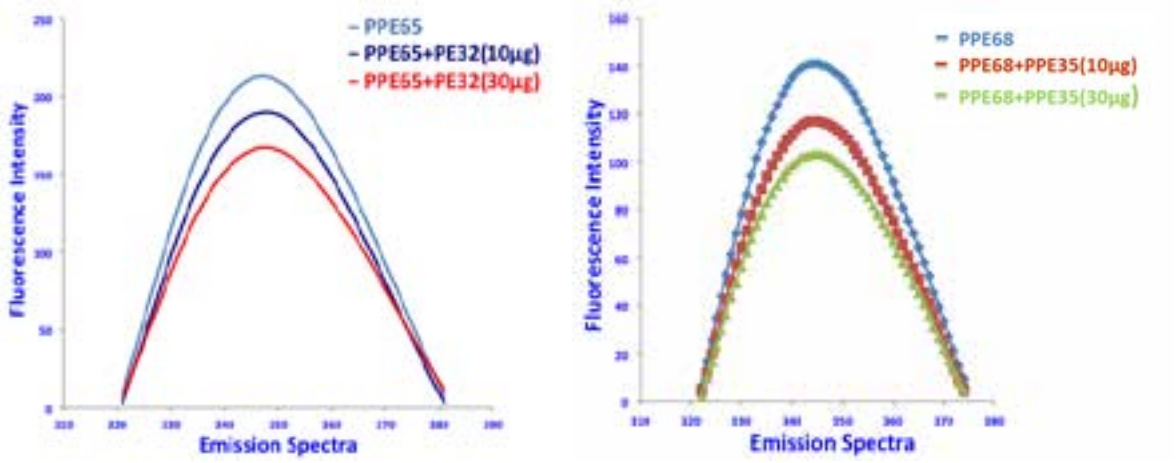
The *M.tb* proteome has a relatively high content of intrinsically disordered proteins (IDPs) primarily due to the presence of unique family of proteins called PE-PPE/PGRS family,

which often contain long disorder region. The role of protein disorder in *M.tb* pathogenicity and host pathogen interaction is still unclear. Our results on two pairs of PE/PPE (PE35, PPE68 and PE32, PPE65) suggests that the presence of intrinsically disordered regions and host-like interaction sites in *M.tb* proteins could be a key strategy employed by the pathogen for rapid adaptive changes, host-invasion, hijacking of host machinery and diversifying the functions of its proteins.

Work done during the year

Structural prediction for each member were investigated and reference was set using the IUPred (long) algorithm. Residues with a predicted disorder propensity >0.5 were considered as disordered. PE-PPE family disorder analysis was also carried out using the RONN prediction tool. For detecting disordered binding regions the ANCHOR was used. Fluorescence emission spectra of all 4 recombinant proteins (0.5 mg/ml) were carried out in a Jasco fluorimeter (FP-6200). The excitation wave length was set at 280nm and emission spectra were measured in the wavelength region 300–340 nm in quartz cuvette of path length 3 mm. All the experiments were carried out at 25±0.1°C with both excitation emission slits set at 5 nm. Thereafter, 3 µl of concentrated proteins were placed on a SensIR ATR reflection element. FTIR spectra were recorded at 4 cm^{-1} resolutions on an Agilent Cary 630 spectrometer equipped with a DTGS detector. Secondary structure analysis was carried out by curve fitting using the OPUS software.

As PE32 and PE35 proteins lack tryptophan residues, fluorescence spectroscopy was used to analyse the interaction of PE32 with PPE65 and PE35 with PPE68, (**Fig. 1**). A concentrations dependent clear shift in the emission spectrum of PPE65 was observed in presence of PE32 (**Fig. 1A**). Similarly, a pronounced change in fluorescence intensity was observed in PPE68 in presence of PE35.(**Fig. 1B**).



Fig, 1: Fluorescence spectroscopy for protein-protein interactions: (**A**) Fluorescence spectra of PPE65 with different concentrations of PE32. (**B**) Fluorescence spectra of PPE68 with different concentrations of PE35.

Using curve fitting analysis on recombinant PPE65 (**Fig. 2B**), more than 66% random coil and unstructured regions content was observed, implying that the protein is loosely folded. The band observed below 1630 cm^{-1} (1630-1615 cm^{-1}) indicated unstructured regions or protein aggregation. The bands near 1643 cm^{-1} (1650-1640 cm^{-1}) and 1675 cm^{-1} (1680-1662 cm^{-1}) arise from random coil conformations. The band near 1653 cm^{-1} (1660-1650 cm^{-1}) signals alpha helical structure, while the band observed above 1680 cm^{-1} (1700-1680 cm^{-1}) is assigned to beta sheets or beta turns. Fair amount of unstructured regions in PE32 was also observed (**Fig. 2A**), however this protein was more structured in comparison to PPE65. PE32 and PPE65 interaction (1:1 ratio) demonstrated more than 66% ordered structure with 36% alpha helices and 32% beta sheets. Thus a structural shift from disorder to order occurred upon complex formation.

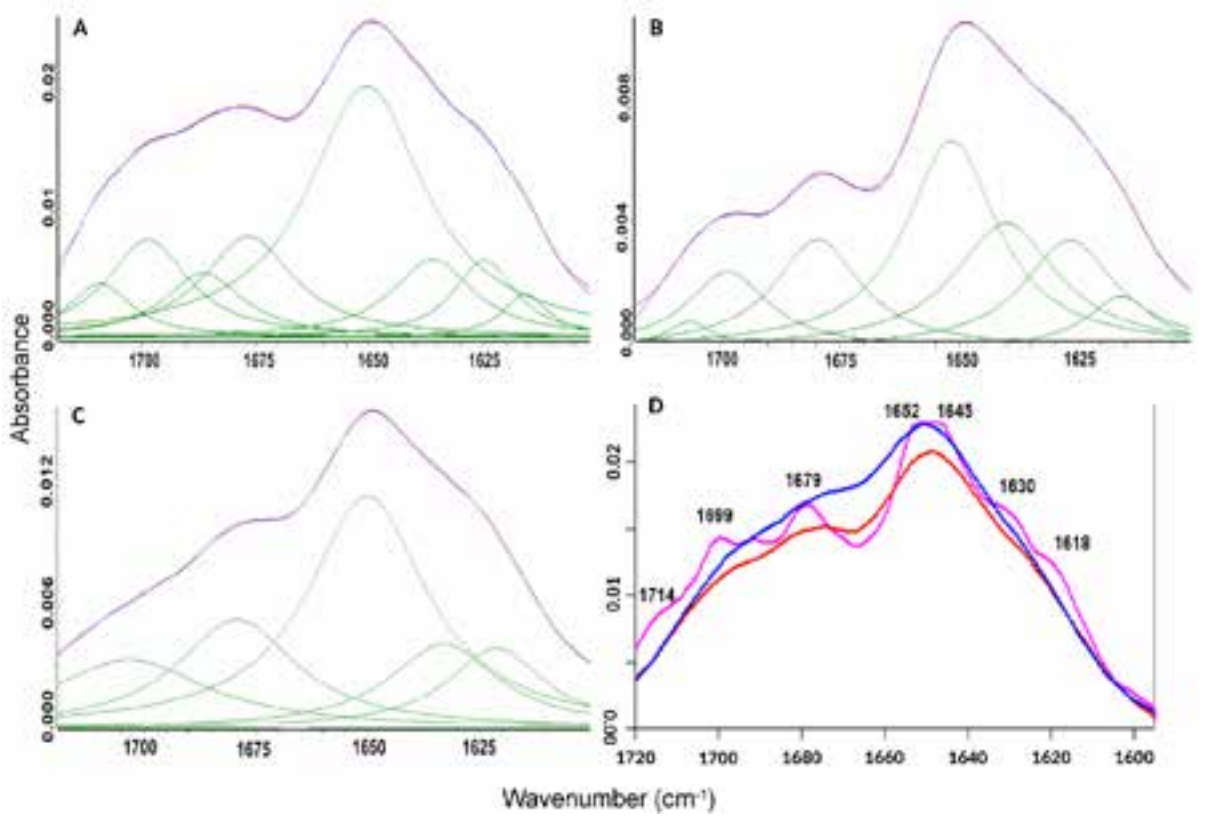


Fig. 2: FT-IR absorbance spectra of the PE35-PPE68 pair: (A) Curve fitting for amide I and amide II regions of PE35 (0.5mg/ml). (B) Curve fitting for amide I region of PPE68 (0.5mg/ml). (C) Curve fitting for amide I region of PE35-PPE68 complex. (D) FT-IR spectra comparison of PE35, PPE68 and PE35-PPE68 complex showing a disorder to order transition upon interaction.

Similarly, PE35 displayed a highly disordered structure with 41% of regular secondary structures (**Fig. 3A**), to which alpha helices and beta sheets contributed 23 and 18%, respectively. The much longer PPE68 had a similar content of secondary structure (43%) as PE35, while in agreement with the applied disorder prediction methods a significant amount of unstructured regions were also observed (**Fig. 3B**). Analysis of the structure content of the PE35-PPE68 complex revealed a major transition from disorder to orde rupon complex formation (**Fig. 3C,D** and Table S2), but with significant regions (40%) still retaining their disordered states in the complex, likely involving the C-terminal disordered half of PPE68. Additionally, in case of PPE68 we observed a high intensity peak near region 1715cm^{-1}, suggesting the presence of lipids. This peak may indicate that the protein is lipidated on some of its known myristoylation sites (**Fig. 3C**), or some

membrane remnants remained attached to it during the purification process. The peak around 1709 cm^{-1} indicates the involvement of charged residues such as Asp/Glu/Asn in the interaction between the two proteins. In all, comparing the spectra of the individual proteins and the corresponding complexes showed major structural shifts towards a more structured state, but also indicated the presence of disordered regions in the complex state for both protein pairs.

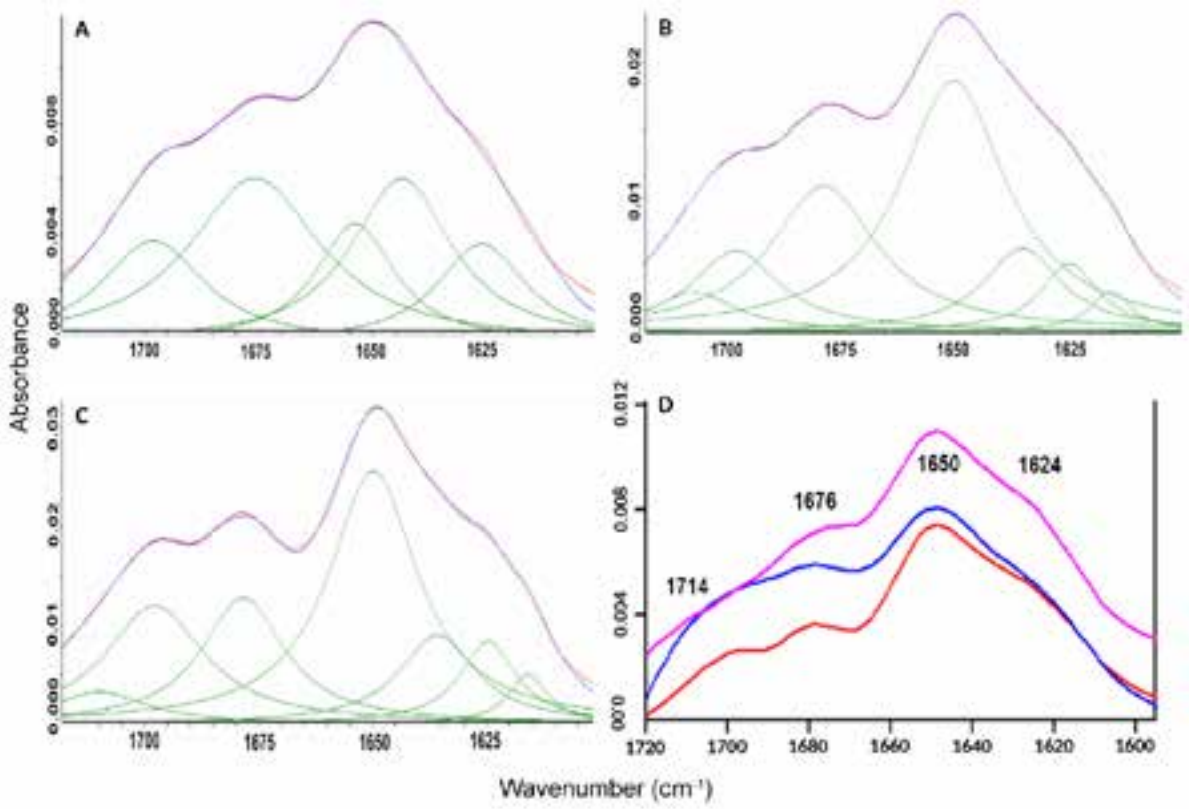


Fig. 3: FT-IR absorbance spectra of the PE32-PPE65 pair: (A) Curve fitting for amide I and amide II regions of PE32 (0.5mg/ml). (B) Curve fitting for amide I region of PPE65 (0.5mg/ml.) (C) Curve fitting for amide I region of PE32-PPE65 complex. (D) FT-IR spectra comparison of PE32, PPE65 and PE32-PPE65 complex showing a disorder to order transition upon interaction.

In summary, infection and intracellular survival strategies of *M.tb* may rely on disordered proteins, as its specific pathogenesis-related PE-PPE/PGRS proteins as well as its secretome are enriched in structurally disordered regions. The repetitive and disordered nature of PE-PPE/PGRS proteins likely enable fast evolutionary changes and thus may mask the pathogen from host immune response. Our results suggest that *M.tb* is able to

counterbalance reductive evolution by employing this specific family of proteins, many of which contain disordered regions which either achieve a more ordered state upon PE-PPE interaction or retain partial disordered states even after the formation of the specific complex. Being exposed on the pathogen surface and showing high levels of functional specialization among family members through variable segments, PE-PPE protein complexes successfully influence the host immune system toward favoring pathogen survival (Figure 4)

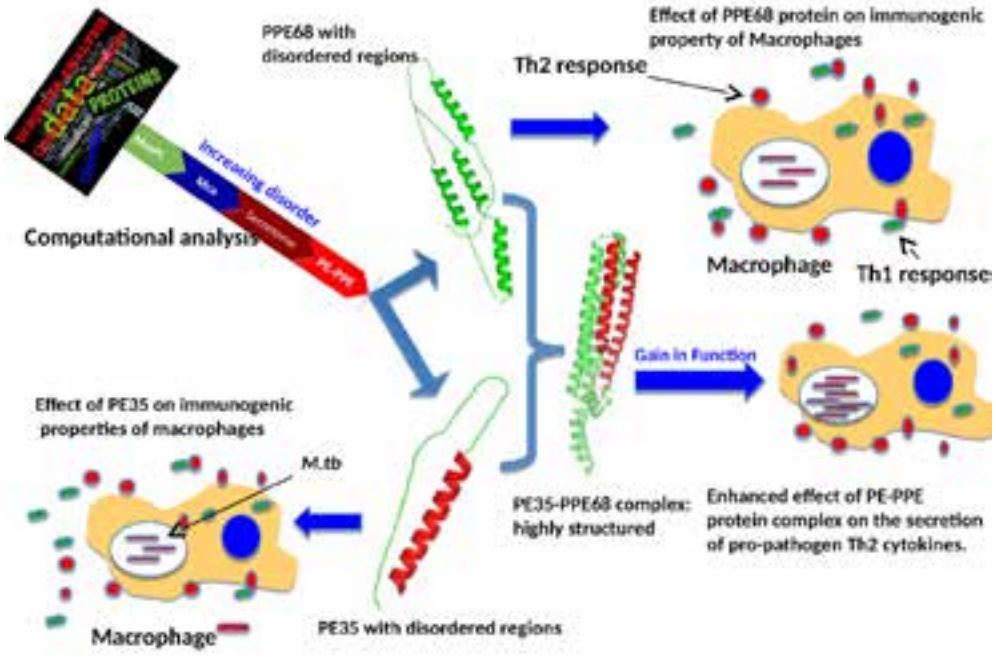


Fig. 4: Proposed model for gain in immunogenicity through protein-protein interaction and disorder-to-order transition of PE-PPE protein pairs. The well-structured PE35-PPE68 complex has an enhanced effect on Th1 and Th2 type cytokines secretion: secretion of Th1 cytokines (green) is decreased, while that of Th2 cytokines (red) are enhanced, which in turn will increase the survival chances of *M.tb.*

4. 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, Dr. Mohd. Khubaib, Simran Kaur Arora

In Collaboration with : Dr. A. Srinivasan, AIIMS and Prof. Seyed E. Hasnain, IIT Delhi Dr. Madan Babu, MRC cambridge

Duration : 2014-2018

Aims, Objectives and Background

One of the important and efficient mechanisms utilized by *M.tb* in host macrophage is by disturbing the Th1 /Th2 balance and switching the balance to pro-pathogen Th2 response. This shift in balance is mainly achieved by suppressing the pro-inflammatory cytokines and enhancing anti-inflammatory cytokines response. One of the protein PPE37 from PE-PPE family, which was found to be highly antigenic and immunogenic, was selected. Earlier studies on PPE37, have shown that this protein is cleaved into N-terminal and disordered C-terminal segments under iron depleted conditions. Full length (PPE37FL) and its N-terminal segment (PPE37N) were expressed and purified for immune studies in mice. These results showed that the protein is involved in immune modulation and suppresses pro-inflammatory cytokines like IFN-γ and TNF-α in immunized mice. The effect of PPE37 full length protein on splenocytes for anti-inflammatory cytokine like IL-10 and IL-4 decreases with increase in protein concentration while as N-terminal segment shows stronger response with increase in concentration of PPE37N antigenic protein.

Work done during the year:

Full length and N-terminal fragment of PPE37 was expressed and purified. Mice were immunized with recombinant PPE37FL and PPE37N proteins along with controls. Finally, mice were sacrificed after 10 days of 2nd booster dose of immunization. Blood samples were collected for serum preparation. The serum were frozen and stored at –80°C. Spleens were isolated and were crushed/perfused-using syringe. Cells were centrifuged, washed and re-suspended in RBC lysis buffer. The cells were centrifuged and finally re-suspended in DMEM containing 10% FBS.

For cell-mediated immune response, splenocytes (1X10^6/well) were seeded in 96 well plate and re-stimulated with 3µg/ml and 5µg/ml of recombinant PPE37FL and PPE37N for 12hr in presence of monensin (BD) GolgiPlug™ and GolgiStop™. Cells were collected, washed with PBS and stained with anti-CD4 and anti-CD8 (CD3a (FITC), CD4 (APC-H7) and CD8 (percycy5.5)). Cells were fixed with 4% paraformaldehyde, permeablized in 0.02% triton X-100, followed by washing and were stained with anti-IFN-γ and anti-TNF-α (APC for IFN-γ, PE-Cy7 for TNF-α) for 1 hrs.

Humoral immune response from serum samples was assayed by enzyme linked immunosorbent assay (ELISA).

Splenocytes isolated from immunized mice were treated with different concentration of recombinant PPE37FL and PPE37N antigenic proteins. After 36 hrs, cells were harvested and intracellular cytokine analysis was done through flow cytometric analysis (**Fig. 1A,B**). Decrease in the number of CD4$^+$ poly-functional IFN-γ and TNF-α cells in comparison to control in rPPE37FL treated cells (**Fig. 1C**) were observed. A decrease can be seen in poly-functional CD8$^+$ cells treated with rPPE37FL protein though the decrease is not statistically significant (**Fig. 1D**). Similar experiments with recombinant PPE37N showed the decrease in number of poly-functional CD4$^+$ and CD8$^+$ IFN-γ and TNF-αcells as compared to control mice samples but the decrease was not statistically significant (**Fig. 1E, 1F**).

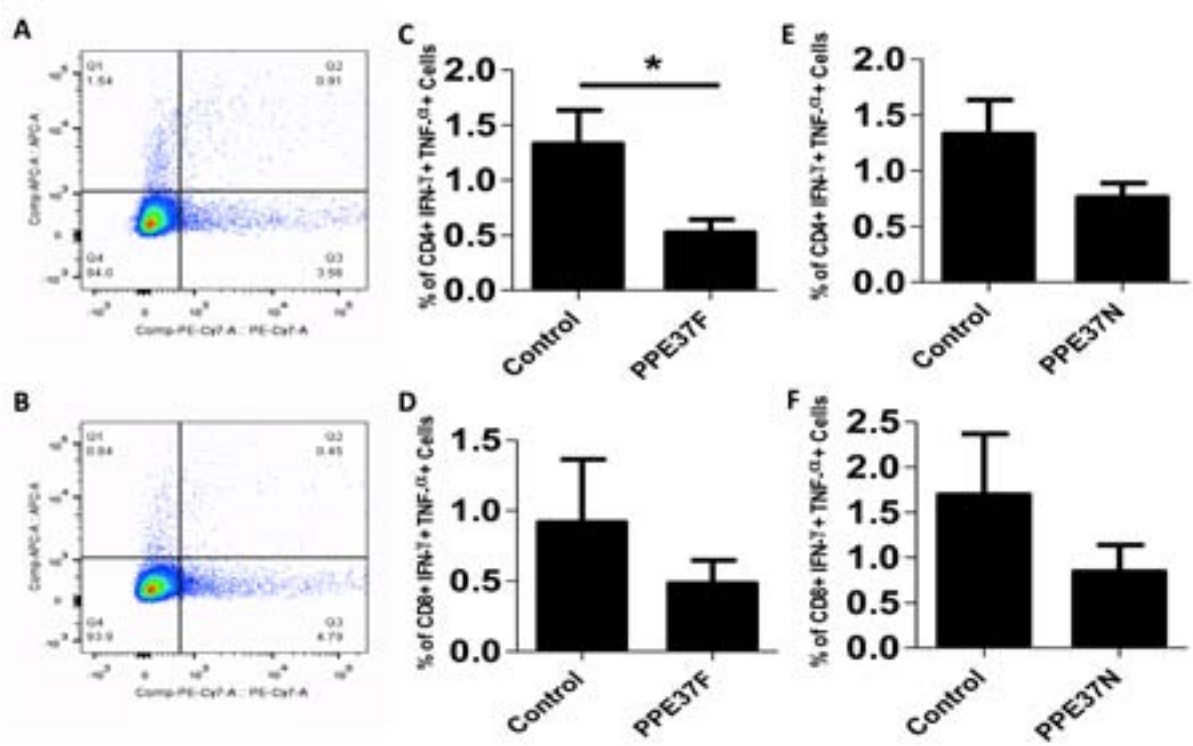


Fig. 1: Recombinant PPE37FL and PPE37N decreases number of poly-functional IFN-γ and TNF-alpha secreting $CD4^+$ and $CD8^+$ cells.

Cytokine profiling was done after 48hrs and 96hrs post-treatment. The results corroborated with our previous analysis showing recombinant PPE37FL and PPE37N protein antigens dampens secretion of pro-inflammatory cytokine response and enhances anti-inflammatory cytokines like IL10 and IL4. Different concentrations of recombinant PPE37FL and PPE37N elicit varied cytokine response. PPE37FL antigenic protein elicits significant secretion of IL10 and IL4 and this effect was decreased with increase in concentration of recombinant antigen PPE37FL (**Fig. 2A, 2C**). However, when PPE37N antigenic protein was used the induction of cytokine secretion like IL4 and IL10 was significantly increased with increasing concentration of protein (**Fig. 2B, D**). The varying effects of PPE37FL and PPE37N on secretion for anti-inflammatory cytokine induction could be possibly due presence of apoptotic c-terminal segment present in PPE37FL protein.

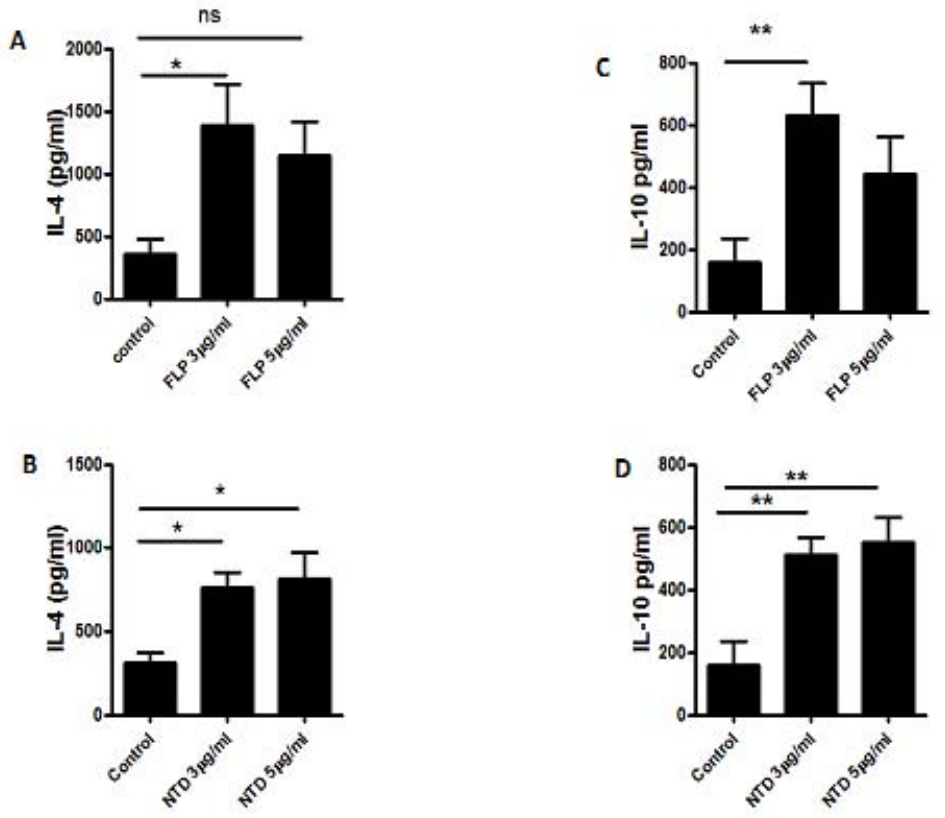


Fig. 2: PPE37FL and PPE37N proteins enhance secretion of Th2 cytokines in re-stimulated splenocytes

After evaluating the IgG immunoreactivity of PPE37FL and PPE37N antigenic proteins in TB patients and healthy controls the humoral immune response of PPE37FL and PPE37N from sera of immunized mice were evaluated and compared with control group of mice.

A highly significant level of IgG humoral response against both antigens in comparison to control mice was observed (**Fig. 3C, D**).

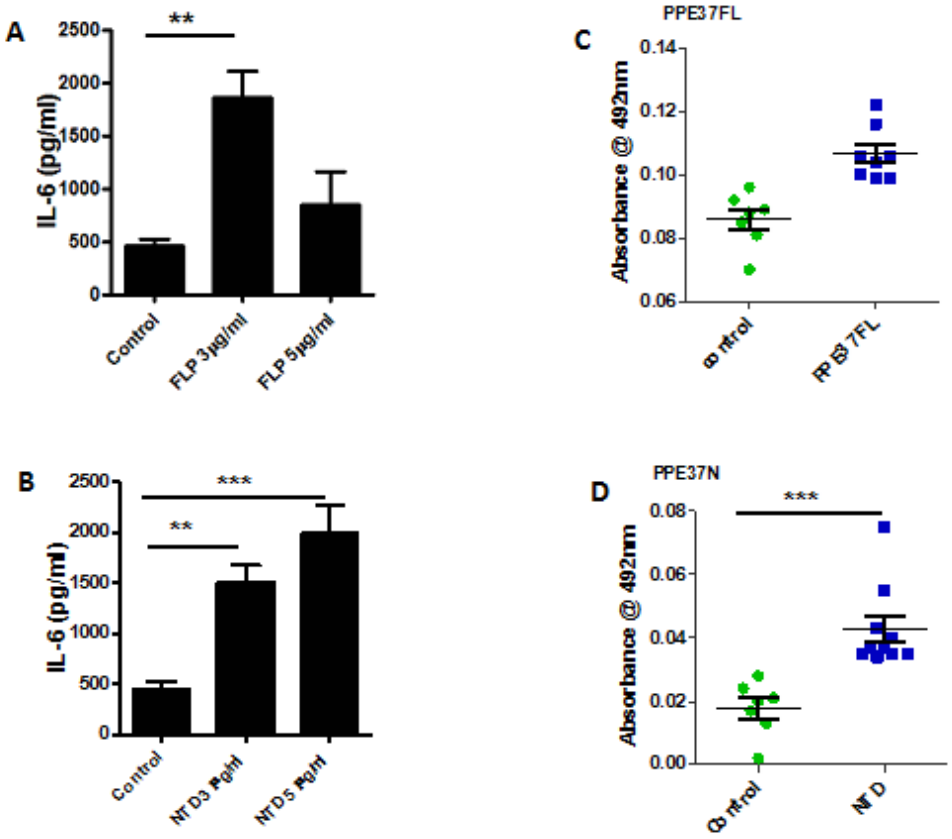


Fig. 3: IL6 induction and B-cell response in Immunized mice

Antigenic Potential of PPE37:

To evaluate the antigenic potential of recombinant PPE37FL and PPE37N, experiments were designed to compare the IgG humoral response in different categories of TB patients such as pulmonary tuberculosis (PTB), extra-pulmonary tuberculosis (EPTB), TB patient contacts and relapse cases of tuberculosis and compared with healthy controls. Statistical analysis demonstrated that PPE37FL and PPE37N elicited a statistically significant immunoreactivity in PTB patients to IgG compared to healthy controls (**Fig. 4A**). Humoral immune response was not found to be significant in EPTB and contacts (**Fig. 4B**). However, in case of relapse cases only PPE37FL antigen elicited significant IgG immunoreactivity (**Fig. 4B**).

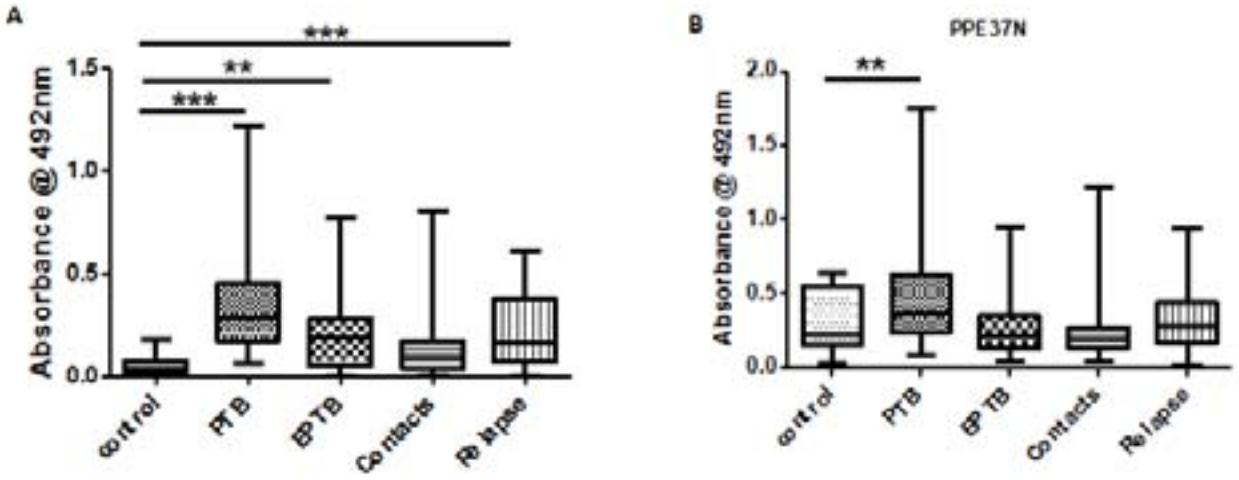


Fig. 4: PPE37FL and PPE37N induce strong B-cell response tuberculosis patients

6. Understanding the role of micronutrients in *Mycobacterium tuberculosis* (*M.tb*) infection using guinea pig (*Caviaporcellus*) as a model

Scientific Staff : Dr. Nasreen Z. Ehtesham, Dr. Javaid Ahmad Sheikh
In Collaboration with : Dr. U. D. Gupta, JALMA; Dr. Seyed E. Hasnain, IITD
Duration : 2014-17

Aims, Objectives & Background

Micronutrient deficiency is known to impair proper functioning of immune system that renders host susceptible to various diseases. Micronutrient deficiency is widespread, more so in developing countries and contributes to high burden of infectious diseases including tuberculosis. There is urgent need to have a proper understanding of relation between isolated or combined nutrient deficiency in relation to tuberculosis, so that a nutritional intervention can be devised to reduce the burden of this disease.

Work done during this year

Male weanling guinea pigs (n=100) were given stock diet for one week. After one week of

acclimatization animals were divided into six groups and fed isocaloric synthetic diet with deficiency as described below. All the diets were prepared as per the NRC recommendations for guinea pigs.

Group-I	n=17	Normal Diet
Group-II	n=16	Calcium Deficient
Group-III	n=16	Magnesium Deficient
Group-IV	n=16	Zinc Deficient
Group-V	n=17	Vitamin D Deficient
Group-VI	n=18	Combined Deficiency

Once the deficient model were generated these animals were infected with *Mycobacterium tuberculosis* H37Rv and 2 animals were sacrificed at day 1 post infection (p.i.) for basal CFU count to confirm the infection dose. Later 4 animals from each group were sacrificed after every two weeks, i.e. 2 weeks p.i, 4 weeks p.i, and 6 weeks p.i to determine the extent of infection progression with various micronutrient deficiencies. 4 animals from each group were used for median survival time. CFU count indicated that guinea pigs exhibited a gradual increase in bacterial load with time. The basal load observed was 1.5 log in lungs, and two weeks after infection, the bacterial load increased to ~ 4 logs. When various micronutrient deficient groups were compared after 2 weeks of infection, there was no significant change in count although Calcium and Zinc deficient animals showed a relatively higher infection as compared to all other groups (Figure 1). After 4 weeks of infection calcium deficiency lead to significant increase in infection as depicted by highly significant increase in bacterial load in lungs. Bacterial count in spleen also confirmed higher infection in Calcium deficient animals. Moreover, higher bacterial load was also observed in Vitamin D deficient animals and animals that were deficient in all the micro nutrients when bacterial load in spleen was measured. Infection status was more pronounced after 6 weeks of infection as visualized by significant increase in the bacterial counts in various groups. Apart from deficiencies that depicted increase after 4 weeks of infection, Magnesium deficiency deciphered increased bacterial load in both lungs

and spleen. This was an important observation as till now only Vitamin D deficiency was being implicated in TB susceptibility. Although there are reports of Magnesium deficiency in TB patients but we hereby directly implicate this as a factor whereby its deficiency can render host to increasingly susceptible to TB.

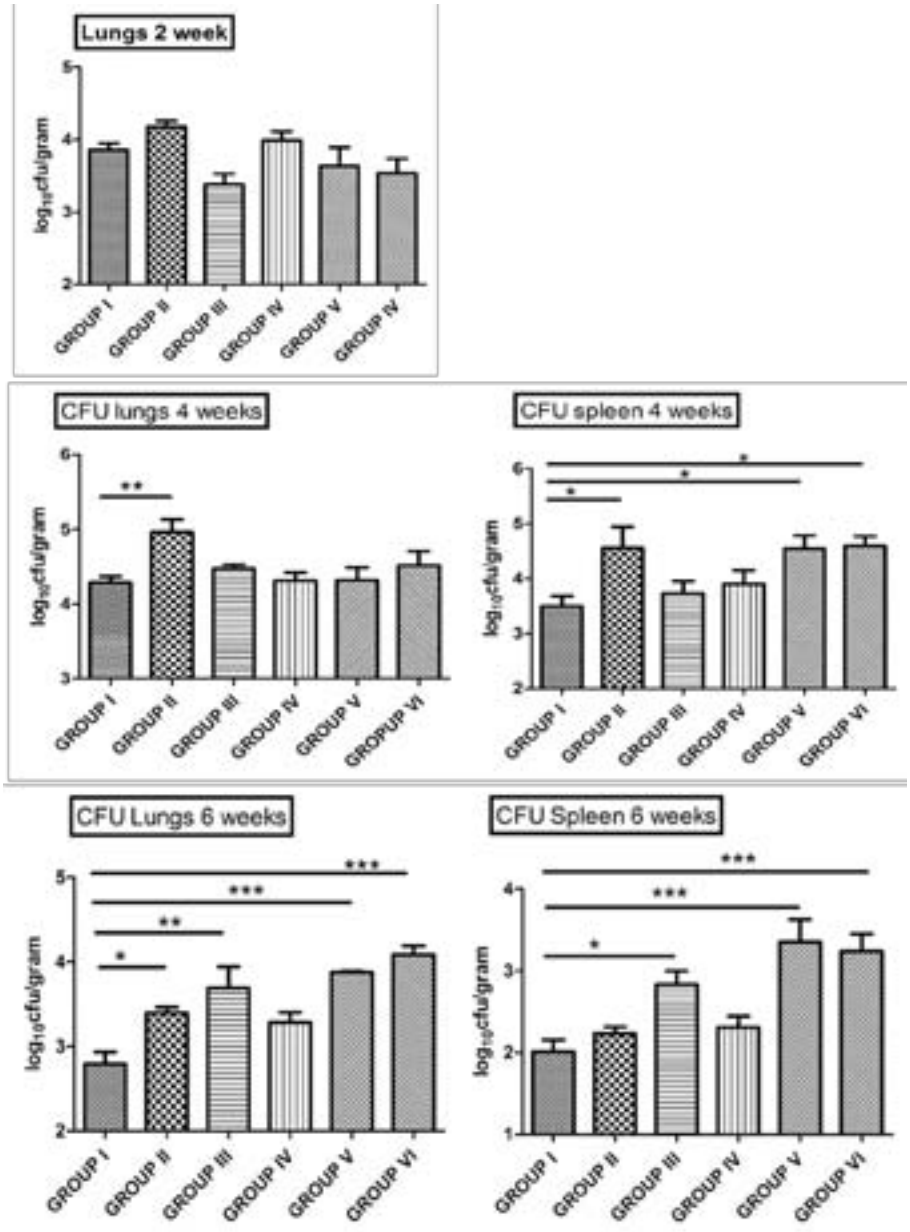


Fig. 1: Determination of CFU in *M. tuberculosis*-infected guinea pigs at various stages of disease. CFU were determined in lung (left) and spleen (right) homogenates derived from *M. tuberculosis*-infected guinea pigs that were deficient in various micronutrients. Protective efficacies are expressed as log10 bacterial counts/gram

Further we evaluated the role of these micronutrients in the survival of infected animals, to depict any altered survival due to the deficiency of any particular micronutrient. The Kaplan Meier survival curve (Figure 2) suggested that Vitamin D deficient animals had least survival and succumbed earlier as compared to other deficient animals. Even though Magnesium deficiency translated in higher bacterial load when evaluated at 6 weeks post infection, these animals survived better as compared to other deficient animals. This discrepancy will be examined once we evaluate all the immunological attributes arising out of these deficiencies.

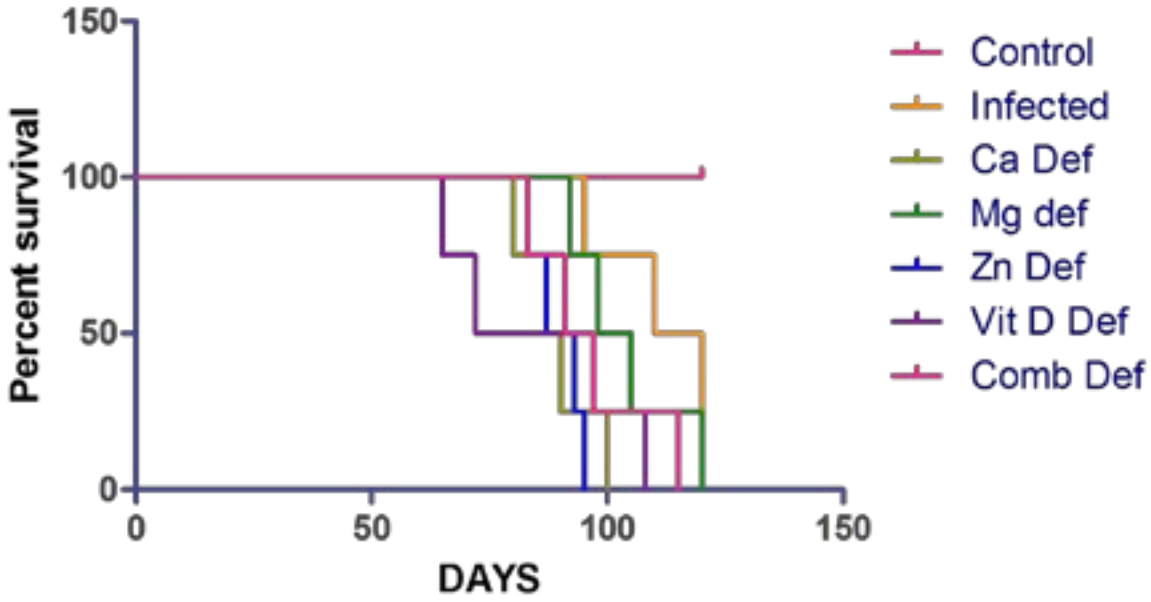


Fig. 2: Kaplan-Meier curves of percent survival of M.tb-infected guinea pigs over the course of disease, post-challenge. Groups of guinea pigs (4 animals per group) were deficient as depicted and their survival rate was determined on a weekly basis up to the termination of study.

Next, we evaluated the lung and spleen sections by histopathology to determine the role of these micronutrients on the extent of pathology. By 2 weeks after infection, discrete, round lesions composed of tight accumulations of epithelioid macrophages admixed with granulocytes could be seen (Figure. 3). These cells have distinct eosinophilic cytoplasmic granules and similar numbers of lymphocytes were also present.

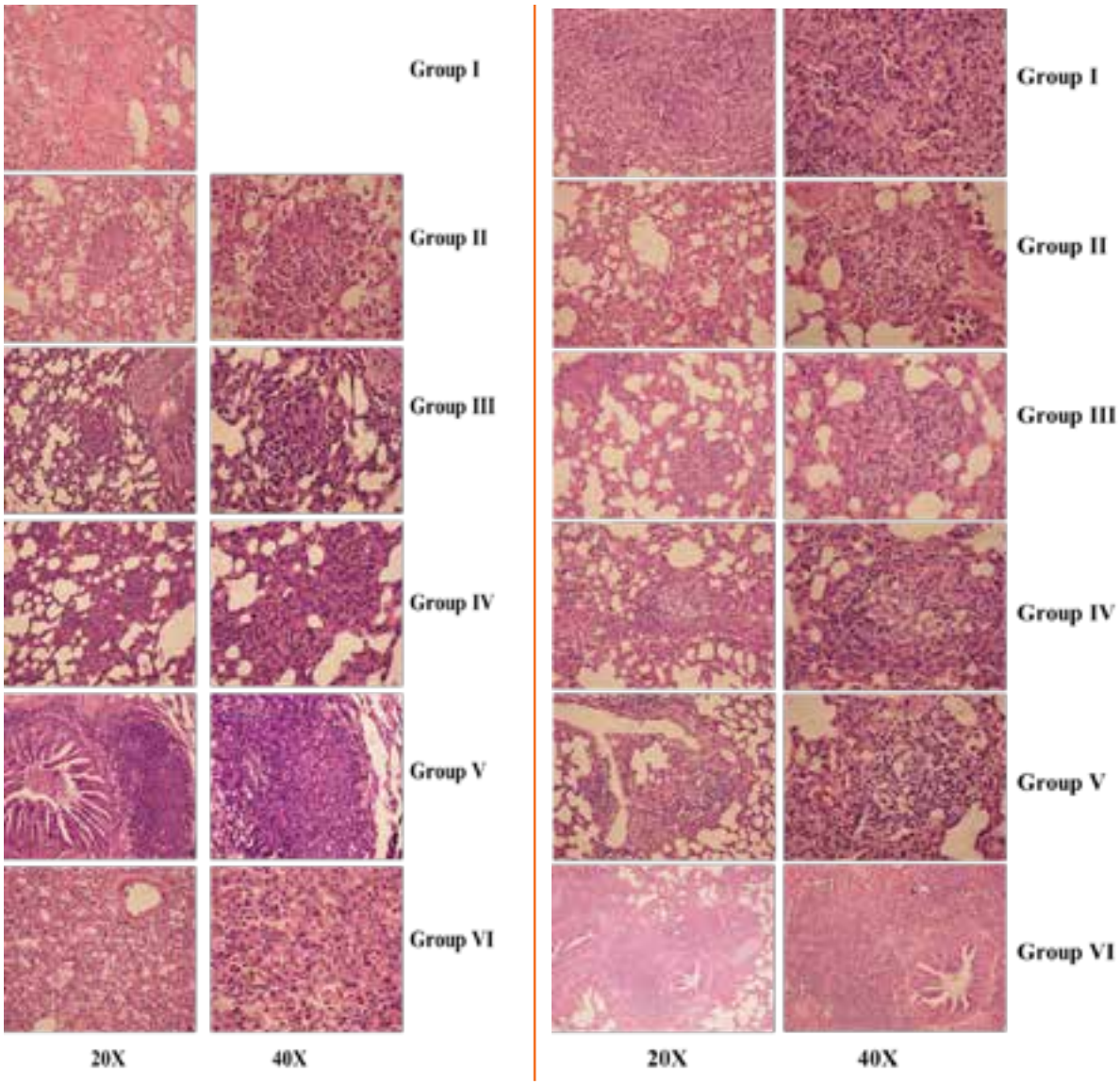


Fig. 3: Representative photomicrographs of lung sections portraying granulomatous inflammation in animals deficient in various micronutrients. Left panel is 2 weeks post infection while as Right panel is 6 weeks post infection.

The lesions were most commonly seen in the parenchyma, close to major airways and blood vessels, effacing and expanding alveolar septa. The infiltration was more pronounced in groups deficient in Magnesium and Vitamin D, which correlated well with CFU load observed in those groups. We were not able to observe any granulomatous inflammation in Zinc deficient animals even though pneumocytic hyperplasia was evident. After 6 weeks of infection, scattered discrete, round lesions with a similar distribution to those after 2

weeks of infection were seen. The diameter of each lesion was substantially increased. While epithelioid macrophages made up the bulk of these foci, neutrophils and lymphocytes were also seen scattered throughout the lesion except in the central core. The cellular identity of the infiltrating cells is currently being studied by immunohistochemistry and will help to visualize the distribution of various cell types in the inflamed sections. The observations if correlated with any micronutrient deficiency will enable us to have a glimpse of cellular fate that in turn translate to immune response in various deficiencies.

STEM CELL BIOLOGY

STEM CELL BIOLOGY

Identification of growth stimulating proteins expressed by the optimal feeder cells

Scientific Staff : L.K. Yerneni, Rishi Man Chugh, Bijendra Kumar
Year in which project started : 2016 (Extramural)
Proposed year of completion : 2018
Funding Agency :

Aims, Objectives and Background

Growth arrested fibroblast feeder cells are indispensable in supporting the proliferation and long term culture of keratinocyte stem cells and embryonic stem cells Alternatively, feeder-less culture systems are not able to fully substitute the feeder functionality. Although, several proteins of importance were identified in irradiated feeder cells, but their growth promoting nature is unknown.

Earlier, we described a novel method of growth arrest in Swiss 3T3 cells for optimizing human epidermal keratinocyte culture for faster formation of epidermal sheets (Yerneni and Chugh 2015 PCT/IN2015/000404; Chugh et al, 2015 PLoS ONE DOI:10.1371/journal.pone.0122056; Chugh et al 2015 Burns 41: 1788-95; Chugh et al 2016 J Pharmacol Toxicol Methods 80: 68-74; Chugh et al 2017 Cytotech 69 391-404). We suggest that the exceptional growth promotion by our technique is the result of expression of specific proteins. Therefore, it is proposed to extract proteins from such feeders, isolate sub-cellular fractions and test them in feeder-free keratinocyte culture. The shortlisted growth promoting fraction would then be subjected to proteomics.

Work done

In view of the SAC recommendation to first test the whole protein extract from 3T3 cells for keratinocyte growth stimulation before testing the sub-cellular fractions, the same was undertaken.

Protein Extraction:

Initially, the proteins were extracted using 1% Triton X from a specific subset of 3T3 cells – the 3K3D cells (sub-culture of 3000 cells/cm^2 every 3 days; Chugh et al 2015a, PLoS One dx.doi.org/10.1371) which were validated for the absence of Mitomycin C resistance. However, the extract was found to be lethal to keratinocytes in vitro. This problem was circumvented by adopting an extraction procedure without detergent. Additionally, the adopted extraction method on the 3K3D cells resulted in protein yields in the range of 0.5 to 0.61 mg per 10^6 cells, while a higher amount in the range of 0.7 to 0.85 mg per 10^6 cells was obtained from a spontaneously transformed clone of 3T3 cells produced previously in the lab (Table 1). Moreover, the total protein yield from the clone cultures was several fold higher than from 3K3D cells due to a higher saturation density of >2 x 10^5 cells/cm^2 in the former against 2.4 x 10^4 cells/cm^2 in the latter. This compelled to first use the protein extract from clones to test for human keratinocytes proliferation since larger amounts of protein are required for experimentation. A single passage of clone cells from a cryo-preserved stock was sufficient to obtain the required protein concentration for a keratinocyte experiment involving two different concentrations, which equivalent to the respective two cell densities.

Table 1. Protein extraction using detergent-free lysis buffer and sonication from 3K3D subset cells and the 3T3 clone cells

Cell Type	Cell No.*	Protein Conc (mg/ml)	Protein (mg) / 10^6 cells
3K3D	4.0 X 10^6	2.00	0.50
	5.5 X 10^6	3.06	0.55
	4.7 X 10^6	2.50	0.53
	6.2 X 10^6	3.80	0.61
Clone	59 X 10^6	50.00	0.85
	89 X 10^6	62.50	0.70
	85 X 10^6	62.50	0.70
	86 X 10^6	62.50	0.70

*Average cell no. in million from triplicate T-75 flasks.

Effect of Protein extract on Keratinocyte proliferation:

The human epidermal keratinocytes were seeded over type IV collagen coated 6-well plates at a density of 10,000 cells/cm^2 per group. The following groups were included:-

i- Keratinocyte cells cultured with feeder-free and serum-free medium supplemented with protein concentration, equivalent to 15000 per cm^2 feeder cells

ii- Keratinocyte cells cultured with feeder-free and serum-free medium supplemented with protein concentration, equivalent to 21000 per cm^2 feeder cells

iii- Keratinocyte cells cultured with feeder-free and serum-free medium supplemented with standard BPE concentration (70 µg/ml)

iv- Keratinocyte cells cultured with 15,000 feeder cells per cm^2

v- Keratinocyte cells cultured with 21,000 feeder cells per cm^2

On day 7, cells were trypsinized and cells counted by Trypan blue exclusion. Further, the cell counts from collagen coated dishes were performed at different passages.

The keratinocyte cells cultured in feeder-free and serum-free culture condition either with protein lysate equivalent to 15 x 10^3 feeders (P15K) or 21 x 10^3 feeders (P21K) group or Bovine Pituitary Extract (BPE) group showed discrete keratinocyte cells proliferation and the overall cell growth in the P21K is higher than in the P15K or BPE groups (Figure 1), where as keratinocyte cells in feeder dependent culture groups 15 x 10^3 feeder per cm^2 (F15K) and 21 x 10^3 feeder per cm^2 (F21K) showed clonal growth of keratinocytes surrounded by disintegrating feeder cells.

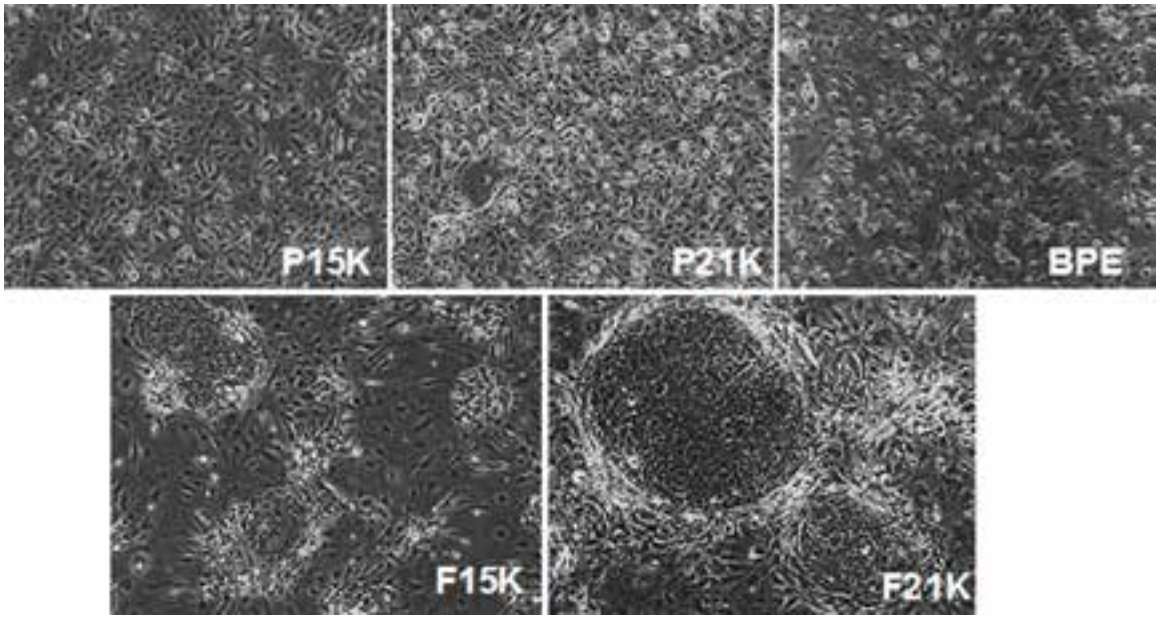


Fig. 1. Human epidermal keratinocyte cell culture in various combinations

The output of keratinocyte cells of protein lysate and feeder groups at various passages were compared with the BPE group. The third passage (P3) cell output of keratinocyte cells in P21K and F21K group was significantly higher ($p< 0.05$) than the BPE group (figure 2). Whereas, the fourth passage (P4) cells showed significant difference ($p< 0.05$) over P21K, F15K and F21K when compared with the BPE group. But the comparative significant difference was nullified in fifth passage (P5).

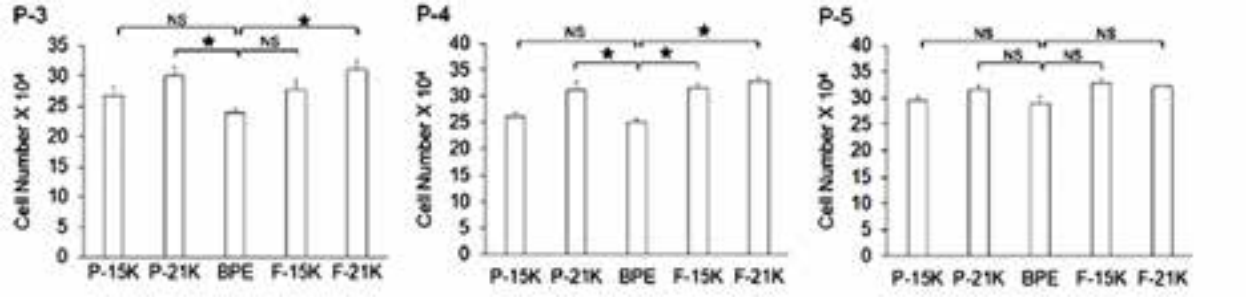


Fig. 3: Cell Number of human epidermal keratinocytes. Bar graph representing the average cell number of keratinocytes at various medium combination and passages. Each medium combination was compared with BPE group by Mann Whitney test and indicated as significant at P<0.05 (*) or insignificant (NS). The cell numbers are presented as the mean with standard deviation of duplicate cultures.

SDS-PAGE analysis:

There are more prominent protein-bands in the protein extract of clone cells than in 3T3 cells (Figure 3).

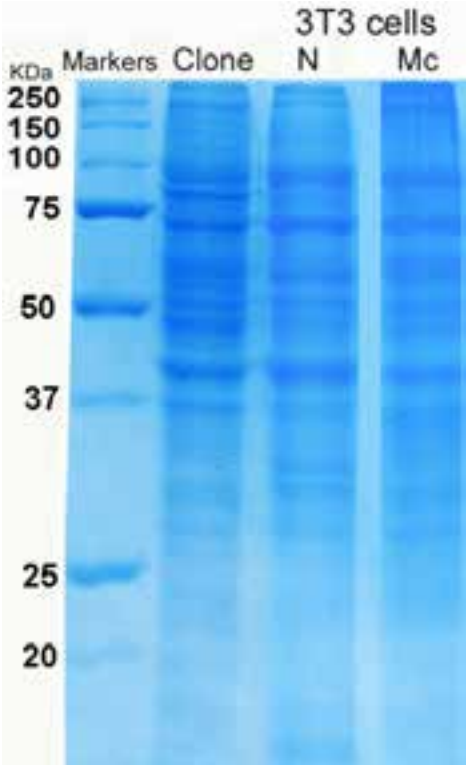


Fig. 3: SDS-PAGE of proteins extracted from Clone cells (a) 3T3 cells (3K3D) Normal (N) and Mitomycin C growth arrested feeders (Mc)

Conclusions

It is concluded that the total protein extract from 3T3 clone cells support the proliferation of keratinocyte cells in feeder-free and serum-free culture conditions as much as in the presence of serum and feeders. This is the first ever study and unreported so far in which the feeder cell proteins were used as the growth promoters of human epidermal keratinocytes. Further in this study, the sub-cellular fractions of the extracted proteins from the feeder cells will be resolved by Gel electrophoresis and segregated and tested for keratinocyte culture. The best performing protein fractions will be subjected to 2D gel electrophoresis and Mass spectrometry to pinpoint the potential protein(s). Simultaneously, the differences, if at all exist, between the growth-promoting activity of protein extracts of normal 3T3 and the clone cells will be evaluated and similarly addressed.

Future course of action

In order to decide the future course of research, it needs to be tested whether or not the significant keratinocyte growth stimulation of clone cell-protein fraction would be reproduced with 3T3 cells that were either growth–arrested or not. In standard co-culture technique the 3T3 cells should be growth-arrested to contain their proliferation allowing growth of only the keratinocyte cells, but this step is unnecessary when extracted proteins are used. However, a differential evaluation will also be undertaken with proteins of normal and growth-arrested feeders.

This work is in progress and involved slow & tedious sub-culture procedures because of low cell yield by the established protocol. This will be followed by the sub-cellular protein fractions of either of the 3T3 feeder cells will be undertaken; after the gel electrophoresis, various sub-cellular protein fractions from the sliced gels will be extracted by Kurian and Scofield, 2012 for the downstream growth stimulation assay using feeder-free and serum-free keratinocyte cell culture; the best performing protein fractions will be subjected to proteomic analysis to identify the potential proteins. The results will then be validated by testing the influence of such identified proteins on keratinocyte proliferation.

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

Scientific Staff : LK Yerneni, K. Amal Dev, Rishi Man Chugh, Bijendra Kumar

Year in which project started: 2017 (Extramural)

Proposed year of completion: 2019

Background

The project is aimed at substituting the murine embryonic fibroblasts with human neonatal, adult or embryonic dermal fibroblasts useful as feeder cells in keratinocyte/stem cell culture. This extramural project was technically approved in 2015 by ICMR under translational research, but funds were released in 2017. However, research work using Neonatal foreskin fibroblasts as feeder cells was initiated through a CSIR direct fellow to work out a method for human epidermal keratinocyte culture to ultimately produce non-xenogeneic Cultured Epithelial Autografts (Annual Reports 2014-15). It was noted that neonatal fibroblasts required a higher concentration of 15 µg Mitomycin C per ml for effective growth arrest in comparison to 4 µg per ml to produce optimally performing 3T3 feeder cells. For inducing irreversible growth arrest, a lower concentration of Mitomycin C is favorable in order to control its traces in the final product of epidermal construct. The other difficulty was that various differential detachment strategies to isolate keratinocytes from the growth-arrested neonatal fibroblast feeders in co-cultures were unsuccessful. It is therefore, necessary to probe the utility of other human fibroblasts sourced from adult or embryonic skin as feeders.

Objectives

1. To find out accurate exposure conditions for a least toxic but effective Mitomycin C induction of growth arrest in primary human dermal fibroblasts adopting the previously established novel strategies.

2. To verify the optimal influence of such growth arrested human dermal fibroblasts on proliferation & the basic characteristics of primary human epidermal keratinocytes.

3. To characterize the human epidermal sheets cultured using growth arrested human dermal fibroblasts.

Work done

The primary cells of adult dermal fibroblasts (Genlantis, USA) were frozen as Master Bank and working bank by passaging 3 times and 2 times, respectively at constant transfer rate of 10,000 cells /cm^2. The working bank cells after 2 more passages exposed a sub-confluent population to Mitomycin C for two hours and re-plated at 10,000 cells/cm^2 exhibited failed growth arrest with 2.5, 5, 7.5 & 10 µg/ml exhibiting random pockets of cell divisions amidst cellular disintegration. The irreversible growth inhibition was observed at 12.5 & 15 µg/ml as revealed by the 40th day post-exposure cell counts (Figure 4) and absence of dividing cells. However, the overnight exposure (16-hours) of confluent population to the same concentrations followed by re-plating at 7,500 cells/cm^2 resulted in effective growth-arrest even with 10 µg Mitomycin C per ml (Figure 5). On the other hand, similar to the difficulty with neonatal fibroblasts, prior exclusive detachment of adult fibroblasts enabling the isolation of the co-cultured keratinocytes was almost impossible.

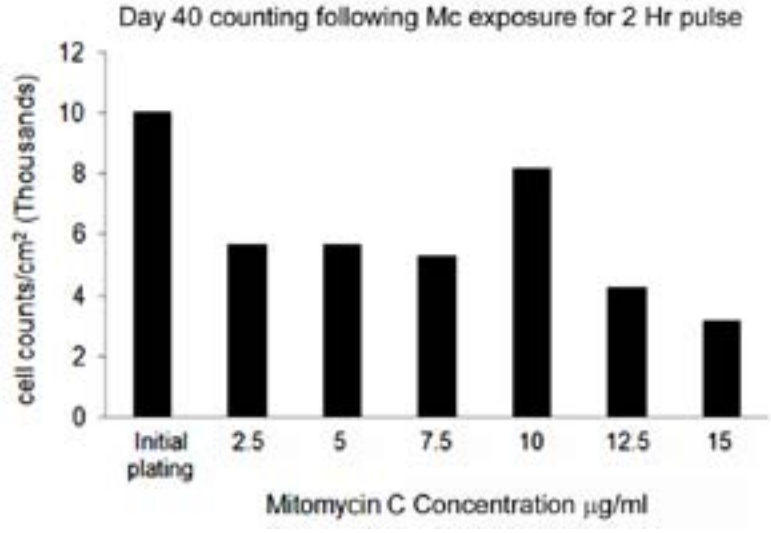


Fig. 4: Viable Adult dermal fibroblast cell count after 40 days of incubation following a two-hour pulse with various concentrations of Mitomycin C

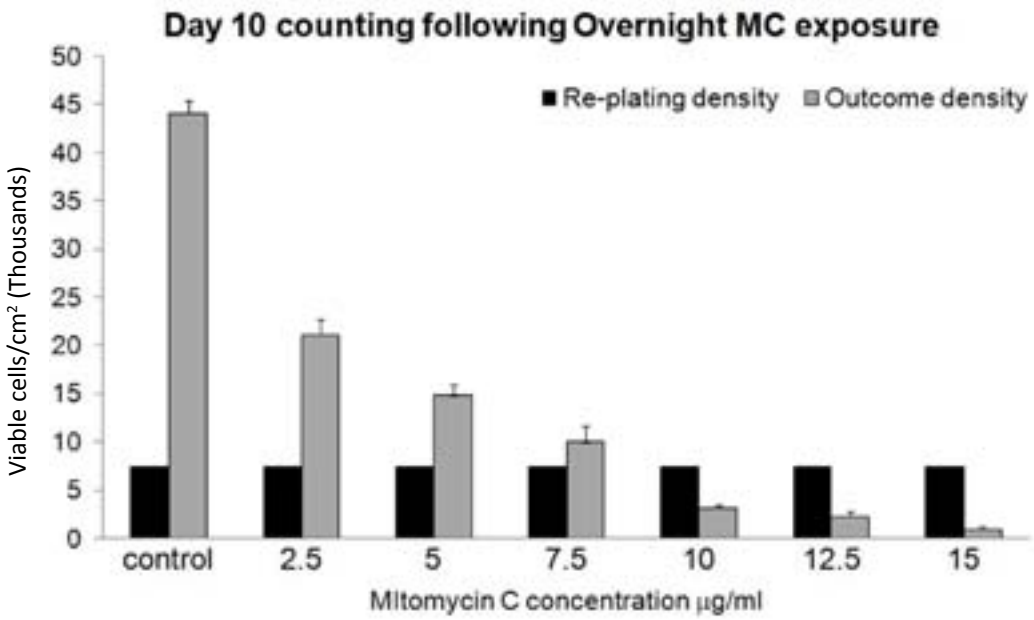


Fig. 5: Viable Adult dermal fibroblast cell count after 10 days of incubation following a 16-hours continuous exposure with various concentrations of Mitomycin C

Before moving on to determining the utility of human embryonic dermal fibroblasts as potential feeders, it was then decided to first solve the issue of differential detachment of feeder cells before trypsinizing the keratinocytes. In a separate experimental set up, it was found that the ease of detaching the embryonic fibroblasts using 1/6-dilution of the standard Trypsin-EDTA was comparable to 3T3 cells while 1/3 dilution was necessary for detaching the keratinocytes, however, EDTA alone was sufficient enough to detach with 3T3 cells leaving behind the keratinocytes.

Conclusions

In a successful feeder dependent keratinocyte culture system, prior detachment of growth arrested feeders enabling isolation of the keratinocytes that remained undetached allowing successive passaging is an essential step which was achievable with embryonic dermal fibroblasts. It was found that the human dermal fibroblasts required higher concentrations of Mitomycin C than the specific sub-set of Swiss 3T3 cells produced in the lab earlier.

Future course of action

The embryonic dermal fibroblasts will be expanded by adopting various sub-culture schemes to generate a more Mitomycin C-susceptible subset similar to the strategies we reported earlier (Chugh et al, 2015 PLoS ONE DOI:10.1371/journal.pone.0122056). Simultaneously, the fibroblasts will be titrated with various concentrations of Mitomycin C at different exposure times and the short-term and long-term post-exposure cell extinctions will be monitored to identify an optimal method.

BIOINFORMATICS AND ENVIRONMENTAL TOXICOLOGY

BIOINORMATICS and ENVIRONMENTAL TOXICOLOGY

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

Duration : 01 April 2013 to 31 Mar 2018

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

Technical staff : Mr. Prince Gautam

Funding Agency : ICMR

Biomedical informatics centre constantly striving to promote and support informatics in biomedical research through National level workshops, training and research facility for biomedical scientists, research scholars and students, since its inception in the second phase of the taskforce. The ongoing research activities of BIC are in line with the primary objectives outlined by the taskforce and requirement of the institute.

Works done in the year:

i) dbGAPs: A comprehensive database of genes and genetic markers associated with psoriasis and its subtypes

Outline: Psoriasis is a systemic hyperproliferative inflammatory skin disorder, although rarely fatal but significantly reduces quality of life. Understanding the full genetic component of the disease association may provide insight into biological pathways as well as targets and biomarkers for diagnosis, prognosis and therapy. Studies related to psoriasis associated genes and genetic markers are scattered and not easily amendable to data-mining. To alleviate difficulties, we have developed dbGAPs an integrated knowledgebase representing a gateway to psoriasis associated genomic data. The database

contains annotation for 202 manually curated genes associated with psoriasis and its subtypes with cross-references. Functional enrichment of these genes, in context of Gene Ontology and pathways, provide insight into their important role in psoriasis etiology and pathogenesis. The dbGAPs interface is enriched with an interactive search engine for data retrieval along with unique customized tools for Single Nucleotide Polymorphism (SNP)/ indel detection and SNP/indel annotations. dbGAPs is accessible at http://www.bmicnip.in/dbgaps/.

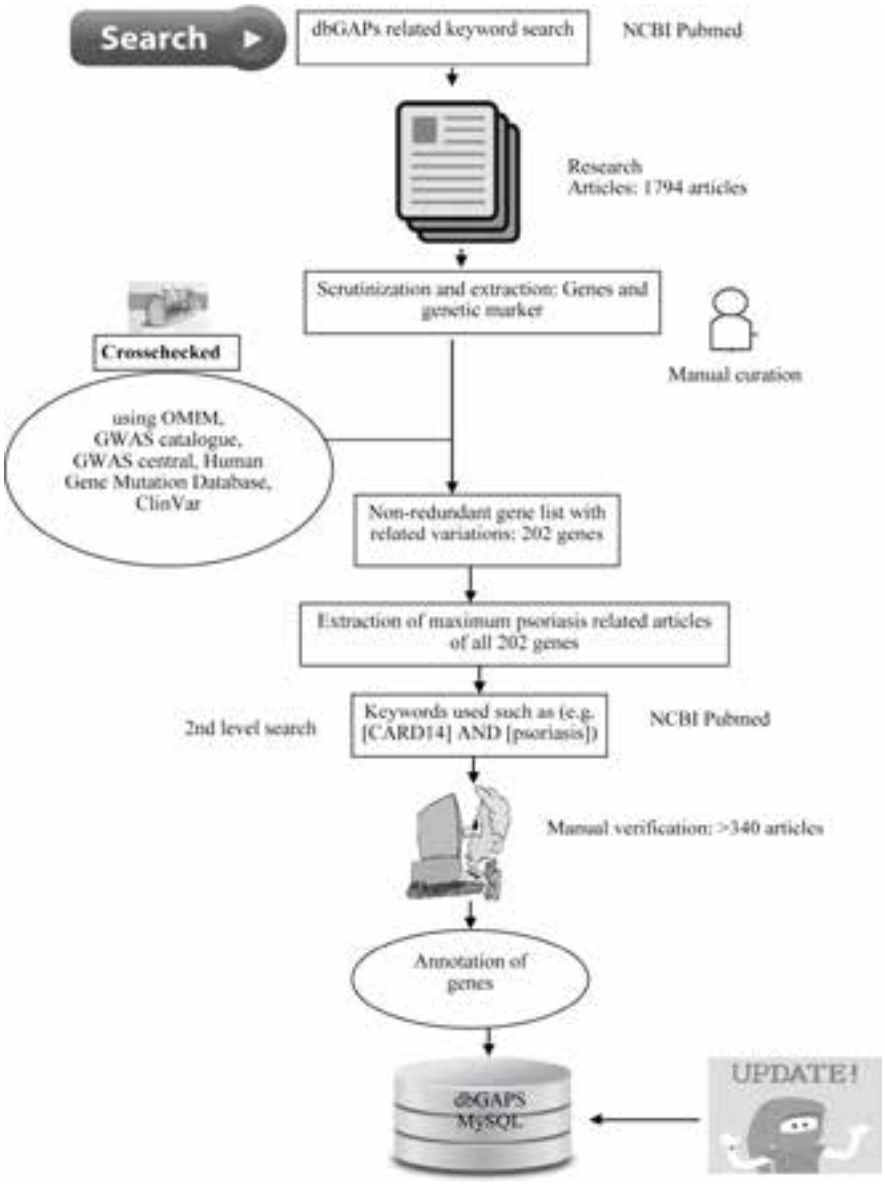


Fig. 1: The workflow of psoriasis associated genetic data collection. The genes and genetic makers are extensively validated prior to their inclusion in the dbGAPs.

Fig, 2: dbGAPs user interface.

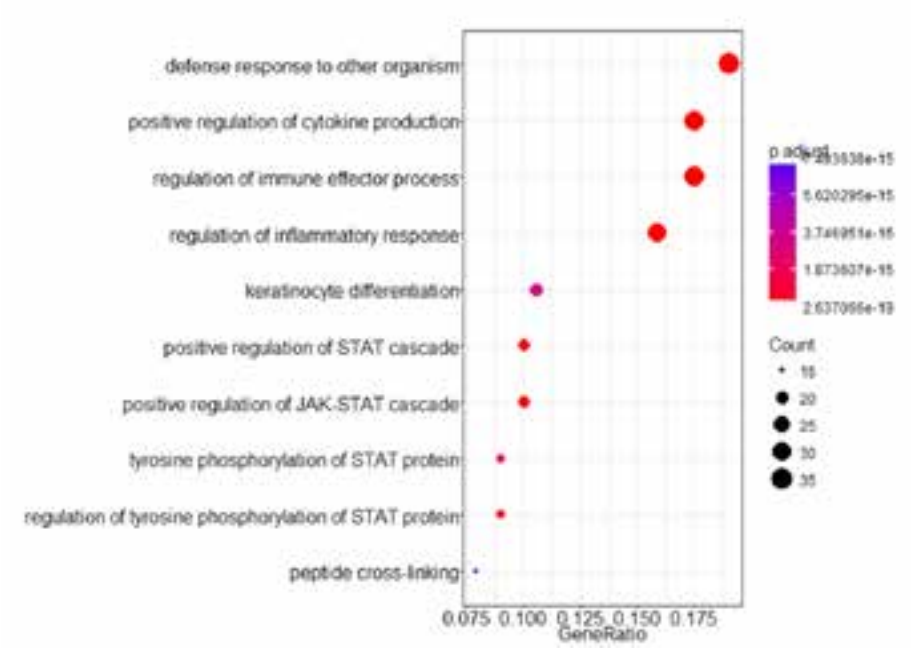


Fig. 3: The enriched gene ontology terms (Biological Process) of psoriasis-associated genes. The horizontal axis represents gene ratio, size of dots indicate the count of genes in each term, and different colours correspond to different adjusted p-values.

Table 1. Genes associated with psoriasis subtypes.

Psoriasis subtypes	Psoriasis associated genes
Based on appearance	
Plaque psoriasis	ACE, **ANGPT2**, APOE, CAMK2G, CARD14, CCHCR1, CDKAL1, CDSN, COG6, **DEFB123**, ERAP1, FBXL19, **FCGR3B**, GJB2, HLA-A, HLA-B, HLA-C, HLA-DQA1, HLA-DQB1, HLA-DRB1, **IFNG**, IFNLR1, IL12B, IL13, **IL18**, IL1B, IL22, IL23A, IL23R, IL36RN, LCE3A, LCE3B, LCE3D, LCE3E, LTA, **MBIP**, MTHFR, NFKBIA, NFKBIZ, NOS2, PLCL2, POU5F1, PTTG1, REL, REV3L, RNF114, RUNX3, SERPINB8, SLC22A5, **TLR4**, TNF, TNFAIP3, TNIP1, TRAF3IP2, VDR, VEGFA, ZNF816, CD226, TYK2, IL1A, IL20, PTPN22, SLC22A4
Pustular psoriasis	CARD14, CSMD1, HLA-C, IL36RN, LCE3B, TNIP1, **AP1S3**, TRAF3IP2
Guttate psoriasis	APOE, CCHCR1, CDSN, HLA-C
Based on age of onset	
Type I psoriasis	ACE, ADAM33, **C17orf51**, CARD14, CCHCR1, **CCL4L1**, CD226, CDKAL1, CDSN, **CLMN**, ELMO1, ERAP1, FBXL19, FLG, HLA-A, HLA-B, HLA-C, HLA-DRB1, IFIH1, IL10, IL12B, IL13, IL1B, IL2, IL21, IL22, IL22RA2, IL23R, IVL, LCE3B, NOS2, PTPN22, REL, REV3L, SERPINB8, SLC22A4, **SMCP**, TNF, TYK2, VDR, VEGFA, ZC3H12C, ZNF816
Type II psoriasis	CCHCR1, CD226, CDSN, HLA-A, HLA-B, HLA-C, IFIH1, IL10, IL12B, IL19, IL1B, **IL1R1**, IL2, IL23A, IL23R, MIF, RNF114, TRAF3IP2, VEGFA, ZC3H12C
Based on location of psoriatic lesions	
Nail psoriasis	HLA-B
Palmoplantar psoriasis	CARD14, CCHCR1, CDSN, HLA-C, IL20
Based on joint complaint	
Cutaneous psoriasis	**ADO**, **FAM27L**, FBXL19, HLA-C, IL1RN, **LINC00643**, MICA, NOS2, RPS26, SDC4, SMARCA4, TSC1
Psoriatic arthritis	**ADAMTS9**, **BARD1**, CARD14, CCHCR1, **CTNNA3**, **DEFB1**, **DENND1B**, ERAP1, ERAP2, FBXL19, GJB2, HCP5, HLA-A, HLA-B, HLA-C, HLA-DQB1, HLA-DRB1, IFIH1, IFNLR1, IL12B, IL13, IL1A, IL2, IL21, IL23A, IL23R, IL4R, LCE3A, LCE3B, LCE3D, LTA, MICA, MICB, MTHFR, NFKBIZ, NOD2, NOS2, POU5F1, **PPARG**, **PSMG3**, PTPN22, PTTG1, REL, RNF114, **RNF39**, RPS26, RUNX3, SDC4, **SLC17A2**, SLC22A5, **SLC46A3**, SMARCA4, STAT2, STAT3, STAT4, **TMPRSS11F**, TNF, TNFAIP3, TNFRSF9, TNIP1, TRAF3IP2, **TRIM39**, TSC1,TYK2, ZNF816

N.B. Genes highlighted in bold are uniquely observed in the specific phenotype.

Citation: Aggarwal et al., (2017). dbGAPs: A comprehensive database of genes and genetic markers associated with psoriasis and its subtypes ***Genomics***. http://dx.doi.org/10.1016/j.ygeno.2017.10.003.

ii) Discovery of T-cell Driven Subunit Vaccines from Zika VirusGenome: An Immunoinformatics Approach

Outline: Emergence of Zika virus epidemics exposed limitations of conventional vaccinology in addressing the immunologic issues created by hypervariable flaviviruses. The immunoinformatics approaches in this context have enlightened scopes for designing T-cell-driven subunit vaccine from conserved regions of genome sequences. Herein, the translated ssRNA (+) genome of Zika virus (Figure 1) was explored in EMBOSS antigenic and VaxiJen to predict 63 peptides as potential antigens. Three MHCII binding peptide prediction tools, viz. NetMHCIIpan, PREDIVAC and immune epitope database (IEDB) were employed in consensus on 63 antigenic peptides to propose14 T-helper cell epitopes (Figure 2; Table 1). Similarly, analysis on 63 antigenic peptides through NetMHC, NetCTL and IEDB MHC-I binding peptide prediction tool led to identification of 14CTL epitopes (Figure 2; Table 2). Seven T-cell epitopes, C:44-66, M:135-149,NS2A:124-144, NS3:421-453, NS3:540-554, NS4B:90-134 and NS4B:171-188, are observed to share overlapping MHC-I and MHC-II binding motifs and hence, are being proposed to constitute minimum T-cell antigens to elicit protectiveT-cell immune response against Zika. Three of them, C:44-66, NS3:421-453 and NS3:540-554 are identified to be conserved across all the selected strains of Zika virus. Moreover, the 21 T-cell epitopes are non-self to humans and exhibited good coverage in variable populations of 14 geographical locations (Figure 3). Therefore, 21 T-cell epitopes are proposed as potential subunit vaccines against Zika virus.

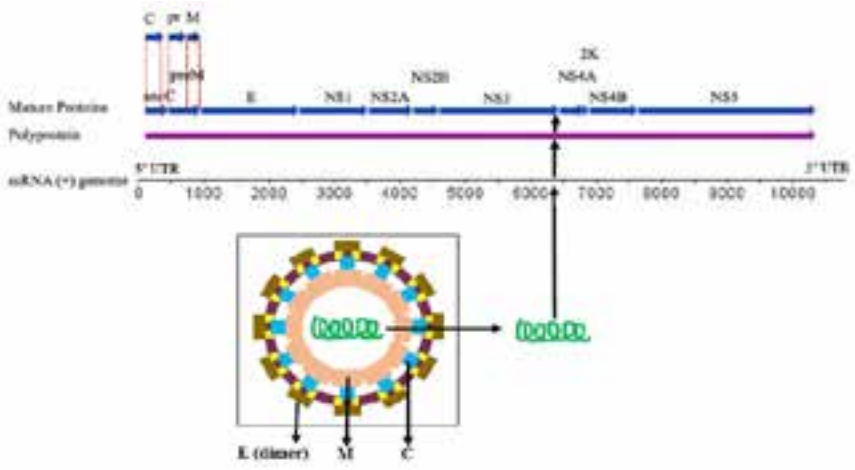


Fig. 1: Genome and proteome of Zika virus.

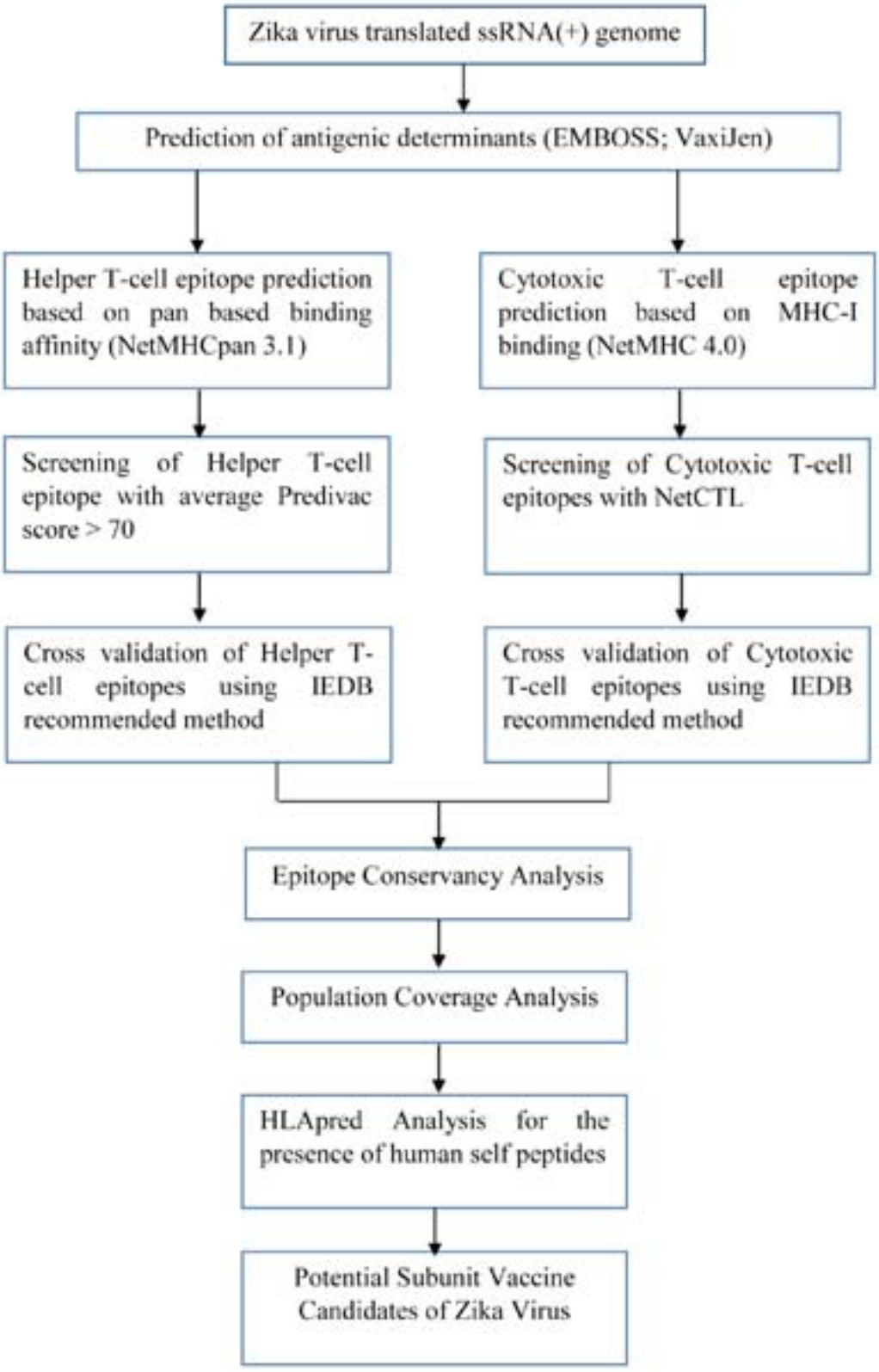


Fig. 1. Schematic representation of protocol used to identify potential subunit vaccine candidates

Table 1: Fourteen proposed helper T-cell epitopes with antigenicity potential in selected immunoinformatics tools

Sl. no	Epitope	Antigenic Score	VaxiJen Score	Net MHCIIpan (NB)	Avg. PREDIVAC Score	Avg. IEDB percentile score	Conservancy (%)
Capsid Protein C (YP_009227196.1) and Anchored Capsid Protein C (YP_009227206.1)							
1	C:19-RGVARVNPLGGLK RLPAGLLLGHG-42	1.143	0.451	13	74.75	10.71	22
2	C:44-IRMVLAILAFLRFT AIKPSLGLI-66	1.179	0.586	13	77.93	3.27	100
Membrane Glycoprotein Precursor M (YP_009227197.1) and Protein pr (YP_009227207.1)							
3	M:22-AISFATTLGVNKCH VQIMD-40	1.143	0.515	10	73.86	14.06	18
Membrane Glycoprotein Precursor M (YP_009227197.1) and Membrane Glycoprotein M (YP_009227208.1)							
4	M:135-FALVAVAIAWLL GSS-149	1.194	0.741	1	71.38	8.81	14
Non Structural Protein NS2A (YP_009227200.1)							
5	NS2A:124-DLMVLINGF ALAWLAIRAMA V-144	1.105	0.4343	10	75.62	5.062857	9
6	NS2A:192- KKNLPFVMA LGLTAVRVVDPINVVGLLLLTR-222	1.234	1.2418	14	77.22929	3.164286	7
Non Structural Protein NS2B (YP_009227201.1)							
7	NS2B:4-PSEVLTAVGLIC ALAGG-20	1.198	0.5818	1	73.51857	19.88357	99
Non Structural Protein NS3 (YP_009227202.1)							
8	NS3:421- DRVIDSRRCLK PVILDGERVILAGPMPVTHASA-453	1.206	0.4198	13	74.02643	6.432857	100
9	NS3:540-DLPVWLAYQV ASAGI-554	1.164	0.6606	9	72.19857	12.52571	100
Non Structural Protein NS4A (YP_009227203.1)							
10	NS4A:54-TIMLLGLLGTV SLGIFFVLM-73	1.156	0.8235	3	73.16	5.382857	100
Non Structural Protein NS4B (YP_009227204.1)							
11	NS4B:90-DLGVPLLMMG CYSQLTPLTLIVAIILLVAHYMYLIPGLQAAAARA-134	1.233	0.5739	13	80.635	2.205	8
12	NS4B:171-GQVLLIAVAI SSAVLLRT-188	1.221	0.4208	13	74.62714	2.316429	15
RNA-dependent RNA polymerase NS5 (YP_009227205.1)							
13	NS5:883-MDYLSTQVR YL-893	1.086	0.9201	6	72.92786	12.03143	98
Protein 2K (YP_009227209.1)							
14	2K:8-AIIIMVAVGLLG L-20	1.159	1.0947	1	73.38357	4.477143	99

NB: Number of binders

Table 3: Fourteen proposed CTL epitopes with antigenicity potential in selected immunoinformatics tools

Sl. no	Epitope	Antigenic Score	VaxiJen Score	Net MHCpan (NB)	NetCTL (NB)	Avg. IEDB percentile score	Conservancy (%)
Capsid Protein C (YP_009227196.1) and Anchored Capsid Protein C (YP_009227206.1)							
1*	C:44-IRMVLAILAFLRFT AIKPSLGLI-66	1.179	0.586	10	4	1.07	100
Membrane Glycoprotein Precursor M (YP_009227197.1) and Membrane Glycoprotein M (YP_009227208.1)							
2*	M:135-FALVAVAIAWLL GSS-149	1.194	0.741	5	4	2.87	14
3	M:151-SQKVIYLVMIL LIAP-165	1.187	0.527	4	4	4.11	96
Non Structural Protein NS2A (YP_009227200.1)							
4	NS2A:73-DVAHLALVAAF KVRPALLVSFIF-95	1.214	1.059	9	4	1.26	20
5*	NS2A:124-DLMVLINGF ALAWLAIRAMA V-144	1.105	0.434	10	4	1.09	9
Non Structural Protein NS2B (YP_009227201.1)							
6	NS2B:112-IAIPFAAGAW YVYVKT-127	1.139	0.579	7	3	2.16	100
Non Structural Protein NS3 (YP_009227202.1)							
7	NS3:236-LRGLPVRYMT TAVNVT-251	1.108	0.704	4	4	1.82	97
8	NS3:357-GKTVWFVPS V-366	1.173	0.863	3	2	13.79	91
9*	NS3:421- DRVIDSRRCLK PVILDGERVILAGPMPVTHASA-453	1.206	0.4198	4	2	5.0	100
10*	NS3:540-DLPVWLAYQV ASAGI-554	1.164	0.6606	6	3	4.91	100
Non Structural Protein NS4A (YP_009227203.1)							
11	NS4A:102-PARIACVLIVV FLLLVVLIPE-122	1.293	0.702	8	3	3.07	100
Non Structural Protein NS4B (YP_009227204.1)							
12*	NS4B:90-DLGVPLLMMG CYSQLTPLTLIVAIILLVAHYMYLIPGLQAAAARA-134	1.233	0.574	9	4	0.52	8
13*	NS4B:171-GQVLLIAVAI SSAVLLRT-188	1.221	0.421	8	4	1.16	15
RNA-dependent RNA polymerase NS5 (YP_009227205.1)							
14	NS5:777-MANAICSAVPV DWV-790	1.21	0.863	5	2	3.61	16

*peptide also identified as Thelper cell epitopes; NB: Number of binders; Number of binders in NetCTL mentioned based on number of supertypes bound with a peptide having NetCTL score > 0.5 and among them at least one supertype should have NetCTL score >1.25.

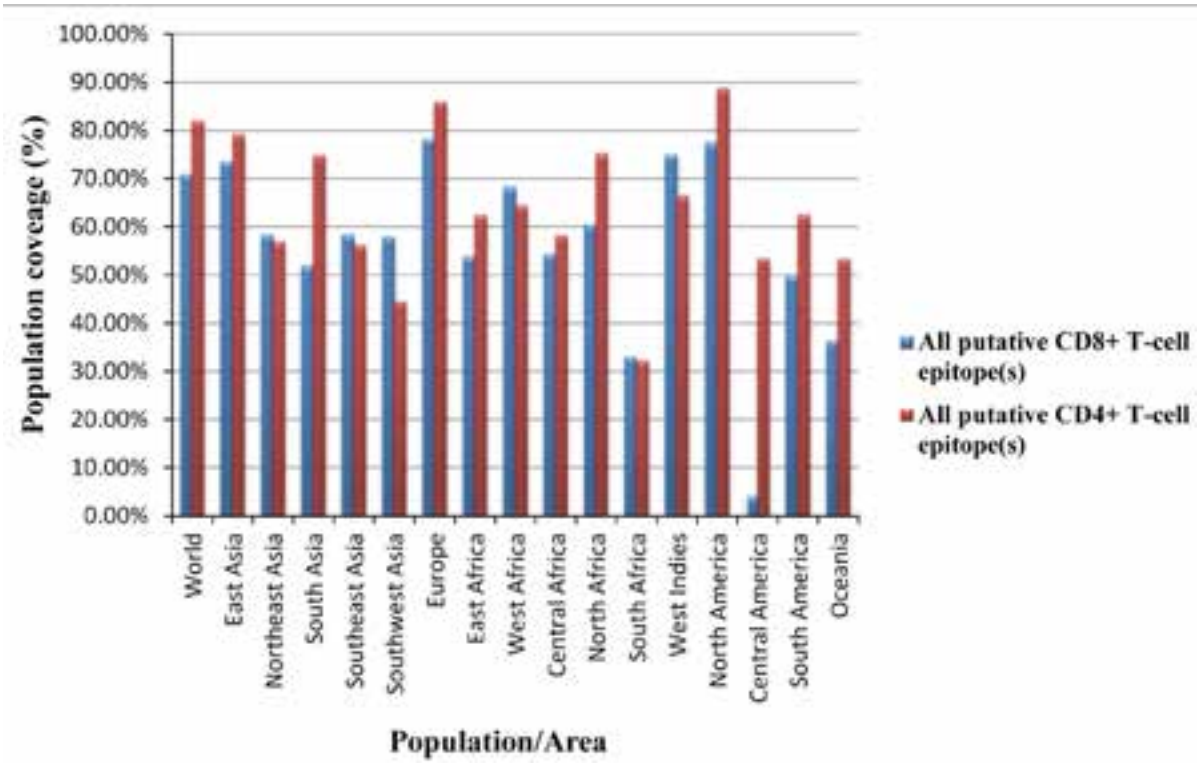


Fig. 3: Population coverage of proposed T helper and CTL epitopes.

These proposed epitopes may be tested experimentally either independently or in combination as subunit vaccine candidates to achieve optimum immunity in host. We anticipate, the *in silico* subunit vaccines proposed in the present study would provide basis for future development of potent vaccines against Zika virus.

Citation: Pradhan et al., (2017). Discovery of T-cell Driven Subunit Vaccines from Zika Virus Genome: An Immunoinformatics Approach ***Interdisciplinary Sci***. https://dx.doi.org/10.1007/s12539-017-0238-3.

iii) Database of esophagous cancer associated genes (dbEcanG) (http://bmicnip.in/dbEcanG/)

Esophageal cancer (EC) is the eighth most common and the sixth most frequent fatal human cancer in the world. About 80% cases of EC worldwide occur in less developed regions. It presents two histologically and genetically distinct subtypes, i.e. adeno- and

squamous cell carcinoma (EADC and ESCC). EC is usually aggressive and invasive in nature, and thus, many patients have a poor prognosis. The overall 5-year survival rate for patients with EC ranges from 15% to 25%. Better outcomes are correlated with early diagnosis, and poor outcomes are associated with the presence of metastases. Most patients are diagnosed at a late stage due to lack of symptoms in the early stages of the disease. Because curative therapy options in patients with advanced disease are lacking.

The literature abounds with the information about genes implicated in EC which has been gained through a variety of high-throughput 'omics' techniques including microarray profiling, proteomics analysis, Deep Gene Sequencing, Exome sequencing etc. However the information is rather sporadic. Esophageal Cancer associated Gene Database (dbE*c*anG) is a repository of the genes contributing to the etiology and pathogenesis of different types of esophageal cancer.

Database of EC risk genes and genetic markers would be helpful in prioritizing vital EC risk genes. The database would be useful in detecting EC genetic markers in an individual's genome through knowledge based homology search tools. We have developed a database esophagous cancer associated genes by screening published literature. The current version of database includes data collated from 1/01/2012 to 31/12/2016. There are 207 genetic markers in 162 genes compiled from 113 articles pertaining to genetic variation studies. As of now, 204 up-regulated and 100 down-regulated genes are curated based on evidences from 141 articles related to gene expression studies. Similarly, 36 genes shown to be hyper-methylated and 13 genes are hypo-methylated. The EC associated genes and gene expression status could be interactively queried and retrieved through a user friendly search engine integrated with the database (Figure 1).

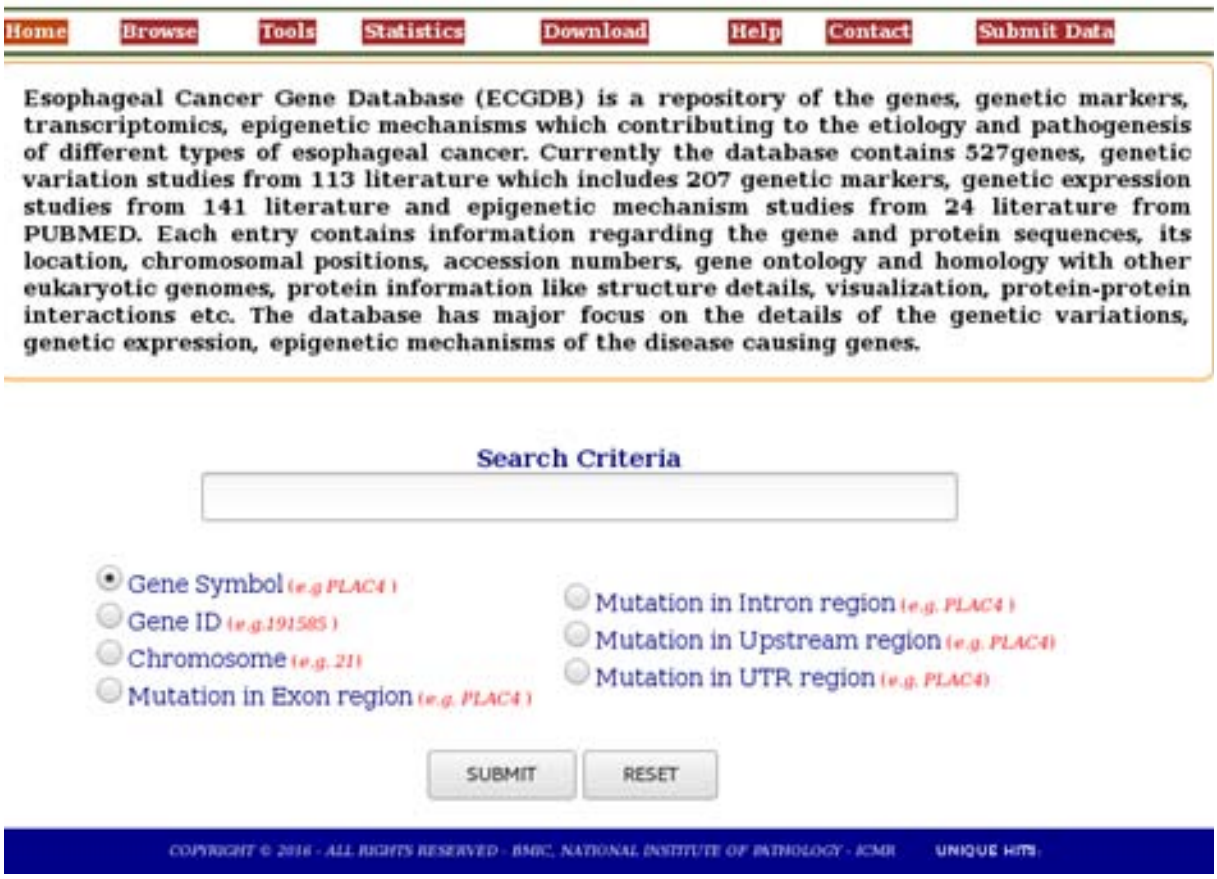


Fig. 1: User interface of dbEcanG

Gene expression data in dbEcanG are mainly populated based on available information in literature. However, availability of raw transcriptomics data in gene expression omnibus provides opportunity for re-analysing these datasets through uniform protocols to identify differentially expressed genes in EC and incorporate the same in dbEcanG.

The gene expression series obtained through microarray and RNA-seq experiments are retrieved and categorized in to three subtypes (BE [Barrett Esophagus], EADC and ESSC). Dysregulated genes in each state with respect to control are identified using LIMMA and listed. PPI network was constructed to identify the key molecule from the dysregulated genes. The observation followed the significant existence of sparsely distributed few hubs, namely, APP (Down-regulated: BE & EAC), SPARC, COL1A1, TRIP13 (Down-regulated: ESSC), CUL4B (Up-regulated: BE), BAG3 (Up-regulated: EAC), CEACAM6 & CEACAM1 (Up-regulated: ESSC).

A selected set of gene expression dataset were used to develop classification of EC subtypes based on gene expression profile. The three datasets having the gene expression values related to three subtypes were used to access the performance of random forest and support vector classifiers. To achieve this, "randomForest" and "e1071" R-packages were used for Random Forest and SVM respectively. In the RF classifier model 32 variables for splitting and 1000 number of trees were chosen via *mtry* and *ntree* parameters, respectively. SVM classifier was trained with radial basis kernel function. A tenfold cross validation was performed to assess performance of both the classifiers. For each fold, accuracy, sensitivity, specificity, AUC- ROC and Mathews correlation coefficient was calculated. To calculate the AUC-ROC was calculated using the AUC R-package. The rest of the metrics were calculated using the following formulae.

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

$$\text{Accuracy} = \frac{TN+TP}{TN+FP+TP+FN}$$

$$\text{Matthews's correlation coefficient} = \frac{(TP)(TN)-(FP)(FN)}{\sqrt{(TP+FN)(TP+FP)(TN+FN)(TN+FP)}}$$

Where TP = True positives, FN = False negatives, FP = False positives, and TN = True negatives. The accuracy measures were estimated as an average over the 10 fold CV given in Table 1

Table 1: Performance of machine learning algorithms in distinguishing EC subtypes

Classifier	Performance metrics	BE	EAC	ESCC
RF	Accuracy	0.92857	0.94362	0.7954
	Sensitivity	0.9	0.95846	0.72035
	Specificity	0.95714	0.91528	0.87
	AUC-ROC	0.92857	0.93687	0.79517
	MCC	0.86143	0.88214	0.59921
SVM	Accuracy	0.92857	0.9433	0.81683
	Sensitivity	0.9	0.95221	0.89405
	Specificity	0.95714	0.92639	0.73939
	AUC-ROC	0.92857	0.9393	0.81672
	MCC	0.86143	0.88219	0.6351

From the table 1, it is observed that SVM is performing better than randomForest in case of ESCC and at par in the case of BE. However, with the EAC dataset RF slightly better than SVM for accuracy and sensitivity while for remaining metrics SVM is performing better. Overall, the performance of both classifiers ESCC is not better than with other two datasets. Though, the differences in the performance metrics of both RF and SVM is not significantly different, due to little accuracy gain of SVM, an interactive and user-friendly R-code was developed (Figure 1) to predict the unknown cases as EC subtype using SVM classifier.

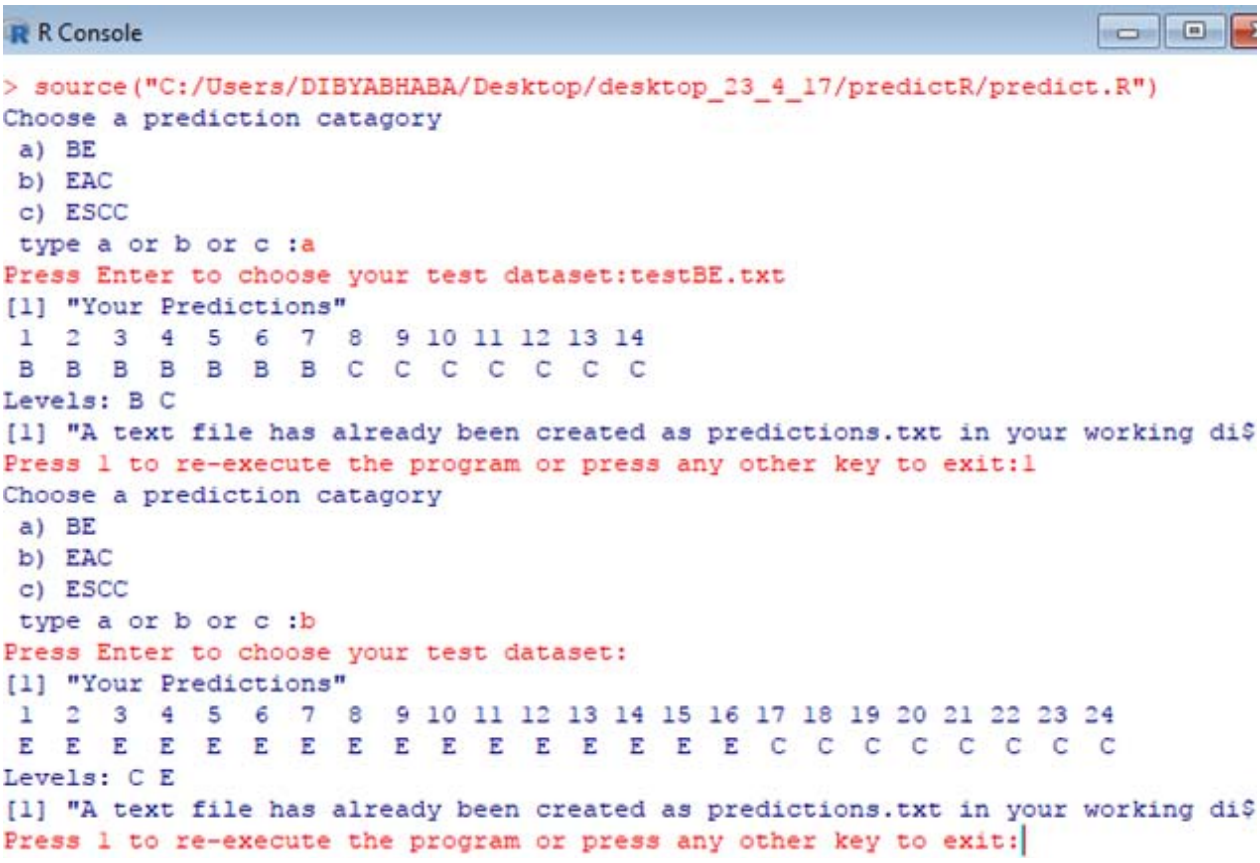


Fig. 1: R code for classification of EC subtypes.

iv) ICMR-NIP Laboratory Information Management System (LIMS)

- An in-house Laboratory Information Management System is being developed for digital reporting of histopathology, cytology, EM and microbiology laboratory test reports generated at NIP. It will improve efficiency, cost reduction and compliance in patient care.
- Patient reports can be uploaded by pathologist through submission form (Figure 1)
- Patients, doctors and researchers can assess digitalized histopathology report through assigned user id and password at the point of patient care.

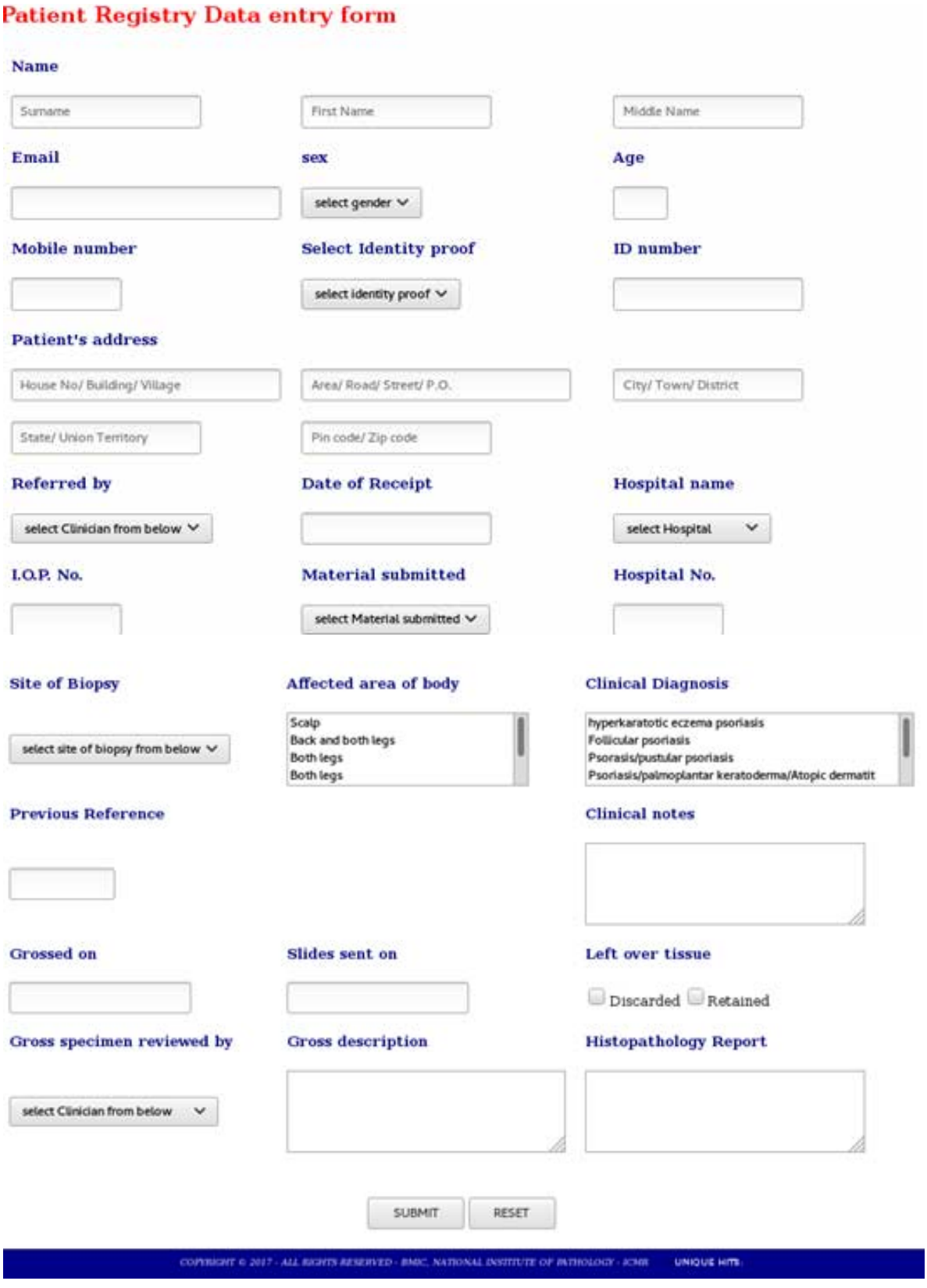


Fig. 1: Report submission form

v) Differential gene expression analysis on 38 samples from Breast cancer patients and controls.

Outline: The study is a part of collaborative work with tumour biology group of National Institute of Pathology. Responsibility of BIC includes, gene expression analysis on 29 breast cancer patients and 9 healthy controls obtained through microarray experiments using LIMMA by classifying the samples based on early stage, late stage and hormones. The cancer subtype predictions were made using genefu. The results were also compared with breast cancer gene expression data from western populations.

vi) Functional annotation and structural characterization of putative A1 protein of *Leishmania*

Outline: The study is a part of collaborative work with infectious disease group of National Institute of Pathology. Responsibility of BIC includes functional annotation of putative A-1 protein. *In silico* tools were used to predict functions from sequence and structure. Structure for the A1 protein is predicted and refined through molecular dynamics simulations. The results were validated experimentally by collaborating research group.

vii) Prevalence of Metabolic Syndrome in Psoriasis Patients: A Meta-analysis of Observational Studies

Background: Psoriasis has been reported to be associated with metabolic syndrome; however, it has still not been established beyond doubt owing to conflicting studies.

Objective: To perform meta-analysis of observational studies to assess prevalence of metabolic syndrome in psoriasis patients.

Methods: The systematic search of the articles published between January 1946 and August 2017 was performed using MEDLINE, PUBMED, EMBASE, EBSCO, Google Scholar and Science Direct. Original observational studies (*i.e.* cross-sectional, cohort and case-control) of psoriasis and metabolic syndrome with its associated risk factors (Hypertension, Raised Triglyceride, HDL Cholesterol, Fasting Plasma Glucose and Waist Circumference)

on human subjects reported in English are included. The association was measured using pooled Odd Ratio (OR) with 95% Class Interval (CI). Statistical heterogeneity was determined using I^2 statistic.

Results: The 48 studies comprising of 14010 psoriasis patients and 100969 controls were included in meta-analysis. Among these, 29.94% subjects in psoriasis patients and 21.61 % subjects in the control group are observed to have metabolic syndrome. Meta-analysis on the psoriasis and control group indicated an increased prevalence of metabolic syndrome among psoriasis patients (OR: 2.20 [95% CI, 1.95 - 2.49]) (Figure 1). The included studies showed significant heterogeneity (I^2 =72.92%). Subgroup analysis confining to geographical locations (Continent wise and Indian subcontinent) also supported increased incidence of metabolic syndrome among psoriasis patients.

Conclusion: The findings of this study support psoriasis patients have higher incidence of metabolic syndrome. Psoriasis patients should be regularly monitored for the complications of metabolic syndrome and its associated risk factor's such as hypertension, raised triglyceride, lowered HDL Cholesterol, increased fasting plasma glucose and waist circumference.

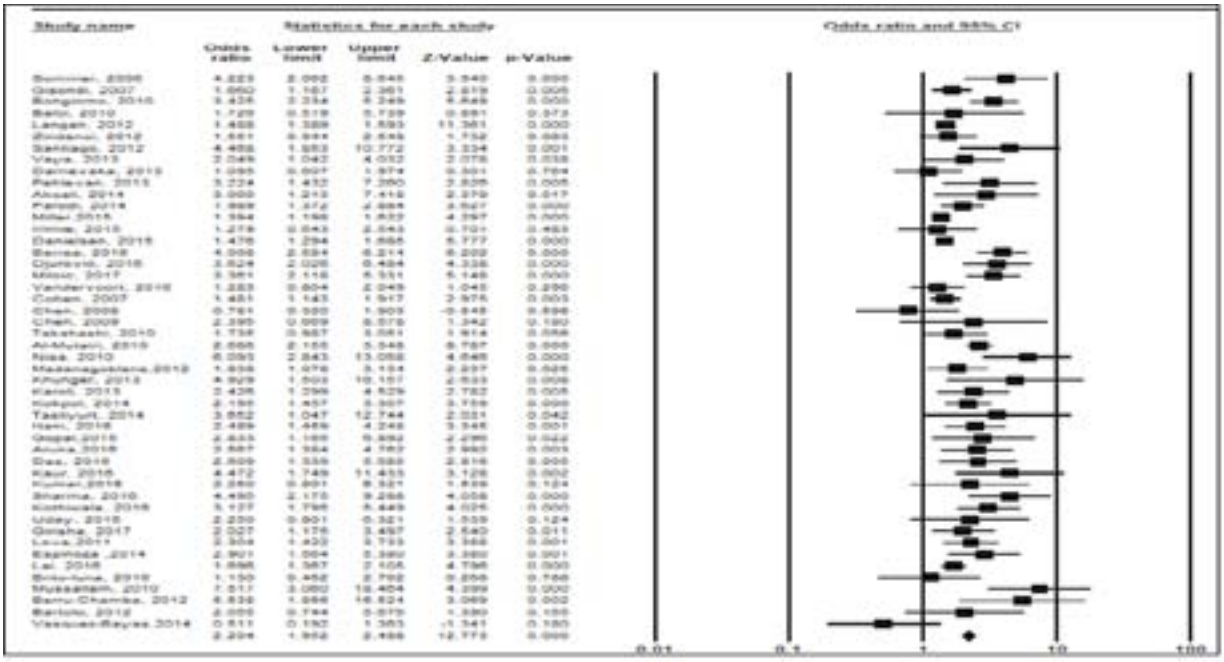


Fig. 1: Forest Plot for prevalence of metabolic syndrome in psoriasis patients.

viii) Identification and Determination of Potent Inhibitors against Cyclooxygenase Receptor using Computer Aided Drug Design Approach

Cyclooxygenase-2 is inducible enzyme and binds to arachidonic acid resulting in the release of metabolites that induce inflammatory responses, pain and fever. Recent studies have shown that strong COX-2 expression is highly correlated with increased the multiple type of cancer risk. The goal of this study is to develop a potent inhibitor that could be used as potential lead molecule for developing COX-2 targeted therapy. Six human COX-2 crystal structures in complex known inhibitors were retrieved from the PDB. An in-house ligand library of huge compounds (12583) was set up through similarity search for six inhibitors from different database. Virtual screening and docking techniques were utilized herein to gain insight the desire outcomes. Binding affinity of prepared ligands and known inhibitors towards COX-2 was approximated through molecular docking and Molecular Machine/generalized Born Surface Area (MMGBSA) free energy calculation using Glide and Prime, respectively. Binding affinities were also computed using AutoDockVina, a widely used program for molecular docking. Based on molecular docking results of COX-2, this study represents 11 compounds (9 from Zinc database and 2 from chembank) that indicate high binding energies to co-crystal inhibitors, range between -10.738 kcal/mol to -7.955 kcal/mol. ZINC000039428234 shows the highest binding affinity among all 11 compounds. The screening experiment successfully identifies the 11 compounds Molecular docking and MMGBSA results gives the informative knowledge of inhibitors with COX-2 based on the binding affinity. The proposed inhibitors of human COX-2 would be synthesized and in vitro experimental work that can be encouraged for new direction as a drug for human COX-2 for the novel class treatment of breast cancer.

ix) Identification of Putative Drug Targets in *C. albicans* through Subtractive Genomics, Metabolic Pathway and Gene Network Analysis

The polymorphic fungus Candida albicans is a member of the normal human microbiome and is used as a model organism for biology. In most individuals, C. albicans resides as a lifelong, harmless commensal. Under certain circumstances, however, C. albicans can cause infections that range from superficial infections of the skin to life-threatening

systemic infections (Sardi et al.,2013). Comparative genomics has off late out-focussed the traditional methods of drug discovery. In this computational approach, whole proteome of C. albicans were retrieved from NCBI database, which was then analysed in CD-hit suite to eliminate the paralogous proteins followed by similarity search against Database of essential genes for identification of essential proteins. Proteins involved in unique pathways were analysed using Kyoto Encyclopedia of Genes and Genome (KEGG) database and Cytoscape. Whole proteome of C. albicans consists of 5000 proteins; out of which 2299 proteins were filtered as non-homologous to human. Essentiality analysis has showed 120 proteins can be considered as putative drug targets. These targets are identified to play vital role in survival and pathogenesis of C. albicans. Prioritization of these targets through choke point analysis, gut flora non-homology, druggability analysis further established potential of these proteins as drug targets. Ten hub proteins are proposed as target of interest based on high betweenness centrality, degree of nodes and closeness centrality. Therefore, these proteins could be ideal target of choice for developing potent future therapeutics against C. albicans infections with subsequent experimental validations.

A drug target identification tool for fungi is proposed to be developed based on methodologies implemented in the above study.

x) Up-regulation of Fibroblast Growth Factor Receptor 1 due to Prenatal Tobacco Exposure Mediates Developmental Defects in New Born

Tobacco-smoking is the most important risk factor for low birth weight, pre-term delivery, congenital anomalies,A3 pregnancy loss and fetal growth. It was estimated that approximately 30% of growth-restricted neonates could be independently associated with maternal smoking. This study was aimed to examine the prenatal tobacco exposure on mRNA expression in the umbilical cord tissue. Gene Expression Omnibus-GSE 11798 data was retrieved to perform gene expression profiling using Bioconductor package LIMMA. Twenty-six genes were found to be differentially expressed (fold change≥ 1.5 and corrected p value<0.05) in tissues obtained from smokers category. Myosin heavy polypeptide-11(MYH11) that is responsible for haematopoiesis is observed to be highly up-

regulated while Carcino-embryonic antigen related CAM6 (CEACAM6) that play significant role in maintaining tissue architecture is substantially down regulated. The dysregulated genes were observed to participate in biological processes / pathways related to growth releasing hormone, angiogenesis and embryonic skeletal and cardiac development. Network analyses of 26 differentially expressed genes were carried out using Cytoscape. Fibroblast Growth Factor Receptor-1 (FGFR1) was identified to be the hub node with 297 interacting partners, which regulates transcription, cell growth, differentiation and apoptosis. The up-regulation of FGFR1 in umbilical cord tissue may lead to future health complications such as encephalocraniocutaneous lipomatosis, osteoglophonic dysplasia and Pfeiffer syndrome in new born. The findings open up possibility of overcoming these adverse health effects through FGFR1 targeted therapies during pregnancy.

A study on ultrastructural changes and oxidative stress-induced apoptosis in placenta exposed to pesticides: A plausible role of TNF-alpha, NF-kB and P38 (MAPK) in LBW babies

Scientific Staff : Dr. Arun Kumar Jain, Dr. Usha Agrawal, Mr. Shashi N. Kumar
In collaboration with : Dr. D. Borgohain, Assam Medical College Dibrugarh
Duration : 2015-2018
Funding Agency : ICMR

The prevalence of LBW (30%) is a major public health issue in India and is the highest among South-Asian countries (Chakraborty and Anderson 2011). Incidentally, high prevalence of low birth weight (42.9%) and prematurity (34.9%,) among infant has been reported in tea garden workers, which has been associated with more than 99% of total neonatal death (Phukan and Mahanta 1998). Due to common usage of chemical pesticides in tea gardens, women workers employed for plucking tea leaves are exposed to these harmful chemicals. Since the workers work even during gestational period, it is likely that pesticide exposure during this period is responsible for LBW and pre-term delivery, fetal death and congenital malformation that reported in tea garden workers. However, exact

mechanism behind the pesticide-induced LBW remains unexplored. In previous studies, it has been found that pesticides were not only deposited in placenta, maternal & cord blood of tea garden workers but were also detected in significant amounts in placenta and blood of unexposed housewives. It also revealed that pesticides present in the environment are able to reach human body through water and food, cross the placental barrier and enter the foetal bloodstream. In addition, some pesticides accumulate in the placenta and causepotential alterations in the development and functions of placental structures resulting in adverse effects on foetal development. One of the well documented adverse effects is low birth weight (LBW) of babies. effects is low birth weight (LBW) of babies. The aim of this study was to understand pathogenesis of LBW due to pesticide exposure in Tea Garden Workers of NE India. Attempts have been made to study histopathological and ultrastructural changes in placenta and to detect the TNF-α, NF-κB, Bcl2/BaX and MAPK expression in placenta.

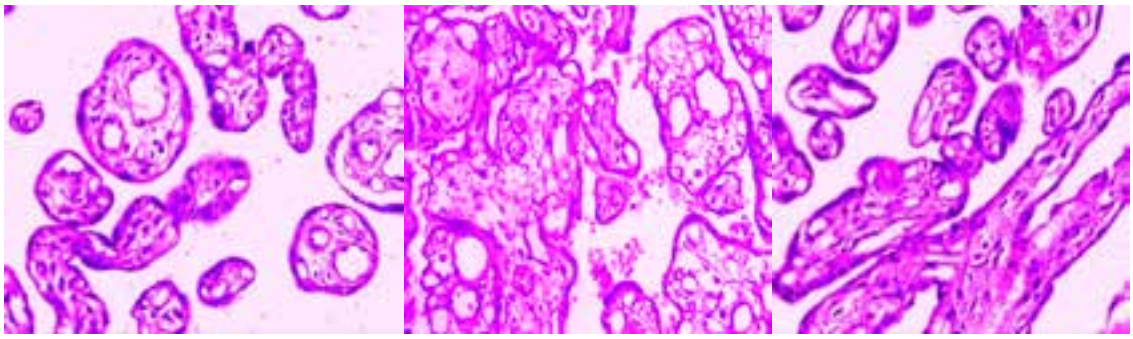
Work done during the year

Quantification of multiple residues by GC/MS/HPLC

Results of the study showed higher level of different classes of pesticides like organophosphates, organochlorine, pyrethroids, carbamates, herbicides, insecticides, fungicides and polyaromatic hydrocarbons in Maternal blood, Cord Blood and Placental Tissue of Tea Garden Worker in comparison to non-tea gardern worker.

Histopathology

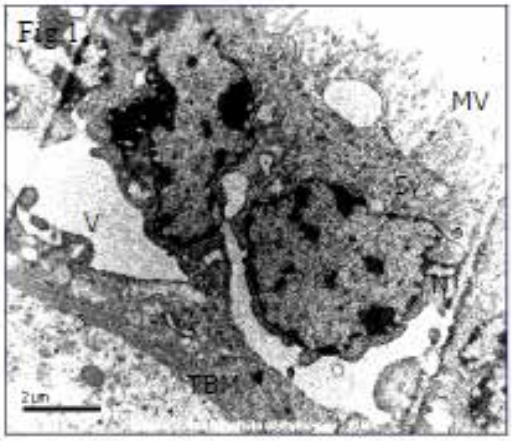
The histological studies revealed decidualvasculopathy in LBW cases of pesticide exposed tea garden workers. The incidence of villous infarct, including focal lesion was significantly higher in the LBW cases of tea garden workers than AGA cases. Furthermore, LBW cases tended to have multifocal infarcts. The incidence of the increase of Syncytiotrophoblast knots (ST) was significantly higher in the LBW than AGA.

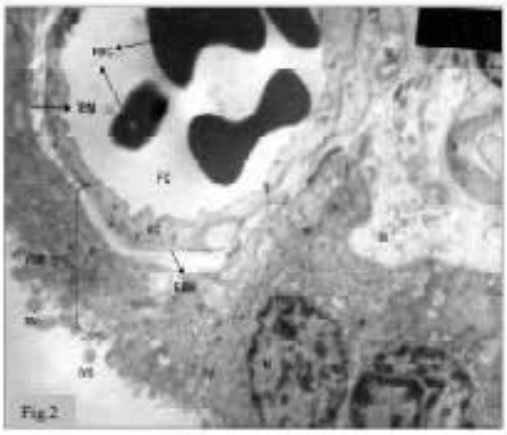


Villous infract:A) decidual vessel shows fibrinoid necrosis of vessel wall and a perivascular mononuclear infiltrate. B. Infracted area shows aggregation of ghost –like necrotic areas. C. Incresed syncytial knots and villous vascular structure in LBW

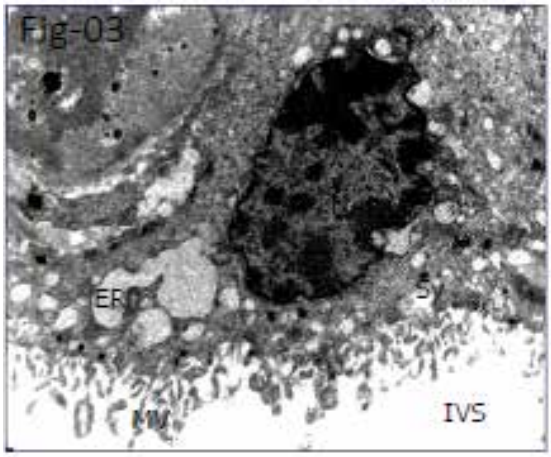
Ultrastructural study

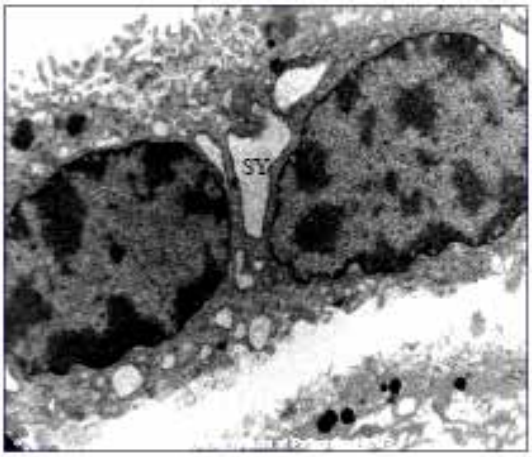
Ultrastructural study of LBW cases among pesticide exposed tea garden workers revealed oedematous endothelial cells in stromal capillaries of the placental villi while the cytoplasm was rich in dilated endoplasmic reticulum, mitochondria, fibrils and fine filaments. Syncytial knots were numerous in exposed workers. In most of the cases of tea garden workers, trophoblasts (especially syncytial trophoblasts) showed dynamic changes in the nuclei such as increased heterochromatin content and nuclear aggregation.





The placental villi shows syncytiotrophoblast (Sy) with irregular microvilli (MV), enlarged vacuoles & degenerated nuclei (N) and thickened TBM (Fig-1). FC was found to be in close approximation with Sy where thinning of Sy, St and EC layer was observed (Fig-2).





The microvilli on the apical surface of the syncytiotrophoblast are short, distorted and the chromatinization within the syncytial nuclei (N) and dilated ER. [3]. Syncytial nuclei are swollen and displayed karyolysis with severe loss of the euchromatin [3&4].

Study of HLA-DR3 and/ or DQ2 restricted CD4+T cells in Type 1 diabetes in North India.

Scientific staff : Dr Neeraj Kumar, Ms Neihenuo Chuzo, Ms Renu Sharma

Collaborator : Dr Nikhil Tandon (AIIMS), Dr Saurabh Verma (NIOP)

Year in which project started: 2016

Proposed year of completion: 2019

Funding Agency :

Background of the project

Type 1 diabetes (T1D) results due to the autoimmune destruction of pancreatic β-cells which is mediated mainly by autoreactive T cells. Immunogenetic association studies have shown a very strong genetic association of HLA-DR3-DQ2 haplotype with type 1 diabetes in North India. However, the exact mechanism behind this association is still not understood and raises the following questions i) What autoantigenic or diabetogenic peptides are presented by the HLA-DR3 and/ or DQ2 molecules to the autoreactive CD4+T cells in the Indian T1D patients and how do these differ from that in the healthy individuals ? ii) Is there a difference in the activation status of CD4 T cells between patients and controls carrying DR3-DQ2 haplotype in response to the autoantigens? Further, our earlier data have shown that HLA-DR3-DQ2 haplotypes associate not only with T1D *per se* but also with the Glutamic acid decarboxylase 65 (GAD65) autoantibodies suggesting an important role of HLA-DR3 and/ or DQ2 molecule in initiating the autoimmune response against GAD65 protein. Therefore, we aimed this study to evaluate the possible role of HLA-DR3 and/or DQ2 molecules restricted and GAD65 specific CD4+ T cells in North Indian T1D patients.

Work done during the year

During the first year, we have been able to standardize most of the techniques involved in the project. These involve i) HLA-DRB1/ DQB1 genotyping by Luminex bead assay using genomic DNA and ii) T cell stimulation assay on peripheral blood mononuclear cells (PBMCs) followed by intracellular cytokine staining.

i) HLA-DRB1/ DQB1 genotyping:

DNA from healthy controls was used for genotyping HLA-DRB1-DQB1 haplotypes by Luminex bead assay. Briefly, exon 2 of HLA-DRB1 and DQB1 genes was amplified using locus specific primer pair. The amplified DNA was denatured and hybridized with the mix of beads carrying the allele specific probes. The samples were acquired on Multiplex system and data was analyzed with HLA Fusion software (Fig. 1). This method is being used to identify HLA-DRB1*03-DQB1*02 positive individuals.

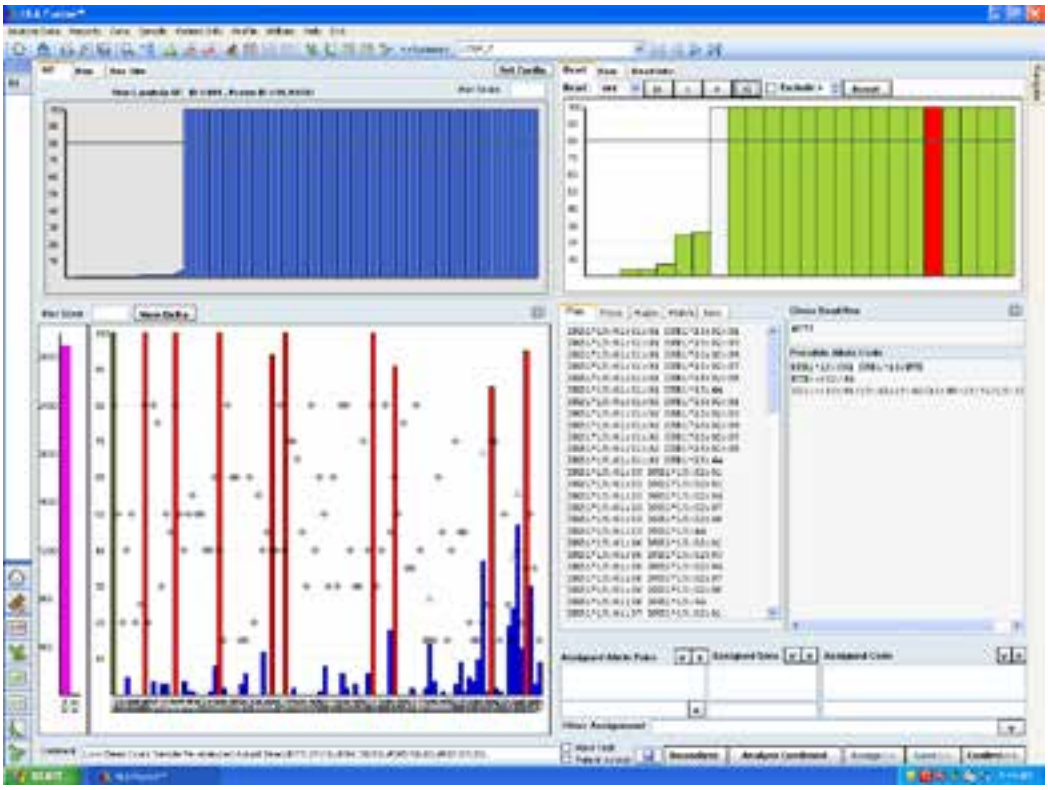


Fig. 1: Snapshot image of actual sample analyzed by the HLA fusion software for the HLA-DRB1 locus. Red bars in lower left panel indicate the probe positivity based on which the alleles are assigned.

ii) T cell stimulation assay:

PBMCs isolated from healthy human blood were subjected to the 16 hours culture with positive (PMA/ Ionomycin) and negative control (PBS). The doses of PMA and Ionomycin were adjusted for 16 hrs culture as the same duration of the culture is going to be used further in culture with GAD65 peptides. The intracellular expression of two pro-inflammatroy cytokines viz. TNF-α and IFN-γ were tested on CD4+ T cells by flowcytometer (Fig. 2).

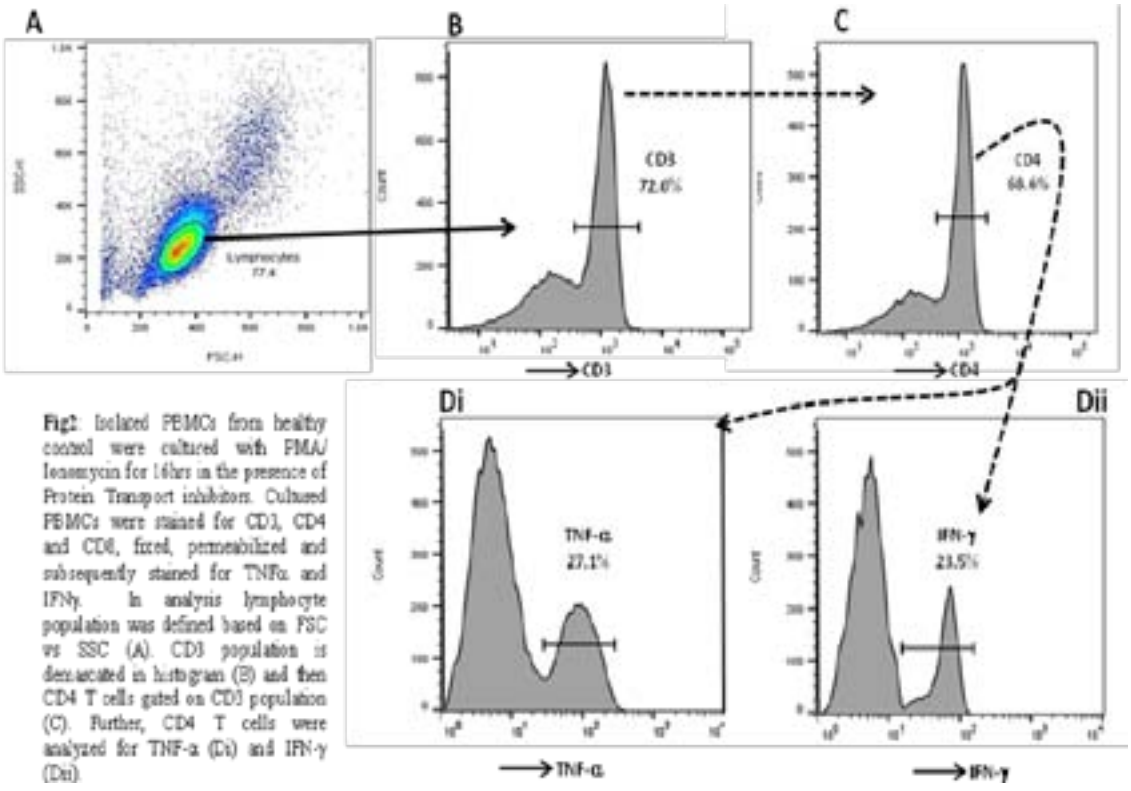


Fig2: Isolated PBMCs from healthy control were cultured with PMA/ Ionomycin for 16hrs in the presence of Protein Transport inhibitors. Cultured PBMCs were stained for CD3, CD4 and CD8, fixed, permeabilized and subsequently stained for TNFα and IFNγ. In analysis lymphocyte population was defined based on FSC vs SSC (A). CD3 population is demarcated in histogram (B) and then CD4 T cells gated on CD3 population (C). Further, CD4 T cells were analyzed for TNF-α (Di) and IFN-γ (Dii).

Future plan of action:

Type 1 diabetic patients will be recruited and genotyped for HLA genes to identify HLA-DRB1*03-DQB1*02 positive individuals. These patients along with the DRB1*03-DQB1*02 positive healthy controls will be tested for T cell stimulation assays using GAD65 peptides, positive and negative controls. The GAD65 peptides will be used in the pool of 5-10 peptides each. Once the immunogenic pool of peptides is identified, the same assay will be repeated with individual peptides of the immunogenic pool.

Exploratory study to identify gut bacterial peptides(s), if any, associated with autoimmune late onset diabetes.

Scientific staff : Dr Sapna Negi

Collaborator : Dr. Harpreet Singh, Indian Council of Medical Research, New Delhi

Dr. Krishna Biswas, HOD, Endocrinology, Safdarjung Hospital, New Delhi

Year in which project started: 2017

Proposed year of completion: 2019

Funding Agency :

Background of the project:

Autoimmunity and inflammation is a common factor responsible for many late onset diseases, including Diabetes, which is commonly known as Type 2 Diabetes (T2D). India has become an epidemic for T2D which is largely believed to be due to changing lifestyle and dietary habits. Diet has also been shown to influence intestinal integrity and microbial composition. Dysbiosis in gut microbiome has been associated with diabetes, but its cause and effect relationship or underlining mechanism remains to be understood. In later ages, although the diet remains similar, health conditions of an individual changes. Apart from diet, ageing too has been shown to have an impact on gut mucosal layer maintenance and secretion. Recent reports indicate that gut microbiota is a potential trigger for antibody production and is associated with many autoimmune and metabolic disorders. This builds inquest of gut bacteria being an autoimmune candidate for development of T2D. Our preliminary bioinformatics analysis shows sequence homology between gut bacterial and human proteins (mainly from basic metabolic and DNA repair pathways) and bidding affinity of these peptides to HLA class II molecules, indicating a possibility. This study is an extension of the preliminary analysis. Here, we will test cross-reactivity of selected gut microbial peptides associated with T2D with human sera from T2D & controls. The positive human sera will then be checked for inflammatory markers, which will suggest its role in causing systemic inflammation in T2D.

Work done during the year

The sequence identity search of gut bacterial (as per HMP) protein (sequence and its annotation taken from Swiss-Prot) and human expressed proteins has been carried out. 1759 peptides of ≥9 amino acid length with complete homology with human peptides and binding affinity with at least one HLA class II allele has been obtained. Further we observed that such autoimmune candidates were enriched in bacterial species belonging to *Firmicutes* and *Proteobacteria* phyla, which led us to conclude that these phyla may have higher disease impact in genetically predisposed individuals. Functional annotation of human proteins homologous to candidate gut-bacterial peptides showed significant enrichment in metabolic processes and pathways. Cognitive trait, Ageing, Alzheimer, Type 2 diabetes, Chronic Kidney Failure (CKF), Chronic Obstructive Pulmonary Disease (COPD) and Various Cancers were the major diseases represented in the dataset. This dataset provides us with gut bacterial autoimmune candidates which can be studied for their clinical significance in late onset diseases.

Results are published (Negi S et al., 2017).

Table 1: Number of bacterial species encoding autoimmune peptides in each gut bacterial phyla of the autoimmune candidate peptides dataset.

Phylum	Bacterial species among autoimmune candidates [n, (proportion)]	Autoimmune candidates [n, (proportion)]	p-value	95% CI
Actinobacteria##	62 (0.16)	628 (0.04)	0	0.195 - 0.341
Bacteroidetes	9 (0.02)	272 (0.02)	NS	
Firmicutes#	146 (0.37)	7012 (0.45)	0.04	1.002- 1.326
Fusobacteria	3 (0.008)	58 (0.004)	NS	
Proteobacteria	175 (0.44)	7567 (0.49)	NS	
Verrucomicrobia	1 (0.002)	19 (0.001)	NS	

"##" Significant p-value on multiple correction.
"#" Significant p-value <0.05.
"NS" non-significant

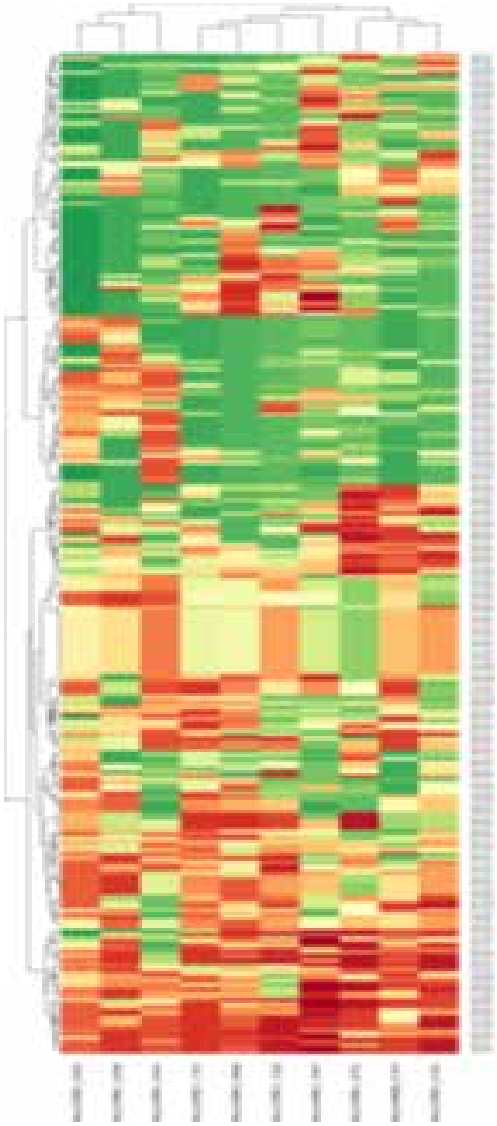


Fig 1: Clustered heatmap of human candidate proteins associated with late onset complex diseases and their binding affinity threshold with common HLA class II alleles. Binding affinity threshold [range: 1(red)± 11(green)]. Lower the threshold higher is the binding affinity with particular HLA class II allele.

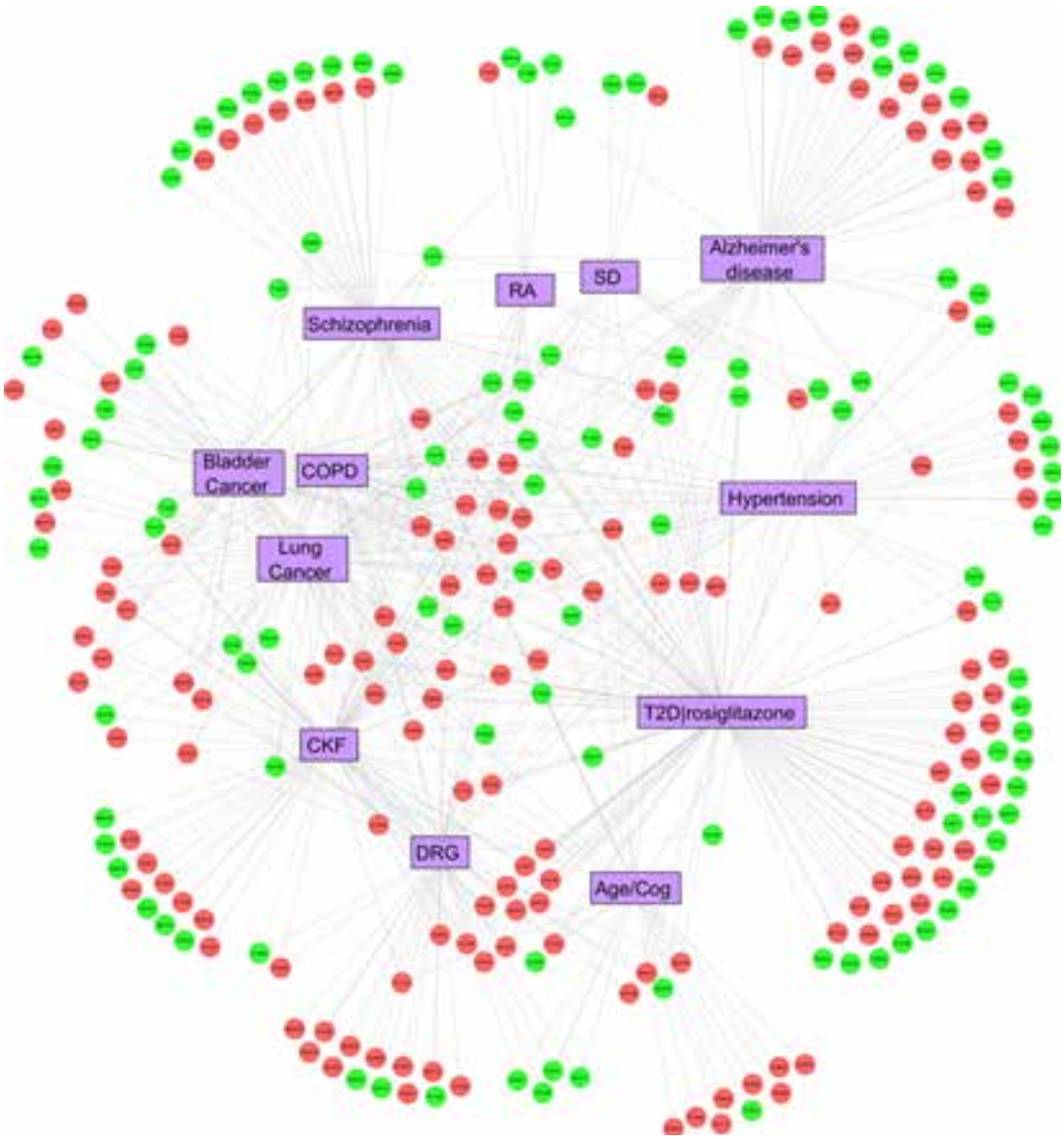


Fig 2: Cytoscape network displaying distance relationship of late onset complex diseases (purple nodes) associated with human candidate proteins (circular nodes). Shorter the distance between disease node and human candidate protein node higher are the number of gut bacterial species possessing autoimmune candidate peptide. Green circular nodes are metabolic genes as defined by KEGG metabolic pathway.

ACADEMIC ACTIVITIES

INSTITUTIONAL ACTIVITIES

- Conducted 3rd workshop at NIP entitled "**Biomedical Data analysis using R**" from 24th -25th January, 2017 exclusively for **Scientists and students of National Institute of Pathology**. **Thirty five** participants including **3 MDs** were trained.

- The 4th training program was organized at NIP entitled "**Structure-based Drug Design**" was organized on **9th February 2017**, as part of **XXI-Quality improvement program on Trends in Pharmaceutical Sciences** (30th Jan – 10th Feb, 2017) to train **30 pharmacy faculties** participated from different states.

- 5th National workshop was organized at NIP entitled "**Next Generation sequencing Data Analysis**" from 15th – 17th March 2017. Twenty five participants including **7 MDs** were trained in the National workshop.


- 16th Smt. Pushpa Sriramachari foundation day Oration was organised by NIP and the guest oration was delivered by Dr. Rajesh S Gokhle, Staff scientist VII, National Institute of Immunology on "Demystifying the Vitiligo Conundrum"

- **DG Visit to National Institute of Pathology on 14th July 2016.**




EXTRAMURAL PROJECTS

NEW PROJECTS

Evaluation of immune status and parasite load in patients of post kala azar dermal leishmaniasis (PKDL) in response to treatment with miltefosine and amphotericin B.

Funded by ICMR (2017-2020). Dr. Poonam Salotra

Development of an affordable, automated and field deployable, point of care and contained system for rapid diagnosis of TB caused by Mycobacterium tuberculosis.

Funded under IMPRINT by MHRD and DHR. Dr. Nasreen Ehtesham

Development of novel quantitative isothermal fluorescence based rapid detection method for diagnosis of malaria"

Funded by ICMR (2017-2020). Dr. Ruchi Singh

Clinical decision support system to identify histogenesis in cases of carcinoma with unknown primary (CUP) (ICMR MIF/BMS)

Dr Usha Agrawal, Dr AK Jain, Dr D Pradhan

Comparative study of Genetic, Clinical and Epidemiological factors of Breast Cancer in Indian population (ICMR Task Force project)

Dr Usha Agrawal, Dr LC Singh, Dr D Pradhan

Molecular profiling of invasive urothelial carcinoma (ICMR ad hoc)

Dr Usha Agrawal

In Vitro Evaluation of Effect of PKCε Silencing on Tumour Invasiveness in Urothelial Cancer (ICMR ad hoc)

Dr Usha Agrawal

Formulation of Formulation of Best Practices for Biofluid Banking- Urine as a Paradigm (DHR).

Dr Usha Agrawal

Exploratory study to identify gut bacterial peptides(s), if any, associated with autoimmune late onset diabetes.

DBT, Dr. Sapna Negi

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

Funded by ICMR Dr Poonam Gautam

To study antitumour potential of Centella asiatica on human urothelial cell carcinoma of bladder" was presented before Project Screening Committee on 15th Nov, 2016 at Ayush Bhawan, Ministry of AYUSH. The project is resubmitted after incorporation of committee suggestions.

Dr. Saurabh Verma

To study the Role of serum micro RNAs in urothelial cell carcinoma (UCC) of human bladder development and tumorigenesis. The project is submitted to DBT.

Dr. Saurabh Verma

ONGOING PROJECTS

Investigations on paromomycin resistance in *Leishmania donovani* using molecular and biochemical tools" funded by ICMR (2015-2018).

Dr. Poonam Salotra

Identification and charecterization of artemisinin resistance associated gene(s) in *Leishmania* funded by ICMR (2015-2018).

Dr. Ruchi Singh

Genome-wide analysis of genetic alterations and gene expression profiles in harmone sensitive and harmone refractory prostate cancer.

Dr. Anju Bansal. Funded by ICMR (2015-2018)

Understanding the role of micronutrients in *Mycobacterium tuberculosis* infection using guinea pig as a model.

Funded by ICMR. Dr. Nasreen Ehtesham

Virtual Centre of Excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis* (Phase I1).

Funded by DBT as Center of Excellence. Dr. Nasreen Ehtesham

Study of HLA-DR3 and/ or DQ2 restricted CD4+T cells in Type 1 diabetes in North India.

Dr Neeraj Kumar, Dr Saurabh Verma, DST-SERB (2016-2019).

Autoantibody Response And Identification Of Tumor-Associated Antigens In Gallbladder Carcinoma - Immunoproteomics Approach. Funded by DST-SERB (2016-2019)

Dr. Poonam Gautam

NEW PROJECTS

POST-DOCTORAL FELLWOSHIP PROJECTS

Role of RipA in inflammation and antimicrobial defence through autophagy.

Dr. Mohd Shariq, Dr. Nasreen Ehtesham, DST-SERB project (2017-2019).

Biophysical and biochemical characterization of essential PE-PPE proteins of *Mycobacterium tuberculosis:* way forward towards novel inhibitors.

Dr. Farha Naz, Dr. Nasreen Ehtesham, ICMR-PDF project (2017-2019).

Development of multiplex loop mediated isothermal amplification assay (m-LAMP) for differential diagnosis of PKDL and Leprosy.

Dr. Shweta; Dr. Poonam Salotra, DST-NPDF project (2017-2019).

Phenotypic and genotypic characterization of polymyxin resistant Gram-negative bacteria isolated from patients with blood stream infections.

Dr. Mudsser Azam; Dr. Ruchi Singh, DST-NPDF project (2017-2019).

In vivo study of gene expression profile and host-pathogen interaction during chronic persistent *Chlamydia trachomatis* infection in reactive arthritis patients.

Dr. Sangita Rastogi, Dr. Praveen Kumar, ICMR-PDF project (2016-18).

New DST- NPDF project: (Approved)

Role of Th40 cells in Type 1 Diabetes in North India.

Dr Gunja Mishra, Dr Neeraj Kumar DST-SERB (2016-2018).

Identification of growth stimulating protein expressed by feeder cells following a novel growth arrest protocol.

Dr. Rishi Man Chugh, Dr. L.K. Yerneni, ICMR-PDF project (2016-18).

Identification and determination of potent inhibitors against cyclooxygenases **????????** using computer aided drug design approach.

ICMR-PDF project (2016-18).

PUBLICATIONS (April 2016-March 2017)

1. Sethuraman G, Bhari N, Salotra P, Ramesh V. (2017) Indian erythrodermic postkala-azar dermal leishmaniasis. BMJ Case Rep. pii: bcr2016217926. doi:10.1136/bcr-2016-217926.

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3. Verma S, Singh R, Sharma V, Bumb RA, Negi NS, Ramesh V, Salotra P. (2017). Development of a rapid loop-mediated isothermal amplification assay for diagnosis and assessment of cure of *Leishmania* infection. ***BMC Infect Dis.*** 17(1):223. doi: 10.1186/s12879-017-2318-8.

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11. Sharma A, Equbal MJ, Pandey S, Sheikh JA, Ehtesham NZ et al. (2017) Immunodominant protein MIP_05962 from *Mycobacterium indicus prani*i displays chaperone activity. ***FEBS J.***;284:1338.

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Accepted Publications

Sandeep Rai, U Bhardwaj, Aroonima Misra. Comparison between photo-stability of alexa fluor 448 (Beckman coulter) and alexa-fluor 647 (Beckman coulter) with conventional dyes- FITC and APC by flow cytometry. ***International journal of lab hemotology***.

Book Chapter

Paris Jafari, Murielle Michetti, Lakshmana K Yerneni, Wassim Raffoul and Lee Ann Applegate (2017). Tissue Engineering, Cell Therapy and Regenerative Medicine- Concepts and Applications in Plastic Surgery. In: Karoon Agrawal**,** Surajit Bhattacharya (Eds.), Textbook of Plastic and Reconstructive Surgery, Volume-I (pp 345-362), NOIDA, India, Thieme Medical and Scientific Publishers. ISBN 9789385062759.

Book chapter entitled "Human fungal pathogens and drug resistance against azole drugs**".** Authors: Preetida J. Bhetariya, Neha Sharma, Pragati Singh, Priyanka H. Tripathi, Santosh K. Upadhyay and Poonam Gautam in a Book entitled "Drug Resistance in Bacteria, Fungi, Malaria, and Cancer" Editors- Andaleeb Sajid, Gunjan Arora, V. C. Kalia. Publisher- Springer, P. 387-428.

Book chapter entitled "Drug Resistance in Cancer". Authors- Santosh K. Upadhyay, Ramesh C. Rai, Rekha Gehtori, Ashutosh Paliwal, Poonam Gautam, Penny Joshi in a Book entitled "Drug Resistance in Bacteria, Fungi, Malaria, and Cancer" Editors- Andaleeb Sajid, Gunjan Arora, V. C. Kalia. Publisher- Springer, P. 449-473.

SCIENTIFIC ACTIVITIES

Dr. Nasreen Ehtesham

1. Elected as an Executive Council member of Kashmir Central University as President's nominee.
2. Awarded Kshanika oration award by ICMR for work done on understanding the biology of *M. tb*.
3. Presented our research work in EMBO tuberculosis conference in Istitute Pasteur, Paris France
4. Member secretary of Institute Bio-safety Committee
5. Reviewer for Short Term Research project for ICMR.
6. Attended meetings as Member of Institute Animal Ethics committee
7. Attended Meeting of IIT Institute Ethic committee as **Chairman**, Institute at IIT, Indore
8. Reviewed several research projects submitted to DBT.
9. Attended meetings for selection of Post Doctoral Fellowship at ICMR.
10. Two students were awarded PhD degree from Hyderabad Central University.
11. External Expert and Doctoral Committee member for PhD students at Kusuma School of Biological Sciences, IIT Delhi.
12. Reviewed research papers submitted to Plos ONE, FEMS Microbiology Letters, Infection Genetics and Evolution, Oncotarget, Cytokines etc
13. Javeed Ahmad was awarded the EMBO fellowship to work for 3 months in MRC, Cambridge, UK

14. Appointed as PhD examiner for students at , National Institute of Immunology, Delhi, Department of Biotechnology, University of Hyderabad and National Institute of Nutrition, Hyderabad

15. Reviewed research proposals submitted to Executive Government Agency of **National Science Centre**, **Poland**

Dr. Poonam Salotra

1. Participated in "Women's Health issues: Broadening the Mandate beyond Maternal Health" meeting at ICMR HQs New Delhi in May 2016.

2. Invited by DNDi for consultation on "Cohort observational study to estimate the prevalence of Post Kala-Azar Dermal Leishmaniasis (PKDL) in Visceral Leishmaniasis patients treated with three regimens in Bihar" at RMRI, Patna in June 2016.

3. Attended meeting on "Health Technology Assessment (HTA) Stakeholder's Consultative Workshop" with DHR-ICMR-iDSI at India Habitat Centre, New Delhi July 2016.

4. Attended meeting on "India Africa Health Sciences meet" at Vigyan Bhawan, New Delhi in Sep 2016.

5. Attended meeting on " The Research Performance Evaluation Analysis of ICMR" at ICMR, New Delhi in Sep 2016.

6. Attended meeting on " Science Promotion Committee Meeting " held at Indian National Science Academy, New Delhi in Sep 2016.

7. Invited speaker on PKDL at "10th Asian Dermatological Congress 2016" held in Oct 2016 at Sahara Star International Convention Centre, Mumbai.

8. Invited as Key Note Speaker in DST Inspire Camp held in Oct at Ch Bansi Lal Univ, Bhiwani, Haryana.

9. Invited delegate at the DNDi consortium meeting on "Setting the Post Elimination agenda for Kala azar in India", held in Nov, 2016 at Vivanta by Taj- Ambassador Hotel, New Delhi.

10. Invited delegate for the Meeting on "National Ethical Guidelines for Biomedical and Health Research involving human Participants" held in Dec, 2016 at ICMR, New Delhi.

11. Invited participant at the Review meeting of Model Rural Health Research Unit (MRHRU) in States, held in Dec, 2016 at DHR, New Delhi.

12. Invited speaker at the "13th Conference on Vectors and Vector-borne diseases" held at Hotel Crowne Plaza, Chennai in Feb, 2017.

13. Invited speaker and chairperson of the session on "New Approaches in *Leishmania* research" at "Mini Symposium on Host Parasite Relationship" under INDO-Belgium Networking project held at Kolkata, India, in Feb, 2017.

14. Chief Guest for Symposium "Emerging Trends in Biotechnology & Drug Discovery" held at CSIR- Institute of Genomics & Integrative Biology, Mall Road, Delhi

Dr. A.K. Jain

Dr. AK Jain delivered lecture on

- "Introduction to R" in 3rd workshop entitled "Biomedical Data analysis using R" from 24-25th January, 2017.

- "Bioinformatics in Medicine" in 4th training program entitled "Structure-based Drug Design" organized on 9th February 2017 at National Institute of Pathology, as part of XXI-Quality improvement program on Trends in Pharmaceutical Sciences (30th Jan – 10th Feb, 2017) by DIPSAR, New Delhi.

Dr. Sangita Rastogi

1. Member Secretary, Institutional Animal Ethics Committee (IAEC) of National Institute of Pathology, New Delhi.

2. Convened IAEC meeting at National Institute of Pathology, New Delhi on 26th May 2016 and 3rd February 2017.

3. External examiner for Ph.D. thesis at BITS, Pilani (July 2016 and March 2017).

4. Attended Selection Committee meeting for selection of Junior Research Fellow at National Institute of Pathology, New Delhi on 18th November 2016.

5. Invited to attend Abcam seminar on '*Optimization techniques for WB, IHC & ICC*' at ICGEB, New Delhi on 23rd November 2016.

6. Convened Library Review Committee Meeting at National Institute of Pathology, New Delhi on 9th December 2016.

7. Assisted in inspection of Central Animal House Facility at National Institute of Pathology, New Delhi on 30th December 2016.

8. Attended a talk by Nobel Laureate Dr. Harold Varmus, Ex-Director US NCI at National Institute of Cancer Prevention & Research (NICPR), Noida on 13th January, 2017.

9. Attended 3rd Professor P.P. Talwar Oration delivered by Professor NK Ganguly, Former DG, ICMR on '*Value of Measurements in Health:Impact on Forecasting and Evaluation*' at ICMR, New Delhi on 16th January 2017.

10. Attended lecture on *'Eliminate Dengue Programme'* by Professor Scott O'Neill, Head of Vector Borne Disease Institute, Monash University, Melbourne, and Director, Eliminate Dengue Programme at ICMR, New Delhi on 7th February, 2017.

11. Attended lecture on *'Understanding inherited breast and ovarian cancer: From gene discovery to precision medicine and public health'* by Dr. Mary-Claire King at AIIMS, New Delhi on 24th February, 2017.

12. Participated in National Workshop on Genome Informatics organized by Genomics Facility and CIIDRET at University of Delhi South Campus on 6th-8th March 2017.

13. Attended ICMR Annual Oration Recognizing Excellence in Health Research delivered by Dr. Glenda Gray and Dr. Salim Abdool Karim on '*HIV vaccines: Progress made to advance candidates to efficacy studies*' and '*HIV prevention in Africa: Challenges and prospects',* respectively at AIIMS, New Delhi on 27th March 2017.

14. Reviewer for Short-term Studentship (STS) projects of ICMR (2016-17).

15. Invited reviewer of manuscript submitted to various journals including *Arthritis Research & Therapy, The Journal of Rheumatology*, *Journal of Infection and Public Health, Critical Care in Obstetrics & Gynecology*.

Dr. Usha Agrawal

- NBE examiner for practicals at Base Hospital, New Delhi on 6th and 7th June, 2016.
- NBE onsite examiner for theory paper evaluation in June 2016.
- NBE examiner for practicals at PGI, Chandigarh on 8th and 9th Sept, 2016
- NBE onsite examiner for theory paper evaluation in Dec 2016.
- Invited and participated in NITI Bill, NBE consultative meeting on 22nd August 2016.
- Nominated to attend Programme on Integrated Scientific Project Management for Women Scientists/Technologists, from Nov 21st-25th, 2016 at Centre for Organisation Development, Hyderabad
- Delivered guest lecture on "Blood and Dental plaque as molecular tools- theories and practices"on 12th December 2016 at ITS Centre for Dental Studies and Research, Ghaziabad.
- Nominated Selection committee member for selection of Research Associate and SRF at NICPR. NOIDA.

- Delivered guest lecture on "Rigours in Medical research" on 23-02-2017 at the International Winter School & FDP on "Grant Proposal & Scientific Writing in Health Services Research", being organised by the Department of Social Work, Jamia Millia Islamia, New Delhi, India and the School of Public Health, University of Minnesota (Twin Cities Campus), Minneapolis, MN, USA from February 23rd-24th, 2017.
- Reviewer for J of Cytology, Molecular oncology
- Institutional Nodal officer for Performance Evaluation Committee
- Coordinator of DNB programme in Pathology at NIP.

Dr Laxmana Yerneni

1. The 51st Annual Conference of Association of Plastic Surgeons of India (APSICON), 24-27 November, 2016, New Delhi.
2. Preparation of designs and elaborate specifications for a process specific cGMP facility as per Schedule M of Drugs and Cosmetic Act for clinical grade stem cell culture and human epidermal construction.

Dr. Anju Bansal

1. Enrolled for UK NEQAS; Breast HER2 IHC Module for 2016 & 2017.
2. Invited participant at DHR-ICMR-iDSI Collaborative "Health Technology Assessment (HTA) - Stakeholders' Consultative Workshop" in collaboration with NICE International, UK and HITAP, Thailand through the International Decision Support Initiative (iDSI), at India Habitat Centre from 25-27th July, 2016.
3. Reviewer for Short term Studentship projects submitted to ICMR, 2017.
4. Appointed reviewer of manuscripts submitted to PloS One, Oschner Journal
5. Biosafety Officer for Institutional Biosafety Committee (IBSC) of NIOP.

6. Appointed PhD Examiner for evaluation of thesis and oral viva for award of degree of PhD in Biochemistry in the Department of Biochemistry, School of Life Sciences, University of Hyderabad.

7. Attended Annual and quarterly meetings of IAPM Delhi Chapter

8. Member of Institutional Animal Ethics Committee

Dr. Ruchi Singh

1. Invited reviewer for manuscript submitted to Journal of Clinical Microbiology, PLOS NTD, Antimicrob Ag Chemoth, Journal of Vector Borne Diseases, Ind J Dermatology.

2. Member of Institutional ethics committee.

3. Member Secretary of Institutional committee for stem cell research.

4. Reviewer for short-term studentship projects of ICMR (2016-17).

5. Reviewer for research project submitted to DST and ICMR.

6. Attended the 'National workshop on systematic reviews' organized by ICMR Advanced centre for evidence based child health, PGIMER, Chandigarh at ICMR, HQ.

7. Participated in the European Research Council's (ERC) 10th Anniversary conference.

Dr. Avninder Pal Singh

1. Attended 31st annual conference of APCON Delhi chapter on 28th Feb 2016 held at University College of Medical Sciences

2. Attended the quarterly meeting of Delhi chapter IAPM held at Vardhman Mahavir Medical College and associated Safdarjung Hospital on 26 Nov, 2016

Dr. Fouzia Siraj

Conferences/workshops attended:

- **Poster presentation** at 32nd Annual Conference of Delhi Chapter IAPM (DAPCON) New Delhi, 26thMarch, 2017

1) Unusual presentation of papillary carcinoma thyroid
2) Determination of molecular subtypes of diffuse large B-cell lymphoma using immunohistochemistry

- **Attended 3rd Workshop on 'Biomedical data analysis using R'.** National Instituite of Pathology, New Delhi, 24th -25th January 2017

Oral presentation:

- **Invited lecture titled "Newer molecular diagnostic tools in CNS tumors". Neuro-sciences Update 2016.** Held at Safdarjung Hospital, New Delhi, 10th December, 2016

Dr. Shruti Sharma

1. Thesis guide for DNB student Dr. Sartaj Ali: "A comparative study to evaluate the use of a liquid detergent as an alternative to Xylene and alcohol in routine Haematoxylin & Eosin and Histochemical staining procedures."

2. Thesis co-guide for DNB student Dr. Jeevan: Role of tumor associated macrophages in Gliomas.

3. Organizing Dermatopathology clinicopathological meeting at National Institute of Pathology every Tuesday.

4. Diagnostic services: Histopathological Reporting of 2000 – 2500 skin biopsies annually.

Dr. Neeraj

1. Dr Neeraj Kumar participated in the 3rd Workshop conducted by Biomedical Informatics Centre on Biomedical data analysis using R held on January 24-25, 2017 at the National Institute of Pathology, New Delhi.

2. Dr Neeraj Kumar participated in the 4th National Conference of Indian Society of Histocompatibility and Immunogenetics (ISHI) organized by Department of Immunopathology held at PGIMER, Chandigarh on December 2-3, 2016 and delivered a lecture on "**Genetic variants mediating risk of Type 1 diabetes in North India".**

Dr. Poonam Gautam

1. Presented a Talk on "WHO: **श्रमिक स्वास्थ्य पर वैश्विक कार्य योजना** (2008-2017)" in two day workshop "2nd National Scientific Hindi Sangoshthi" held from **21st-22nd March 2017** at NIOH, Ahmedabad.

2. Participated in the **workshop** on "Biomedical data analysis using R" from **24-25th January, 2017**, organised by Biomedical Informatics Centre, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi.

3. Attended **8th Annual meeting of Proteomics Society, India (PSI)** alongwith 3rd meeting of Asia Oceanica Agricultural Proteomics Organization (AOAPO) organized by NIPGR, Delhi at Hotel The Grand from **14th -17th Dec. 2016.**

4. Attended the conference in **"Cancer In Asia: Bridging the Gaps"** organized under 12th International conference of the Asian Clinical Oncology Society (ACOS), 35th Annual Convention of Indian Association for Cancer Research (IACR) and mid-Term Conf. On IASO to be held from **8th-10th April, 2016** at The Ashok Hotel, New Delhi.

Dr. Saurabh Verma

1. **As Guide:** Dr. Ankita Sahu, ICMR Post Doctoral Fellow joined in project titled, "Identification and determination of potent inhibitors against cyclooxygenases using computed aided drug design approach".

2. **As Investigator:** MSc dissertation titled, "Flowcytometric analysis of CD44+/ CD24- expression in breast cancer stem cells submitted by Leena Arora, student of Indian academy of Sciences.

STUDENTS ACTIVITIES

Dr. Dibyabhaba Pradhan

- "Computer Aided Techniques for Target Based Drug Design" in 19th Workshop on Computational and Structural Bioinformatics, organized by Bioinformatics Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram during January 9 - 10, 2017.
- "Differential gene expression analysis" in 3rd workshop entitled "Biomedical Data analysis using R" from 24-25th January, 2017.
- "Protein structure modelling and validation" in 4th training program entitled "Structure-based Drug Design" was organized on 9th February 2017, as part of XXI-Quality improvement program on Trends in Pharmaceutical Sciences (30th Jan – 10th Feb, 2017) by DIPSAR, New Delhi.
- "Role of molecular docking in Drug design" in XXII-Quality improvement program on Current Scenario in Pharmaceutical Sciences (30th Jan – 10th Feb, 2017) held at DIPSAR, New Delhi.
- "Alignment and variant calling" in 5th National workshop entitled "Next Generation sequencing Data Analysis" from 15th – 17th March 2017.
- "Differential gene expression analysis: RNA-Seq" in 5th National workshop entitled "Next Generation sequencing Data Analysis" from 15th – 17th March 2017.

Dr. Shweta Aggarwal

- "Visualization of Bimolecular structures" in 4th training program entitled "Structure-based Drug Design" was organized on 9th February 2017, as part of XXI-Quality improvement program on Trends in Pharmaceutical Sciences (30th Jan – 10th Feb, 2017) by DIPSAR, New Delhi.
- "NGS file formats and QC" in 5th National workshop entitled "Next Generation sequencing Data Analysis" from 15th – 17th March 2017.

Mr. Deepak Kumar Deep

- Mr. Deepak Kumar Deep participated and presented work in MICROCON 2016 conference, held from 23rd-27th November, 2016 at PGIMER, Chandigarh on the topic "Comparative Transcriptome Profiling Identifies Genes Associated With Experimental Paromomycin Resistance in *Leishmania donovani*". He also received travel grant support to attend this conference.

Ms. Aditya Verma

- Ms. Aditya Verma participated and presented work in MICROCON 2016 conference, held from 23rd-27thNovember, 2016 at PGIMER, Chandigarh on the topic "In-vitro susceptibility towards artemisinin and induction of artemisinin resistance in *Leishmania donovani* isolates". She also received travel grant support to attend this conference.

Ms. Keerti Kaumudee Dixit

- Ms. Keerti Kaumudee Dixit participated and presented work in ICGEB Workshop on "Molecular Biology of *Leishmania*", held from 24rd-26thOctober 2016 at ICGEB, Trieste, Italy on the topic "Application of LAMP in rapid diagnosis of VL and PKDL using Direct Blood Lysis". She also won FEBS Journal award along with cash prize for best oral and poster presentation.

Ms. Vanila Sharma

- Ms. Vanila Sharma awarded doctorate degree

Mr. Himanshu Kaushal

- Mr. Himanshu Kaushal submitted his PhD thesis.

Dr Nitu Kumari

- Dr Nitu Kumari has presented a poster entitled "**Stability of Urinary biomarker in lyophilized urine sample of Urothelial bladder cancer**" in conference

"Global Biobank Week - Towards Harmony in Biobanking, held in Stockholm from 13th to 15th September 2017"

- Dr Nitu was awarded with International Travel grant from ICMR and DST
- Dr Nitu Kumari awarded PhD degree from BITS, Pilani.

Mr. Praveen Kumar

- **Mr. Praveen Kumar** Awarded Ph.D. (Biosciences) by BITS, Pilani (2016).
- **Mr. Praveen Kumar** Awarded Post-Doc fellowship by ICMR under the mentorship of Dr. Sangita Rastogi (2016).
- **Mr. Praveen Kumar** Participated in hands-on training on "*Gene Expression Microarray*" at National Institute of Pathology (ICMR), New Delhi on 21st-22nd April 2016.

Ms Namita Singh

- **Ms Namita Singh** Awarded Ph.D. (Biosciences) by BITS, Pilani (2017).

Ms Priya Prasad

- **Ms Priya Prasad** Participated in hands-on training on "*Gene Expression Microarray*"at National Institute of Pathology (ICMR), New Delhi on 21st-22nd April 2016.

Mr. Arnab Nayek

- "Major Biological database resources and sequence homology search tool" in 4th training program entitled "Structure-based Drug Design" was organized on 9th February 2017, as part of XXI-Quality improvement program on Trends in Pharmaceutical Sciences (30th Jan – 10th Feb, 2017) by DIPSAR, New Delhi.
- "Linux basics and R commands" in 5th National workshop entitled "Next Generation sequencing Data Analysis" from 15th – 17th March 2017.
-

Ms. Rashi

- Ms. Rashi presented lecture on "Protein Preparation and Molecular Docking by using AutoDock Tools" in 4th training program entitled "Structure Based Drug Design" was organized on 9th February 2017, as a part of XXI-Quality improvement program on trends on Pharmaceutical Science (30th Jan – 10th Feb, 2017) by DIPSAR, New Delhi.

Dr Gunja Mishra

- Dr Gunja Mishra (Post Doctoral Fellow) attended the 4th National Conference of Indian Society of Histocompatibility and Immunogenetics (ISHI) organized by Department of Immunopathology held at PGIMER, Chandigarh on December 2-3, 2016. For this, she was awarded the travel grant from ISHI.

- Dr Gunja Mishra (Post Doctoral Fellow) was selected to participate in Advanced Course in Basic and Clinical Immunology held at Arizona, USA on February 19-22, 2017, for which she was awarded travel grant from Gender Equality and Career Development Committee (GECD) of International Union of Immunological Societies (IUIS) and Federation of Clinical Immunology Societies (FOCIS).

Ms Nehineuo Chuzo

- Ms Nehineuo Chuzo (Ph D Student) participated in the 3rd Workshop conducted by Biomedical Informatics Centre on Biomedical data analysis using R held on January 24-25, 2017 at the National Institute of Pathology, New Delhi

Ms. Priyanka H. Tripathi

- Presented **poster** on "Expression of gonadotropin releasing hormone receptor in Glioblastoma cell line-derived exosomes and potential as circulatory biomarker" in conference on **"Cancer In Asia: Bridging the Gaps"** organized under 12th International conference of the Asian Clinical Oncology Society (ACOS), 35th Annual Convention of Indian Association for Cancer Research (IACR) and mid-Term Conf. On IASO to be held from **8th-10th April, 2016** at The Ashok Hotel, New Delhi

- Participated in the **workshop** on **"Assays for cell migration and invasion"** organized by AIIMS, New Delhi and Indian Associaton of Cancer Research (IACR) on **7th April 2016** in the Department of Biochemistry (Convergence Block), **AIIMS, New Delhi**.
- Participated in the **workshop** on "Biomedical data analysis using R" from 24-25th January, 2017, organised by Biomedical Informatics Centre, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi
- Attended "**Education day Seminar"** in **8th Annual meeting of Proteomics Society, India (PSI)** and 3rd meeting of Asia Oceanica Agricultural Proteomics Organization (AOAPO) organized by **NIPGR, Delhi** at Hotel The Grand on **13th Dec. 2016.**

Mr. Javed Akhtar

- Participated in the **workshop** on "Biomedical data analysis using R" from 24-25th January, 2017, organised by Biomedical Informatics Centre, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi.
- Attended "**Education day Seminar"** and participated in **8th Annual meeting of Proteomics Society, India (PSI)** alongwith 3rd meeting of Asia Oceanica Agricultural Proteomics Organization (AOAPO) organized by **NIPGR, Delhi** at Hotel The Grand from **13th** and **14th -17th Dec. 2016.**

Ms. Ratna Priya

- Participated in the **workshop** on "Biomedical data analysis using R" from 24-25th January, 2017, organised by Biomedical Informatics Centre, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi.
- Attended "**Education day Seminar"** in **8th Annual meeting of Proteomics Society, India (PSI)** and 3rd meeting of Asia Oceanica Agricultural Proteomics Organization (AOAPO) organized by **NIPGR, Delhi** at Hotel The Grand on **13th Dec. 2016.**Mr. Javed Akhtar

Mr. Javed Ahmad

- Mr Javeed Ahmad participated in the workshop on, "Biomedical data analysis using R" from 24-25th January 2017, organised by Biomedical Informatics Center, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi.
- Mr. Javeed Ahmad presented a poster entitled "Intrinsically disordered Mycobacterium tuberculosis protein PPE37 induces proliferation of tolerogenic immune cells and apoptosis by N and C terminal domains, respectively" in the conference "International Conference of Immunology, 2016" held in Melbourne Australia from 21-26 August 2016.
- Mr Javeed Ahmad was awarded SERB-DST travel grant to attend the conference "International Conference of Immunology, 2016" held in Melbourne Australia from 21-26 August 2016.

Ms. Rashmi Borah

- Ms. Rashmi Borah attended NGS file format and QC" in 5th National Workshop entitle "Next Generation Sequencing Data Analysis" from 15th-17th March 2017.

Mr Manjunath Pichipalli

- Mr Manjunath Pichipalli participated in the workshop on, "Biomedical data analysis using R" from 24-25th January 2017, organised by Biomedical Informatics Center, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi.

AWARDS/PATENTS

1. **Dr. Poonam Salotra** was awarded J.C. Bose National Fellowship- 2017.

2. **Dr. Poonam Salotra** was awarded Drs. Kunti & Om Prakash Oration Awarded by ICMR.

3. **Dr Nasreen Ehtesham** was awarded **Kshanika Oration** award by ICMR**.**

4. **Dr. Poonam Salotra** was appointed Member of RTAG (Regional Technical Advisory Group), SEARO, WHO, 2017-2019.

5. **Dr. Poonam Salotra** was appointed member of the WHO Advisory Panel on Parasitic Diseases, 2017 to 2018.

PATENTS

1. Patent granted: Genetic Markers for Diagnosis of Tuberculosis caused by Mycobacterium tuberculosis Inventors: Hasnain SE, Rahman Syed Asad, Thornton Janet M, Ahmad J, Ehtesham NZ
   Europeon Patent application no. PCT/IB2014/066469/ 2015.

2. Patent granted: LK Yerneni and Ashok Kumar. A culture system for the growth of stem cells. Indian Patent File No. 2086/DEL/2009, Dt. 13/08/2010, Issue 33/2010, p 24092; Grant No. 274456, Dt. 26th July 2016; Post-grant Publication Dt. 29/07/2016.

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