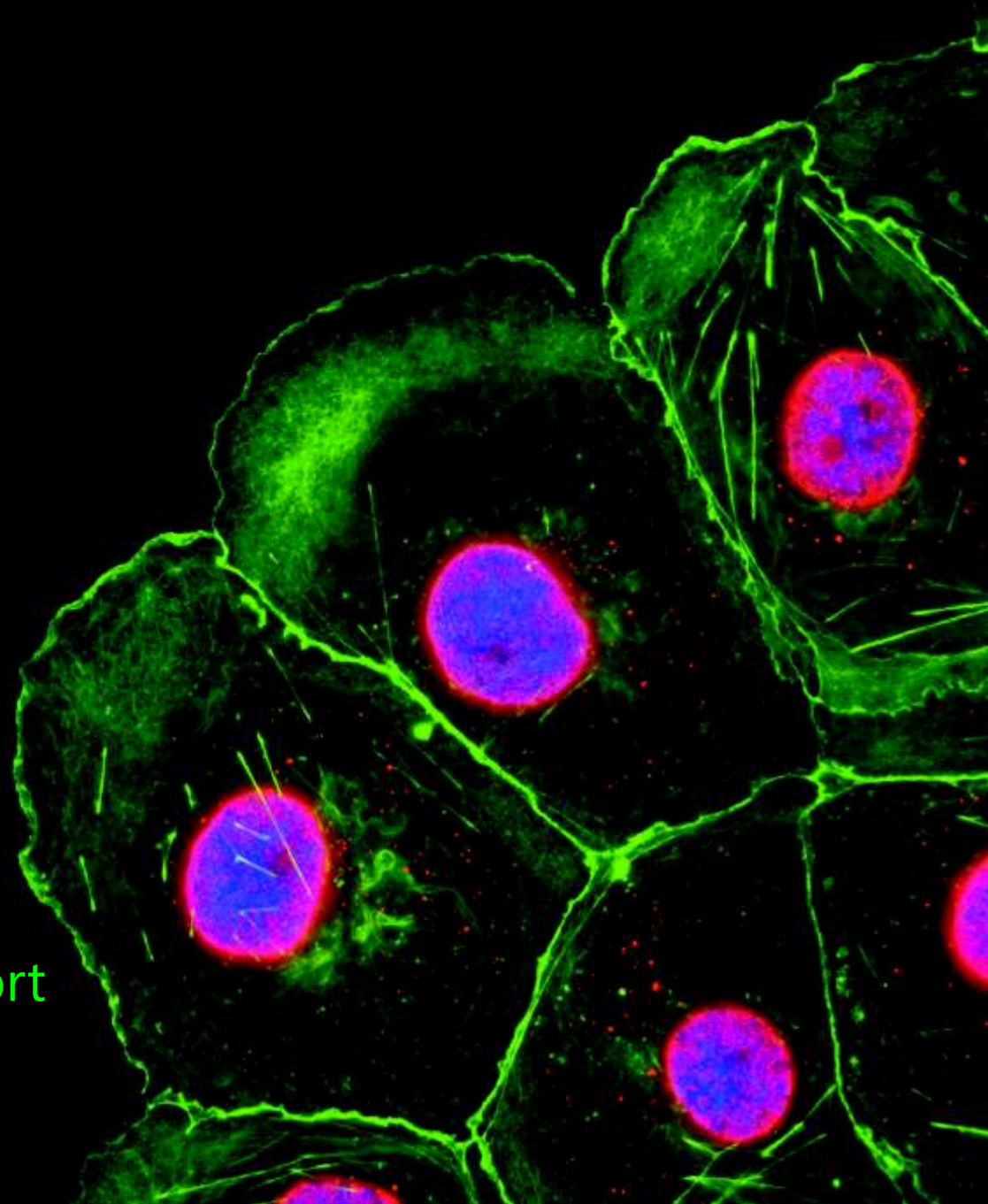
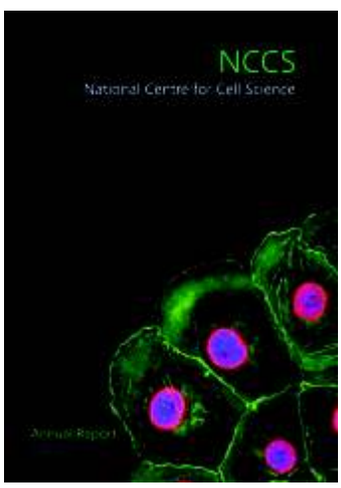
NCCS

National Centre for Cell Science



Annual Report

2012-2013



Cover page image

COS-7 cells immunostained for endogenous Nup358 (red) and F-actin (green).
DNA is stained with Hoechst dye (blue).
(Image courtesy: Dr. Jomon Joseph)

National Centre for Cell Science

Annual Report 2012-2013

Contents

Index of Research Reports

Mandate of NCCS

- To receive, identify, maintain, store, grow and supply:
  - Animal and human cell cultures.
  - Newly developed and existing (typed) cell lines.
  - Hybrid cells including hybridomas.
  - Tissues, organs, eggs (including fertilized ones) and embryos.
  - Unicellular, obligate pathogens, parasites and vectors.
  - Plasmids, genes and genomic libraries.

- To develop, prepare quality control and supply culture media, other reagents and cell products independently and in collaboration with industry and other organizations.

- Research and development.

- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

- To serve as a National Reference Centre for tissue culture, tissue banking and cell products, data bank etc., and to provide consultancy services to medical, veterinary and pharmaceutical institutions, public health services and industries etc. in the country.

- To provide and promote effective linkages on a continuous basis between various scientific and research agencies/laboratories and other organizations, including industries within the country.

- To participate in programs conducted for the betterment of society and advancement of science and technology in the country.

- To collaborate with foreign research institutions, laboratories and other international organizations in the areas relevant to the objectives of the facility.

From the Director's Desk

It gives me immense pleasure to present the Annual Report of the National Centre for Cell Science (NCCS), Pune, for 2012-13, this being the 25th year of NCCS. This special year was commemorated with various activities, such as Silver Jubilee orations and other talks by eminent researchers and academicians including the Nobel laureate, Prof. Jules Hoffmann, and different competitions like students' debate, essay-writing, photography and T-shirt design contests. We are proud to have reached this important landmark in the history of NCCS by shining like a beacon of high standards of research, service and capacity-building over 25 years.

The national cell repository of NCCS, one of its kind in India, has been providing valuable service and support to many research institutions, universities and industries all over India. During this year, NCCS continued this tradition of commitment to service-related activities by providing 3290 cell lines to 135 organizations in the country.

In recognition of the fact that progress can only be made by encouraging young scientific talent and curiosity, NCCS admitted twenty-four research scholars into its Ph.D. programme, under the mentorship of various faculty members, bringing the total number of scholars to 124. This pool was strengthened with registration of twenty five scholars as Ph.D. students under the University of Pune. Furthermore, twenty-one project trainees and fourteen summer trainees also received guidance and training through various programmes run at NCCS throughout the year.

NCCS continues to pursue research towards resolving human health issues. In one such study, scientists at NCCS addressed the issue of non-healing diabetic ulcers arising due to dysfunctional endothelial progenitor cells (EPCs) present in the diabetic patients' marrow, which poses a major clinical challenge in the management of diabetes. They developed a polycaprolactone-gelatin (PCG)-based direct "growth-cum-delivery" system for the application of normal EPCs

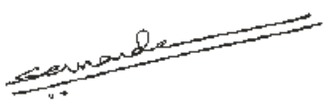
on diabetic wounds. In vivo studies carried out by this research group clearly showed that application of this 'biological dressing' results in an accelerated and fibrosis-free wound healing in diabetic mice. A patent has been filed for this system, in collaboration with IITB, Mumbai. Other studies carried out by this group revealed that an oral administration of simvastatin, a drug used to control cholesterol levels, affords radio-protection to hematopoietic stem cell (HSC) niche cells and also reduces radiation-induced adipogenesis in the marrow compartment, leading to a significant increase in the efficacy of stem cell transplantation.

In a quest to identify novel therapeutic strategies against HIV-1, NCCS has initiated studies to gain a deeper understanding of the interactions between the viral proteins Nef and Tat, with the host cell. Studies with Nef protein have unraveled the critical role of cellular heat shock proteins, specifically HSP40 and HSP70, in the viral replication cycle. In addition, the research group carrying out these studies has shown the importance of HSF-1 in viral replication, which seems to be a positive regulator of the virus, thus making it a potential target for inhibiting the virus. Furthermore, genome-wide recruitment analysis by this group showed that the HIV-1 transactivator Tat protein also regulates cellular gene expression by modulating the activity of cellular promoters. A search for novel anti-HIV molecules from natural products and synthetic sources, undertaken by the same group, has resulted in the identification of a few novel lead molecules with potential for use as anti-HIV microbicides.

Recent studies have suggested that metabolic disorders like diabetes and obesity may alter the risk of developing a variety of cancers. In a first-of-its-kind study undertaken in India, scientists at NCCS compared the mortality from malignancies in diabetic and non-diabetic populations to understand the inter-relationship between diabetes and cancer. Their findings suggest that the interplay between diabetes and cancer in the Indian population is complex, providing impetus for a cohort study to be undertaken further, for an in depth understanding of this relationship.

A group of scientists at NCCS has taken the initiative for another study on similar lines, involving an investigation of the microbiome of the Indian population, using both culture-based as well as metagenomic approaches. These studies revealed that the microbial community composition of teenagers in India is distinctly different from that of the Finnish population. The Firmicutes/Bacteroides ratio was found to consistently decrease with age in the Indian families studied, which was different from the trend observed in the European population. In culture-dependent studies, as many as 27% of the isolates were found to be potentially novel taxa. Metabolic adaptations for survival in the gut environment were identified through comparative genomics of two of these isolates with their closest neighbors. Studying the correlation between gut flora and disease, this group found that the gut microbiota profile of patients suffering from celiac disease (CD) and their first degree relatives (FDR non-CD) is different, even though both are genetically susceptible to developing this disease. Following six months of gluten-free diet, the gut microbiota of patients suffering from celiac disease was seen to resemble that of FDR non-CD individuals. This emphasizes the need for further detailed studies on the gut microbiota in Indian populations, to decipher its contribution to health and disease.

As we proudly cross the Silver Jubilee milestone, we at NCCS resolve to further expand our excellence in innovative basic and translational research in diverse areas of modern cell biology, especially those addressing paramount human health issues. I am confident that NCCS will enjoy many more years of success in unraveling the mysteries of the cell and promoting science for the benefit of society, through the concerted efforts and dedication of the entire NCCS team.



Shekhar C. Mande
Director

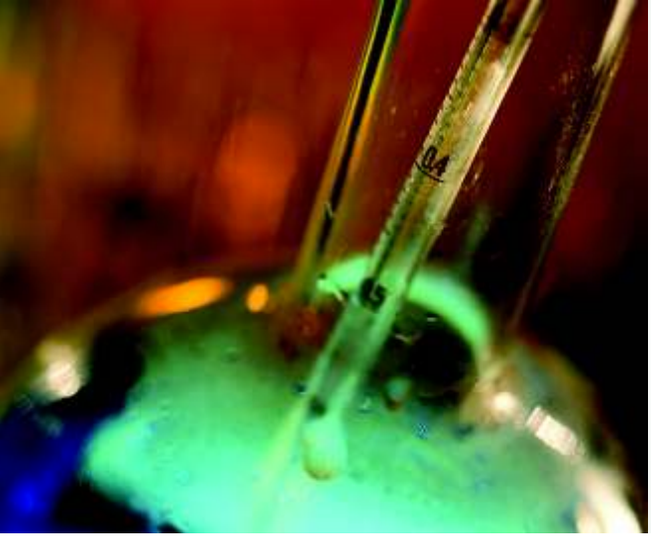
Human Resource Development

During the year 2012-13, twenty-four students joined NCCS for pursuing Ph.D. under the guidance of various faculty members. During this year, twenty five students were registered as Ph.D. students under the University. The total number of Ph. D students as on 31st March 2013 was 124. During this year, eighteen students submitted their theses to the University & twenty one were awarded Ph.D. degrees.

The project training programme is conducted twice in a year i.e. during January-June and July-December, while the summer training programme is conducted during the month of May every year. The number of students who received training under these programmes during the year 2012-13 was:

Project Training: 21

Summer Training: 14



Repository

NCCS serves as a national cell bank for animal cell lines. The repository manages the expansion, cryo-preservation and distribution of cell lines. In the year 2012-13, we have supplied 3290 cell lines to 135 research institutions in the country.

Technical Officers

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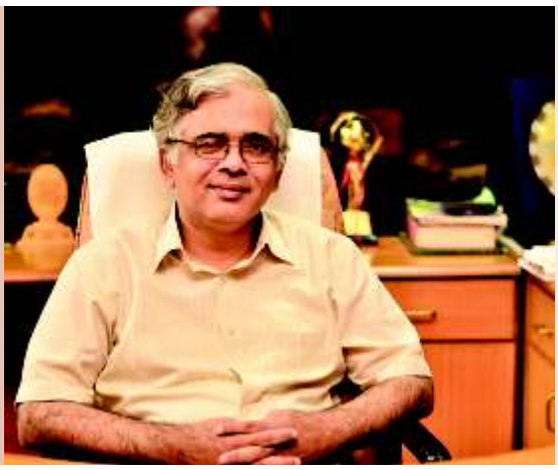
Research Report

Bioinformatics, Proteomics & Structural Biology



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Structure-function properties of *M. tuberculosis* proteins

Background

Our laboratory has been involved in two broad areas of work, namely, (1) determining and analyzing protein structures derived from *Mycobacterium tuberculosis* and (2) developing novel computational methodologies in the analysis of large and desparate data. In the former, our focus has been on heat shock proteins of *M. tuberculosis* and those involved in redox processes. In the latter, we are attempting to develop computational methods to understand the dynamics and evolution of protein interaction networks.

Aims and Objectives

1. To identify important proteins of *M. tuberculosis* involved in redox reactions and in heat shock response. Purify these recombinant proteins, as well as relevant protein complexes and undertake structure-function analyses on them.
2. Analyze large scale data arising from studies such as gene expression, deep sequencing, proteomics, available in literature to find common patterns, if any.
3. Evolve methodologies for analysis of protein: protein interactions, especially the dynamics of changes in these interactions and their relevance on biological processes.

Work Achieved

Crystal structure of *NrdH*:

Ribunucleotide reductases (RNRs) catalyze conversion of ribonucleotides to the corresponding deoxyribonucleotides, required for DNA synthesis. RNRs are thus essential enzymes in DNA replication and repair. Among the three main classes of RNRs in all known organisms (class I, class II and class III), which differ in their subunit composition, co-factor use and oxygen requirements, *M. tuberculosis* genome harbors one copy of the Class Ib RNR. These are encoded by the *nrdE*, *nrdF*, *nrdH* and *nrdI* genes. We have undertaken work to characterize all of these proteins structurally and biochemically. We propose to determine structures of these enzymes, in isolation and in complex forms.

Participants

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Ashwani Kumar, *JRF*
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In the period under review, we have determined the structure of the NrdH protein that is involved in the reaction chain of RNRs. *M. tuberculosis* NrdH protein (encoded by the Rv3053c open reading frame) is a 79 residue long thioredoxin like protein with a glutaredoxin like sequence. At the end of each cycle of ribonucleotide reduction, RNR needs to be reduced in order to be primed for the next cycle of reduction. An external co-factor in the form of glutaredoxin/thioredoxin usually performs this step for the Class1a RNR. The oxidized glutaredoxin/thioredoxin is then reduced by the glutaredoxin/thioredoxin reductase which can further be reduced by NADPH. In the case of a class1b RNR this co-factor is a protein called NrdH with a glutaredoxin like sequence. Interestingly, NrdH even though appears to be glutaredoxin like, behaves like a thioredoxin and can accept electrons from thioredoxin reductase. Thus, NrdH in *M.tuberculosis* is speculated to supply electrons required for the biochemical reaction of ribonucleotide reduction.

The crystal structure of NrdH from *M. tuberculosis* was solved and refined to crystallographic R-factor of 12.8% (Rfree 14.6%) at 0.87Å resolution. The 3D structure is similar to the structures of other NrdH proteins from *E.coli* and Cornybacterium, and has a typical thioredoxin fold. Structural superimposition of *M. tuberculosis* NrdH over Thioredoxin A (TrxA) and *M. tuberculosis* Thioredoxin reductase (TrxR) over the already solved structure of *E.coli* TrxR - TrxA complex explains the ability of *M. tuberculosis* NrdH to accept electrons from *M. tuberculosis* TrxR. This also raises an important question as to why Glutaredoxins are unable to do so. Statistical coupling analysis of NrdH, Glutaredoxins and Thioredoxins reveals different sets of coevolving contiguous clusters of amino acid residues which might probably explain the differences in the biochemical properties of these structurally similar yet functionally distinct sub classes of proteins. Similarly, the conserved sequence motifs in the neighbourhood of Class Ib NrdH proteins also explain their role in maintaining integrity of the NrdH structure (Fig. 1).

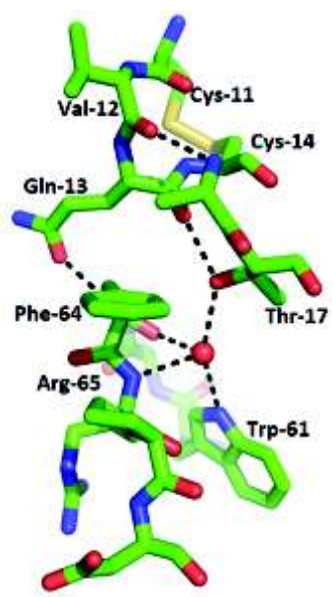

Fig. 1: View of the Active site area of MtNrdH. The active site of NrdH contains the canonical CXXC sequence motif that is frequently found in thioredoxin-like proteins. NrdH sequences are characterized by a WSGFRP motif. This image shows both the two conserved motifs, i.e. CVQC and WSGFRP, along with a conserved water molecule. The network of hydrogen bonds stabilizes the redox active site of MtNrdH, and also presents a distinct hydrophobic patch for its interaction with substrates.

Functional studies on *M. tuberculosis* GroEL1:

The chaperonin GroEL is known to assemble into the essential tetradecameric architecture with two heptameric isologus rings each enclosing a cavity for sequestering non-native substrate proteins. However, recombinant *Mycobacterium tuberculosis* GroELs, GroEL1 and GroEL2, exist as dimers and thus fail as molecular chaperones *in vitro* and *in vivo*, when expressed in *Escherichia coli*. We had earlier reported that GroEL1 exists in multiple oligomeric forms, including single-ring and double-ring variants and the switch between these forms is mediated by Seryl phosphorylation. One of the Serine residues that we had identified employing Mass Spectrometry was Ser393. Multiple sequence alignment of GroEL homologues identified five residues that map to inter-subunit space have been altered from different residues to serine in the *M. tuberculosis* GroELs, indicating potential phosphorylation sites. During the period under review, complementation studies of the corresponding phosphomimetic GroEL1 derivatives in *E. coligroEL* conditional mutant strain showed partial restoration of GroEL activity with S393D mutant and significantly enhanced activity with a double mutant S393D + S75D, respectively (Fig. 2). Complementation was carried out by cloning the *groEL1* gene and its mutants in the pBAD24 vector in the *E. coli* strain LG6.

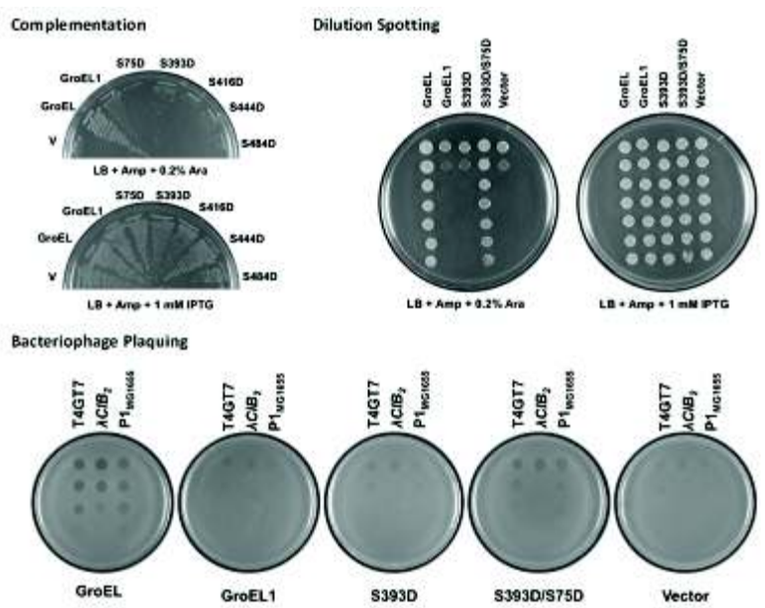

Fig. 2: *Surface phosphomimetics restore chaperone activity in GroEL1*. A. *E. coli* LG6 cultures expressing the indicated *groEL* variants were streaked on the LB agar plates supplemented as indicated and the plates were incubated at 30°C. B. Serially diluted cultures of *E. coli* LG6 expressing the indicated *groEL* genes were spotted onto the surface of LB agar plates supplemented as indicated, and the plates were incubated 30°C. C. Lawns of *E. coli* LG6 expressing the indicated *groEL* genes were prepared on LB agar plates supplemented with 0.2% L-arabinose. Serial 100-fold dilutions of the indicated bacteriophages were spotted onto the lawns, followed by incubation at 30°C.

Future Work

1. To clone, express, purify and crystallize RNR subunits. To generate relevant protein:protein complexes of NrdH and other components of ribonucleotide reductase assembly.
2. To analyze the functional properties of GroEL1 protein based on microarray and other biochemical studies.

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Identification and characterization of novel potential biomarkers for breast cancer using gel-based 2-D DIGE & LC-based iTRAQ-LC-MS/MS proteomic approaches and bioinformatic tools

Background

In women, breast cancer is the most common malignancy and the second most common cause of cancer-related mortality. Three subtypes of breast cancer are identified ER and PR positive, HER2 positive and triple negative. As yet very few drugs are available for breast cancer, which remains a major cause of morbidity and mortality in women. Early diagnosis of breast cancer improves the likelihood of successful treatment and can save many lives. But, it requires successful strategies for early detection and screening of the disease. However, current techniques like mammography to detect breast tumor has intrinsic limitations. Thus early diagnostic biomarkers are critically important for detection, diagnosis, and monitoring disease progression in breast cancer. There is an urgent need to discover novel biomarkers of breast cancer for early detection and diagnosis. Quantitative proteomic profiling of body fluids, tissues, or other biological samples to identify differentially expressed proteins represents a very promising approach for discovering novel potential biomarkers. Proteins associated with breast cancer identified through proteomic profiling technologies could be useful as biomarkers for the early diagnosis, assessment of prognosis, prediction of therapeutic effect and treatment monitoring.

Mass spectrometry (MS) based proteomic technologies have experienced major improvements in recent years for simultaneous analysis of thousands of proteins on the basis of differences in their expression levels and characterization of post-translational modifications involved in cancer progression. The most commonly used proteomic approach is a combination of either two-dimensional electrophoresis (2-DE) or liquid chromatography (LC) to separate proteins/peptides and MS to identify and characterize them. Such rapidly developing technology enables us to discover novel cancer biomarkers to a greater extent. Such biomarkers may have broad applications, such as early indication of disease, monitoring of disease progression, and monitoring of drug targets.

Participants

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Snigdha Dhali, *Technician*

Collaborator

Dr. Sanjeeva Srivastava, *IIT Bombay*
Dr. Manas Santra, *NCCS Pune*

Already, several putative breast cancer biomarkers with potential clinical applications have been reported using proteomic technology. Estrogen receptor, progesterone receptor, Her-2/neu, CA125, CEA, CA15.3 are among reported biomarkers and have been widely accepted for routine clinical use. These biomarkers have been serving as prognostic and predictive markers for targeted therapy. However, their detection sensitivity, and specificity to predict metastasis potential is limited. With reported breast cancer biomarkers, much is left to be desired in terms of clinical applicability. We need novel breast cancer biomarkers that will further enhance our ability to diagnose, prognoses, and predict therapeutic response. Because biomarkers can be analyzed relatively noninvasive methods and are economical as compared with other expensive techniques, hence it is worth to investigate novel biomarkers. In this work, we plan to identify novel potential biomarkers for breast cancer using high throughput mass spectrometry based proteomic approaches in Indian scenario. In addition, identified biomarkers will be subjected to bioinformatic tools to understand the various physiological pathways.

Aims and Objectives

1. Identifying novel potential biomarkers for breast cancer using gel-based 2-D DIGE and gel-free iTRAQ LC-MS/MS proteomic approaches
2. Understanding the various physiological pathways and disease pathogenesis of the identified novel potential biomarkers using bioinformatic tools

Work Achieved

Comparison of serum proteome profile of breast cancer and healthy subjects using 2-D DIGE

The principal aim of this study was to perform a comparative serum proteome analysis of breast cancer patients and healthy subjects to identify the differential expression pattern of serum proteins in breast cancer. Firstly, we developed a 2-DE method to obtain maximum coverage of the serum proteome by removing the highly abundant proteins. This optimized protocol was used to process the patient and healthy control serum samples for further 2-D DIGE proteomic analysis. A comprehensive serum proteome analysis was performed on 24 samples (8 malignant, 8 benign and 8 control) using 2-D DIGE. Individual serum samples from the two groups (malignant vs control as one group and benign vs control as second group) were labeled with Cy3 and Cy5 and pooled internal standard containing the equal amounts of all the samples in the experiment was labeled with Cy2. To eliminate bias resulted from dye labeling (either Cy3 or Cy5), the dye swapping approach (alternative labeling of test and control with Cy3 and Cy5 in different experimental sets) was used during the labeling of the protein samples. Three images corresponding to three samples labeled with different CyDyes were generated from each gel, and a single merged image was obtained combining the three (Fig. 1). Around 900 protein spots were detected on each 2D-DIGE gels when analyzed using DeCyder 2D software. Pair-wise comparison of each control and maliganant/benign samples to the mixed internal standard present in each gel was performed using the DIA module of the software. Files generated in DIA were further analyzed in Biological Variation Analysis

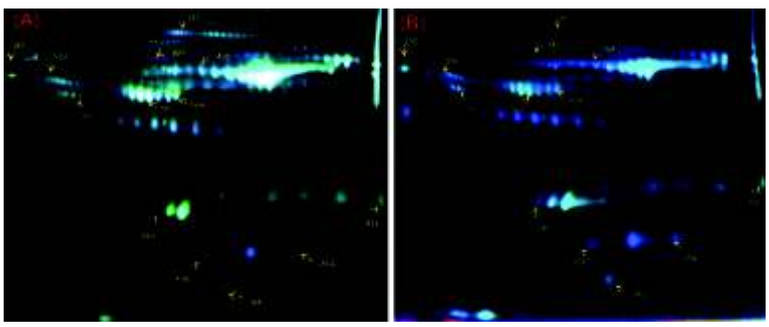

Fig. 1 : Representative 2D-DIGE images comparing the serum proteome of healthy subjects and breast cancer patients. (A) 2-D DIGE image of healthy control vs malignant, (B) 2-D DIGE image of healthy control vs benign.

(BVA) module to obtain the differential protein expression between the two experimental groups across all the gels. 305 protein spots were found to be significantly up-regulated and 316 proteins down-regulated in the case of malignant and 261 proteins were up-regulated and 307 proteins were down-regulated in the case of benign.

Comparison of breast cancer tissue proteome and its control tissue proteome using 2-DIGE

In this study, we evaluated different protein extraction protocols from published papers to obtain maximum coverage of the breast tissue proteome. A combination of T-PER buffer extraction, Trizol method and desalting offered reproducible separation of over 1000 spots.This optimized protocol was used to process the breast cancer malignant, benign and normal tissue samples for 2-DE and 2-D DIGE. Proteome analysis was performed on 24 samples (8 malignant, 8 benign and 8 control) using 2-D DIGE as mentioned in the earlier section (Fig. 2). Around 1500 protein spots were detected on each 2D-DIGE gels when analyzed using DeCyder 2D software. Pair-wise comparison of each control and maliganant/benign samples to the mixed internal standard present in each gel was performed using the DIA module of the software. Files generated in DIA were further analyzed in Biological Variation Analysis (BVA) module to obtain the differential protein expression between the two experimental groups across all the gels. 607 protein spots were found to be significantly up-regulated and 576 proteins down-regulated in the case of malignant and 568 proteins were up-regulated and 567 proteins were down-regulated in the case of benign. Differentially expressed and statistically significant (Student's t-test; $p<0.05$) protein spots were identified by MALDI-TOF/TOF mass spectrometry.

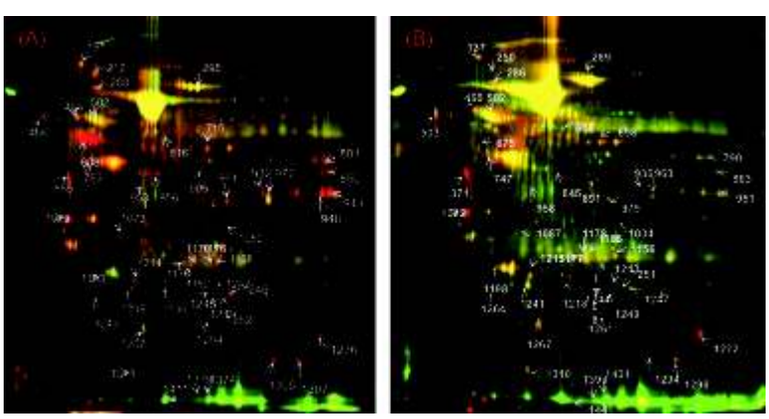

Fig. 2: Representative 2D-DIGE images comparing the tissue proteome of healthy subjects and breast cancer patients. (A) 2-D DIGE image of healthy control vs malignant, (B) 2-D DIGE image of healthy control vs benign.

Comparison of breast cancer tissue proteome and its control tissue using LC-based iTRAQ-LC-MS/MS proteomic approach

We also subjected same tissue samples labeled with isobaric mass tags for relative and absolute quantitation (iTRAQ) to identify proteins that are differentially expressed in breast cancer in relation to non-cancerous breast tissues (controls) for novel potential biomarker discovery. The proteome was extracted from tissue samples by optimized T-PER Trizol method, and desalted by Millipore Amicon 3 KD MWCO tubes. The pellet was reconstituted in 0.5 M TEAB. The protein was quantified using 2D-quant kit, and 100ug of each sample was taken in duplicates. Digestion of the protein sample was performed according to manufacturer's protocol and were labeled with 4-plex iTRAQ reagents. The samples were pooled after labeling and were fractionated using SCX-HPLC. Fractions were collected and subjected to separation by nano LC and were spotted onto the MALDI plate. The sample spots were subjected to MALDI-TOF/TOF and the data was analyzed using Protein Pilotsoftware. 187 protein spots were found to be significantly up-regulated and 38 proteins down-regulated in the case of malignant and 136 proteins were up-regulated and 24 proteins were down-regulated in the case of benign. We identified non-redundant differentially expressed proteins including structural proteins, transcription factors, receptors, signaling components, enzymes, and chaperones. A panel of proteins showing consistent differential expression in breast cancer relative to the non-cancerous controls was discovered. Some of the proteins include protein disulphide isomerase, calreticulin, tropomyosin alpha-3 chain, heat shock protein 90, tubulin beta chain, 78kDa glucose-regulated protein, heat shock 70 kDa protein, alpha keratin 14, collagen alpha-1 chain, vimentin, annexin A2, immunoglobulin heavy chain, were over expressed in breast cancer. Similarly, fatty acid-binding protein, membrane primary amine oxidase, polymerase I and transcript release factor were under expressed in Breast cancer. A representative spectra of down-regulated and up-regulated peptides are shown in Fig 3. The work is under progress.

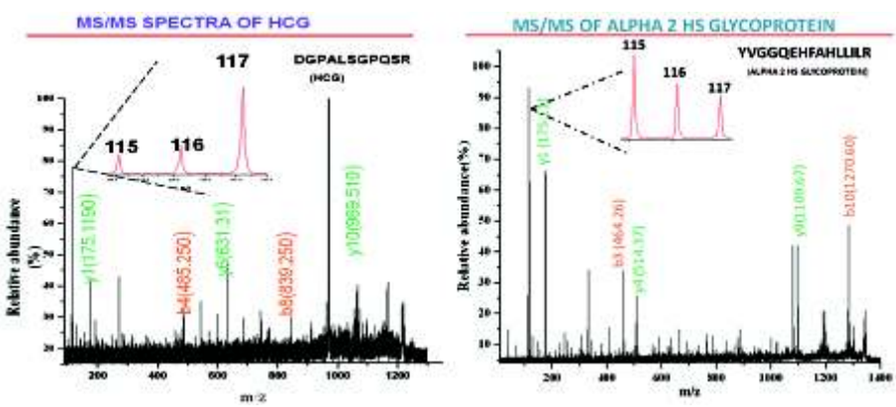

Fig. 3 : Representative spectra of up-regulated and down-regulated peptide / protein analyzed by iTRAQ LC-MALDI-MS/MS. The inset shows a zoom into the m/z region of the MS/MS spectrum where reporter ions appear.

Future work

1. Validating identified differentially expressed proteins using western blotting and MRM based LC-MS/MS.
2. Identification and characterization of phospho proteins involving in breast cancer.
3. Identifying metabolites involving in breast cancer using mass spectrometry.

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Molecular Simulation to Biochemical Network Perturbation in Infectious Disease: Stability and Stochasticity in Synthetic Circuit

Background

Systems biology provides a deeper understanding of the complex host pathogen interactions providing an insight into the initiation and progression of the infectious disease. Parasites like Leishmania modulate a wide variety of host protective responses to facilitate their survival inside the host. The pathogenicity of such parasites is often associated with a wide variety of molecules and hence it becomes essential to explore, develop and invent new strategies to reduce the parasite burden inside the host. IPC (Inositol phosphorylceramide) forms the major sphingolipid in several parasites like *'Leishmania'*. IPC is generated by the transfer of a phosphoryl inositol head group from PI to ceramide, a process catalyzed by the Inositol phosphorylceramide synthase (IPCS). IPC plays a key role in maintaining the infectivity and virulence of *Leishmania.* A systems level understanding of the parasite sphingolipid metabolism has already shown the relative importance of IPCS considering it as a choke point enzyme in the pathway. (Mandlik et al, 2012) IPCS therefore serves as a druggable target for the treatment of *Leishmania.*

Synthetic devices such as toggle switch and repressilators can modulate the expression of several genes within a system under study. Such devices can be created by the assembly of standard biological parts which can be used to create more and more complex devices and novel biological systems. Development of a bistable genetic circuit has been reported for the IPCS protein in *Leishmania* where the circuit exhibited bistable oscillatory behaviour along with robustness to the introduced perturbations. (Mandlik et al, 2012). Towards this end we now report the construction of a tristable genetic circuit for the IPCS protein in *L.major*. Site specific delivery of this circuit into the parasite using suitable drug delivery systems is expected to alter the sphingolipid metabolism of the parasite thereby reducing its infectivity as a whole.

Participants

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PruthviRaj Bejugam, *JRF*
Sonali Shinde, *Project JRF*
Sneha Maria, *Project Assistants*
Mayur Tanna, *Project Assistants*
Shruti Sharma, *Project Assistants*
Amruta Munje, *Project Assistants*
Virashree Jamdar, *Technician*

Collaborator

Dr. M S Patole

Aims and Objectives

1. Design of an tristable synthetic device for regulating the concentration of the IPCS protein
2. Quantitative and qualitative analysis of the designed circuit, understanding the behavioural response of the circuit with and without the addition of an inducer molecule
3. Possible regulation of the sphingolipid metabolism of the parasite by delivery of the synthetic circuit using appropriate drug delivery systems

Work Achieved

1. Construction of the tristable genetic switch

The construction of synthetic devices is based on the principle of modularization and incorporation of standardized biological parts whose connectivity and cooperativity decide the end behaviour of the designed network. Construction of such genetic devices would lead to the development of new therapeutic regimens for the treatment of *Leishmaniasis*. The tristable genetic circuit designed for the IPCS protein includes a toggle switch coupled to a repressilator. (Fig 1a) The toggle switch comprises of three mutually repressible genes (SLS1, AUR1 and SLS4). Sustained oscillations are introduced into the genetic circuit by coupling a repressilator with the toggle switch. The modules in the repressilator are arranged in a manner such that the three genes (LacI, TetR and LamdaR) exhibit cyclic repressibility. Coupling of the toggle switch with the repressilator is achieved by introducing a transcriptional repression reaction between the first gene of the repressilator (LacI) and the toggle switch. Autoactivation plays an important role in maintaining the tristability of the circuit. The tristable genetic switch is a Brusselator model in which one species (SLS4) in the model serves as an auto-activator in the circuit. To understand the switching behaviour of the circuit between the steady states, simulation is performed. Parameters like concentration of genes, proteins and promoters; their

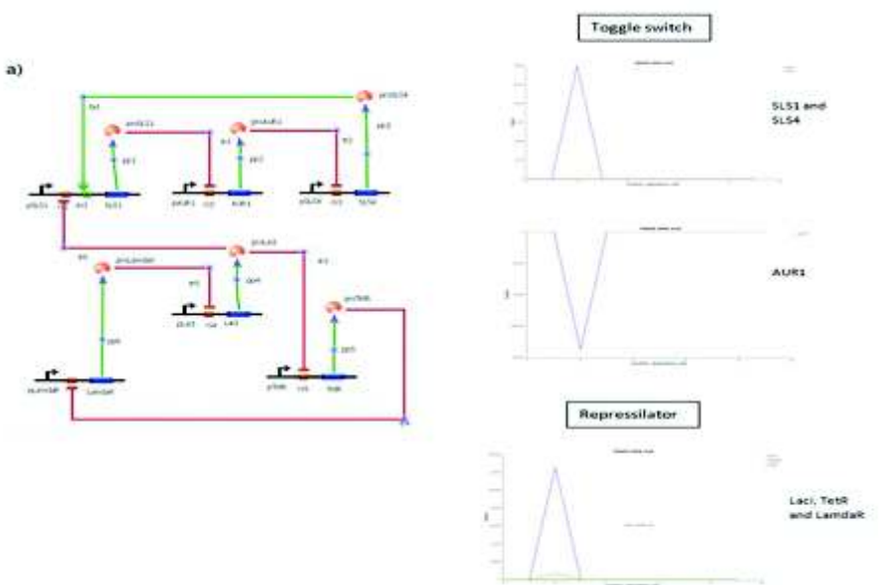


Fig. 1a: Simulation results for tristable genetic circuit without inducer

transcription and translation reaction rates; protein degradation rates; Hill's coefficient and dissociation constants form a set of random variables in the circuit which inturn regulate the behavior of the circuit. The complex interactions between various modules in the circuit account for the stochastic nature of the synthetic network. The sudden rise or fall in the protein levels as a consequence of 'transcriptional bursting' or 'transcriptional pulsing' phenomena can be associated with the switching behavior in the circuit.

Addition of small molecules such as the chemical inducer (IPTG) produces a transient chemical input driving the expression of inducible promoters in the circuit. This is expected to reduce the repressive effect of the repressilator over the toggle switch. (Fig 1b) It was observed that relative concentration of AUR1 protein and LacI protein determine the switch behaviour in the circuit. Prior to the addition of an inducer, the repressilator was under the control of the toggle switch. Addition of an inducer molecule generated a switching behaviour in the circuit.The repressilator now controlled the toggle switch and gave rise to robust tunable oscillations in the circuit. Higher degradation rates of AUR1 lead to an increase in the concentration of SLS1 and SLS4. SLS4 (SMSase) would

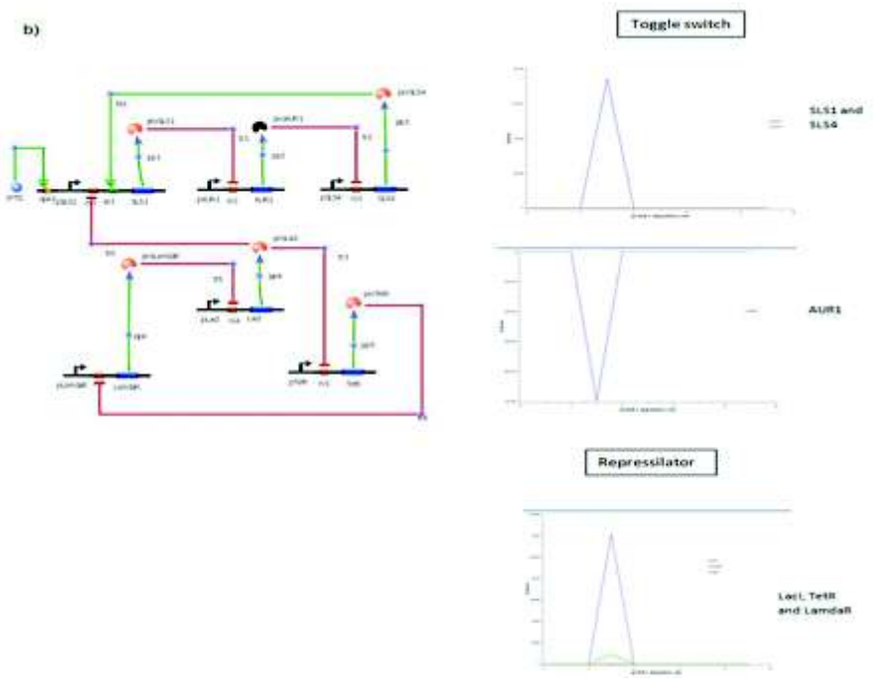


Fig. 1b: Simulation results for tristable genetic circuit with inducer

repress the production of IPC and also serve as an autoactivator in the circuit. Addition of an inducer molecule was thus found to improve the overall functioning of the circuit.

2. Validation of the designed circuit

Synthetic circuits are highly dynamic in nature as they incorporate a number of different modules within them. To understand the behaviour of the system, it is necessary to understand the multistate attractor dynamics of the system. The state dependent expression pattern of components of the circuit, their connectivity over a period of time needs to be estimated. The network has to be validated both quantitatively and

qualitatively to further understand the network dynamics. The designed circuit was validated using the R statistical package to study the network connectivity and map the transitions between the attractor states. Linear network construction with and without the addition of an inducer indicated that AUR1 and LacI proteins appear to be the major regulators in the circuit. Posterior probability analysis highlighted the fact that AUR1 appears to be connected with LacI alone. TetR on the other hand shows connectivity with both SLS1 and SLS4. Upon addition of an inducer, the connectivity of repressilator (LacI and TetR) with SLS1 and AUR1 of the toggle switch is decreased while the connectivity of the repressilator with SLS4 appears to increase. Thus the addition of a chemical inducer appears the switch the circuit functioning towards increasing the concentration of SLS4 as compared to AUR1 and SLS1.

The time course evolution of the system is represented by the switching 'ON' and 'OFF' states of genes in the attractor states as shown in Fig 2a and 2b. The system as a whole had 32 attractor states each with 2 basins of attractors. Robustness analysis indicated that in both the systems (with and without inducer) 60% of the original attractors remained unperturbed to external perturbations.

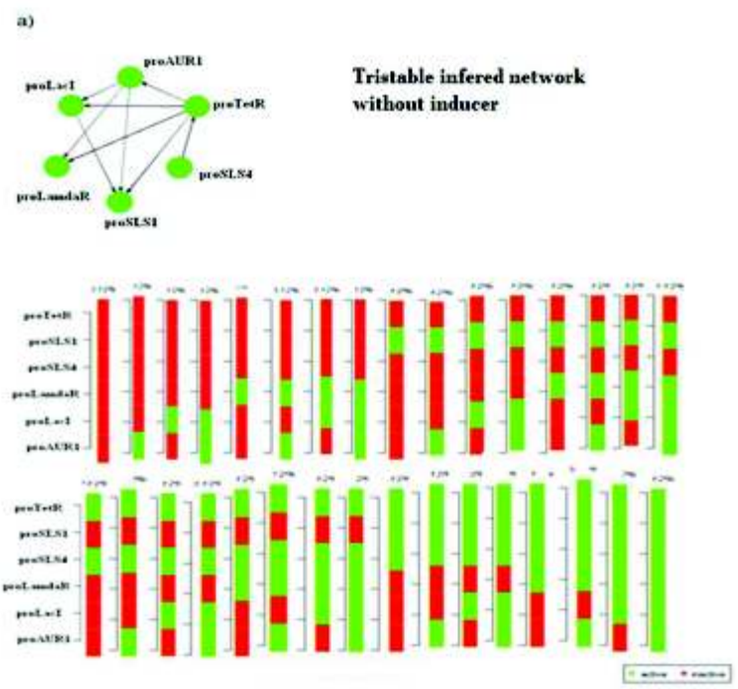
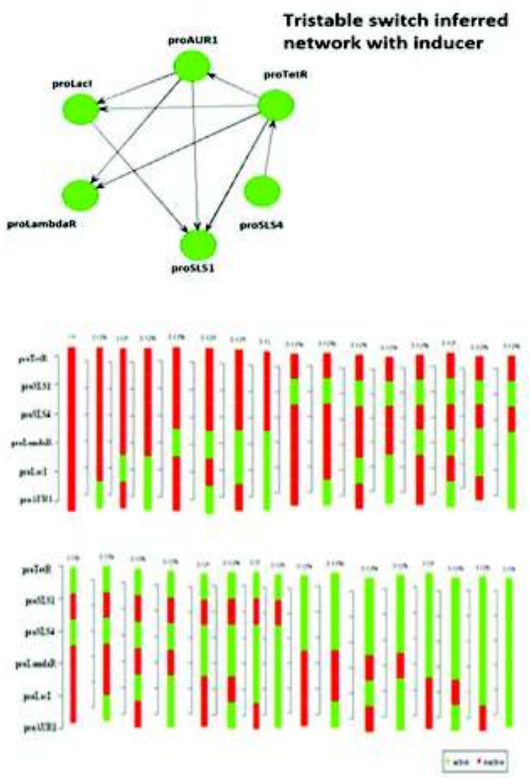


Fig. 2a: Network inference and attractor states for tristable circuit without inducer [Active (ON) state of a gene is represented by green color while the inactive state (OFF) state is represented by red color]

3. Time course evolution of the system

The synthetic circuit is highly dynamic in nature and the network dynamics are often richer than the dynamics of individual modules. The parameters of the circuit determine the qualitative behavior of the system. The properties of the system are described in a deterministic framework using ordinary differential equations (ODEs) that describe the time-evolution of each of the species included in the circuit. The ODE's are solved and

Fig. 2b: Network inference and attractor states for tristable circuit with inducer [Active (ON) state of a gene is represented by green color while the inactive state (OFF) state is represented by red color]



eigenvalues obtained are represented in the form of a jacobian matrix. Nullclines within the system are the solution curves for which all of the differential equations are equal to zero i.e. the points at which d(SLS4)dt=0 and d(AUR1)/dt=0. Three nullclines were obtained for the tristable circuit. A total of 3 steady states along with 2 unstable states were obtained in the phase plane diagram. The intersection of nullclines represents the saddle point. At the saddle point, hopf bifurcation was seen which arises when the limit cycle surrounding the equilibrium point (comprising of a pair of complex eigenvalues), arises or goes away as the parameters (SLS4 and AUR1) are varied. The intersection of the three nullclines at the saddle point was much sharper after the addition of an inducer as compared to that without the inducer where the intersection of only two nullclines was seen. (Figure 3a and 3b)

For nonlinear dynamical systems with multistate attractor dynamics, the probability and direction of transitions between the attractor towards a metastable attractor in a phase space can be studied in a quasi potential landscape. Quasi potential in a system relates to the force required for crossing the transition barriers during state transitions between stable attractor states. The ODE are ranked in order of their best (lower) fitness to worst (higher fitness) and the quasi potential is then determined. Lower the quasi potential, the higher would be the probability of that state to be occupied as a system transits from one state to another. The transitions towards the metabstable attractor state were much smoother after addition of an inducer into the circuit. Attractor states with a higher concentration of SLS4 and a lower concentration of AUR1 were favoured. At the saddle

Fig. 3a: Phase plane diagram showing the nullclines for tristable circuit without inducer

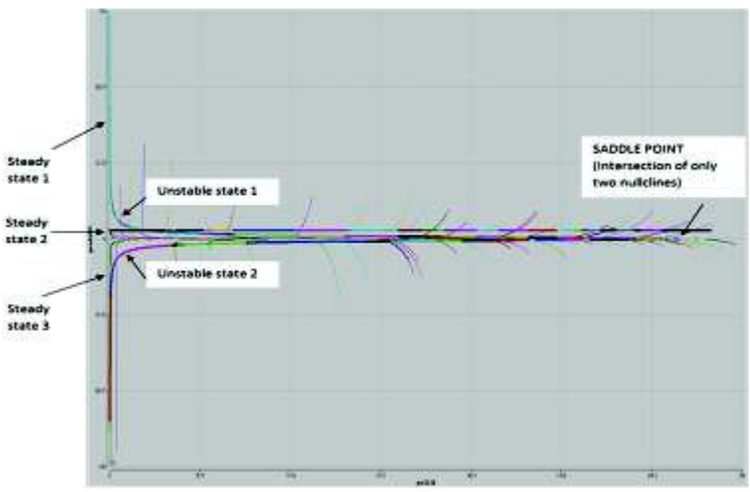
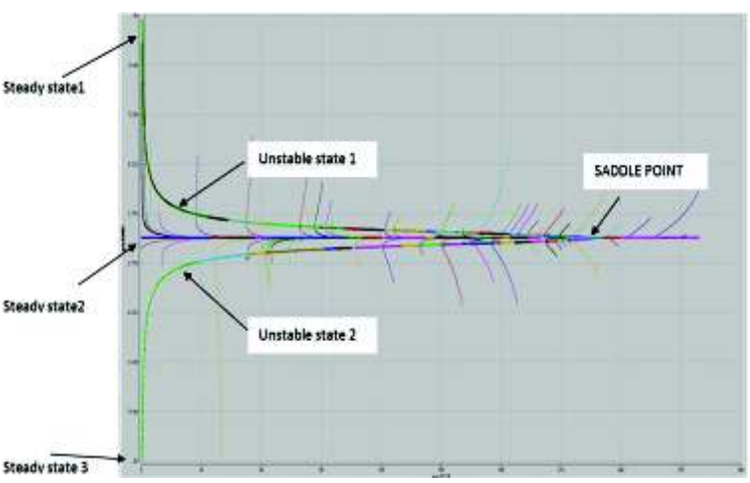


Fig. 3b: Phase plane diagram showing the nullclines for tristable circuit with inducer

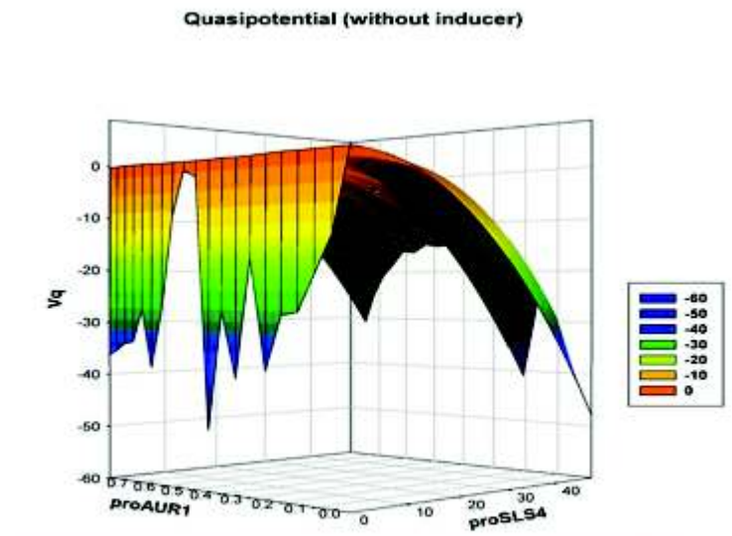


point, the system relaxed to its attractor state much faster upon addition of an inducer. (Figure 3c,3d) As the existence of multiple stable states is a common feature of synthetic circuits, it is essential to not only understand the dynamics involved in the attractor state transitions but it is also proves essential to understand the stablility of the attractor state with respect to the mean residence time or the time span that a system stays in a particular attractor state. This would provide more insights into the gene expression of each of the modules in the circuit, the associated transcriptional delay, the rate of switching 'ON' and 'OFF' of the genes involved in the circuit.

Future Work

1. The analytical approximation made so far would be compared with the geometric mean residence time at which a particular attractor state stays. This would provide a deeper insight into the actual behavior of the circuit.
2. Construction of a series of plasmids *in vitro*, nanomotor design and delivery of these plasmids to understand the functioning of the synthetic circuits constructed.

Fig. 3c: 3D Quasipotential landscape of the tristable circuit without inducer

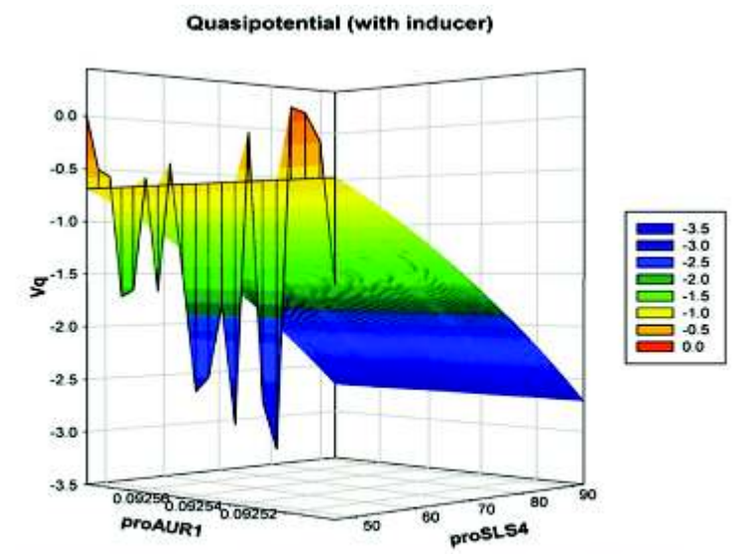


d(proSLS4)/dt = (pp3 translation rate * pSLS4 strength * [SLS4]) – (SLS4 degradation rate * [SLS4])

d(proAUR1)/dt = (pp2 translation rate * pAUR1 strength * [AUR1]) – (AUR1 degradation rate * [AUR1])

ΔVq = -(((d(proSLS4)/dt)^2)+((d(proAUR1)/dt)^2))* ΔT

Fig. 3d: 3D Quasipotential landscape of the tristable circuit with inducer

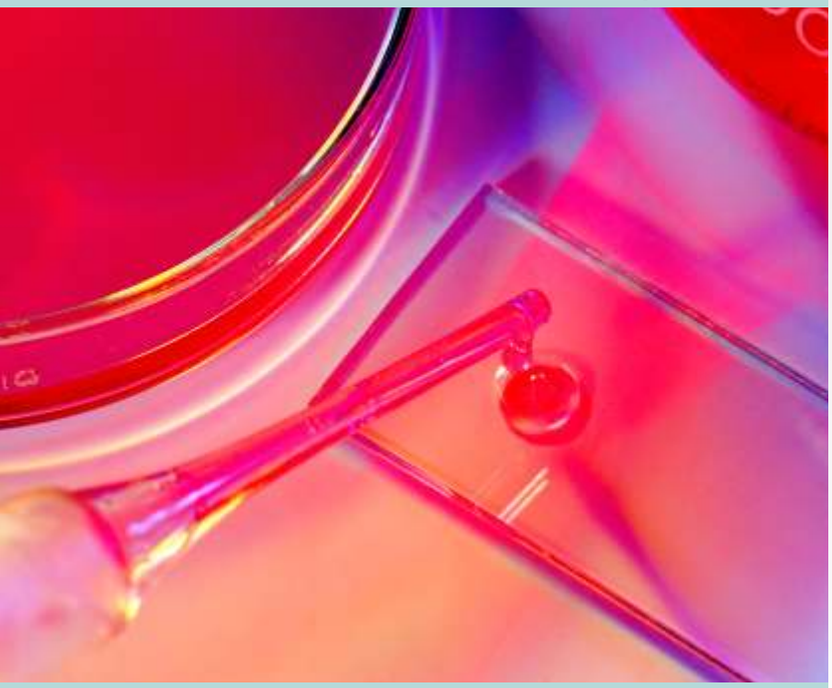


d(proSLS4)/dt = (pp3 translation rate * pSLS4 strength * [SLS4]) – (SLS4 degradation rate * [SLS4])

d(proAUR1)/dt = (pp2 translation rate * pAUR1 strength * [AUR1]) – (AUR1 degradation rate * [AUR1])

ΔVq = -(((d(proSLS4)/dt)^2)+((d(proAUR1)/dt)^2))* ΔT

Research Report



Cancer Biology

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Resolution of tumor heterogeneity based on cancer stem cell hierarchies and genetic instability

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Background

The progression of human cancers is classically thought to develop through clonal expansion of a single mutated cell, with further evolution being driven by genomic changes. The continued acquisition of such genetic alterations often results in the emergence of an array of tumor subclones with varying adaptive changes that enable survival of such cells in a stressful tumor microenvironment. It is increasingly appreciated that this intra-tumor heterogeneity may possibly extend to virtually all measurable properties of cancer cells, ranging from size, extent of differentiation, proliferation, migratory or invasive capacity, etc. Intra tumor heterogeneity is also generated by differentiation of cancer stem cell (CSC) population leading formation of various lineages. CSCs differ from their non – CSC counterparts in several features including self-renewal, regeneration, DNA repair, invasion / metastases, etc. Such heterogeneity is presents a major therapeutic hurdle with the mechanisms underlying its emergence remain poorly understood.

With establishing a lead by demonstrating the involvement of CSCs in ovarian cancer, we also mapped this abberant stem cell hierarchy using membrane labeling dyes to explore the slow cycling nature of CSCs. In the current study we have further quantified the discrete cell populations that contribute to intra-tumor heterogeneity based on resolution of CSC hierarchy, genetic instability and cell cycle phases. Such a step makes way for further understanding the cross-talks between the various populations that is an important determinant in drug resistance and tumor recalcitrance.

Aims and objectives

Flow cytometery based resolution of heterogeneous cell fractions within tumors based on CSC hierarchy (proliferative differences), genomic instability (ploidy changes) and cell cycling.

Work Achieved

Differences in cellular proliferative capabilities were combined with quenching dynamics of vital membrane labeling dyes (PKH26/67) towards a cell-surface-marker free resolution of the CSC hierarchy in a serous ovarian adecocarcinoma xenograft model (A4) established earlier in the lab. Three subsets were thus delineated *viz*. PKH^{hi} (label-retaining), PKH^{lo} (dervied from 3-4 PKH^{hi} cell divisions as identified through label-chase kinetics) and PKH^{neg} cells (total label quenching). PKH^{hi} cells represent reversibly quiescent CSCs, PKH^{lo} cells comprise of activated CSCs and progenitors, while PKH^{neg} cells represent the large tumor bulk and are generated by rapidly cycling xenografted cells and host-derived cells. Regenerative potential and functionalities associated with these cell subsets are reported earlier by our group. Such proliferation-associated hierarchies with differential label-retention capabilities were enumerated through flow cytometry (Fig.1a-i).

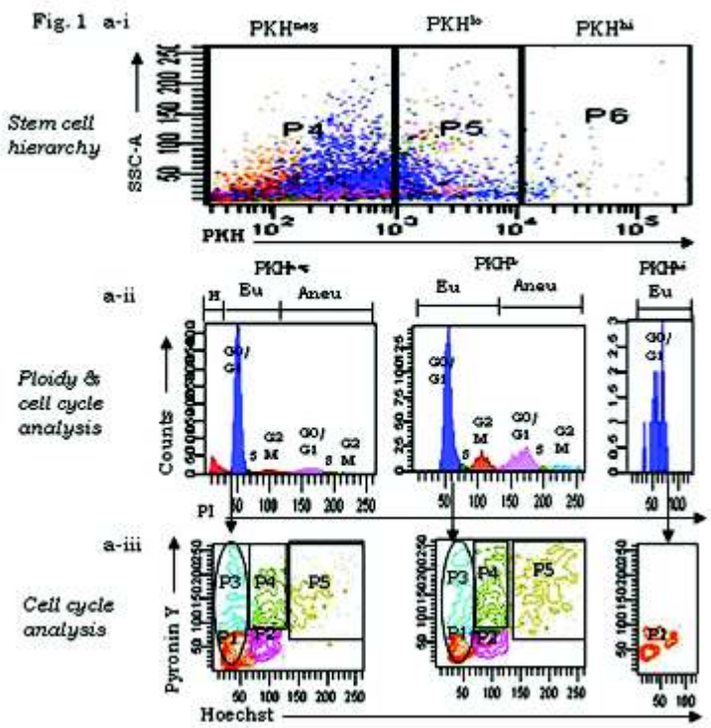

Fig.1: a-i Representative FACs profile of A4 tumor of PKH staining. a-ii Representative FACs profile of PI staining for cell cycle phases of PKH based subsets of A4 tumor, a-iii Representative FACs profile of Hoechst pyronin Y staining of PKH derived subsets where P1 – EuG0, P2- AneuG0, P3 EuG1, P4 –Mixed fraction (Eu SG2M and Aneu G1), P5- Aneu SG2M.

Besides resolving CSC-generated cellular diversity, genetic instability in terms of events associated with increased ploidy levels can also be identified through flow cytometry. Propidium iodide (PI) stained xenograft-derived profiles demarcated three discrete populations based on their varying DNA content as-(i)euploid, (ii) aneuploid derivatives of and (iii) host derived cells identified with a lower DNA content (NOD/SCID mice, 2n=40) than that of human xenografts. An important value addition in PI staining is of basic cell cycle phase resolution (G0/G1,S,G2/M), that was achieved through combined DNA-RNA content based cell cycle analysis. G0 phase (quiescent and other non-

replicative cells) was demarcated from G1 (entry into cell cycle) based on Hoechst and PyroninY staining (DNA and RNA binding respectively).

When superimposed across the PKH hierarchy, ploidy and cell cycle based tumor fractions resolve a total of 18 discrete cell groups (Fig.1a-i, a-ii, a-iii), all of which were represented at defined frequencies (Fig.2a). We also affirmed such a definition of cellular heterogeneity of being cell line independent, since similar profiles were generated in xenograft models of OVCAR3 cell line as well (Fig.2b).

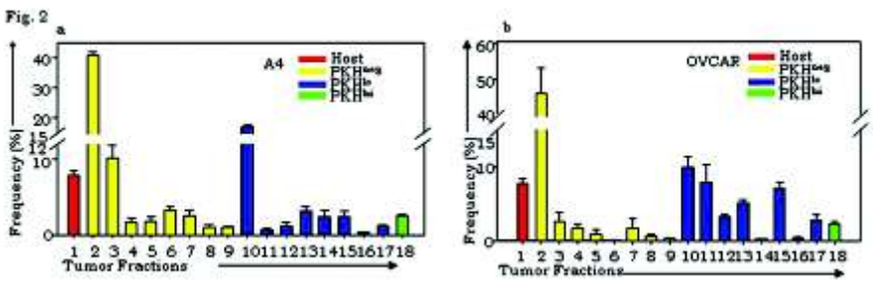

Fig. 2: Frequency of 18 A4 tumor fractions (±SEM) based on proliferative hierarchy, ploidy and cell cycle analysis of A4 tumors (a) and OVCAR tumors (b) where 1- host, 2- PKHneg Eu G0, 3- PKHneg Eu G1, 4- PKHneg Eu S, 5-PKHneg Eu G2M, 6- PKHneg Aneu G0, 7-PKHneg Aneu G1, 8-PKHneg Aneu S, 9- PKHneg Aneu G2M, 10- PKHlo Eu G0, 11- PKHlo Eu G1, 12- PKHlo Eu S, 13- PKHlo Eu G2M, 14- PKHlo Aneu G0, 15- PKHlo Aneu G1, 16- PKHlo Aneu S, 17- PKHlo Aneu G2M, 18- PKHhi Eu G0.

In conclusion, the CSC (PKH^{hi}) fraction represents self-renewing cells undergoing rare divisions; their quiescence was affirmed through an association with G0; PKH^{lo} fraction contains activated CSCs, progenitors and growth-arrested aneuploid events. We have earlier shown that such aneuploid cells retain a capability of cell cycle re-entry on exposure to drug-induced stress through selection/adaptation of intrinsically generated genetic rearrangements. The PKH^{neg} fraction is dominated by euploid-differentiated cells (in G0/G1) with few cycling and/or aneuploid cells. Host cells recruited in the xenograft towards its establishment, survival and modulation of microenvironment-immune cross-talk during disease progression are also associated with PKH^{neg} fraction. We are currently using this definition of tumor heterogeneity to study the modulation of different populations in response to specific chemotherapeutic drugs.

Future Work

This approach of mapping intra cellular heterogeneity makes possible in-depth analysis of cellular diversity and molecular cross-talks. Modulation of the above mentioned 18 tumor fractions in response to drug exposure in which further cell associations is being identified by profiling additional, relevant cell surface markers would provide insights drug response to cellular heterogeneity.

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Cancer, Chemotherapy, and Metabolic disorders

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Background

There is a medical need for advances in cancer treatment since surgery; radiotherapy and conventional cytotoxic chemotherapy have made only a modest overall impact on mortality. Thus, the significance of discovering new targets, pathways and strategies for therapeutic intervention in cancer is extremely vital. The understanding of molecular events that contribute to cancer development as well as those which enhance drug-induced cell death will not only help in better indepth inter-relationship between cancer and chemotherapeutic drugs but also will improve sensitivity and specificity of the treatment.

Reports suggest that chemotherapy has been used to treat more than half patients diagnosed with cancer. Some of the drugs, which reformed and set standard therapy for the treatment for malignancies, are paclitaxel, 5-fluorouracil, methotrexate, cisplatin, vinblastine, doxorubicin. Major impediment for the effectiveness of systemic chemotherapy is the frequent development of drug resistance in cancer cells. Development of resistance has been attributed to altered transport of drugs across the plasma membrane, enhanced DNA repair mechanisms, alteration and non-functionality of the target molecules, genetic responses to growth factors and diminished/limited access to target cells. Because of these, chemotherapy has turned out to be increasingly complicated and therefore, the anticipated success rate is much below than expected.

Hepatocellular carcinoma (HCC) is one of the most common malignant tumor and third leading cancer causing death worldwide. According to reports of World Health Organization (WHO), there are approximately 626,000 new cases of HCC diagnosed with and 600,000 deaths related to it every year, across the world.Surgery is the most appropriate option for HCC, but in most of the cases HCC are diagnosed at advanced stage when surgery is not feasible.Therefore, for the larger part of HCC, chemotherapy remains to be the alternative treatment strategy though with insignificant

benefits.However, therapeutic outcome of chemotherapy is generally poor due to inherent resistance of cancer cells to the treatment or due to development of acquired resistance. Cancer cells exhibit inherent resistant phenotype because of certain genetic alteration or they acquire resistance following exposure to drug(s).There has been a great deal of work been done on P-glycoprotein (P-gp) and its role in development of resistance. However, the role of other molecules is inadequately investigated. New factors contributing to resistant phenotype may be targeted to overcome the resistance and restore chemosensitivity. Therefore, identification and better understanding of novel molecules or exploring the pathways associated might be helpful to overcome resistance.

Overcoming drug resistance, whether inherent or acquired, is a major challenge for improving the outcome of conventional and newer chemotherapy. Therefore, targeting molecules associated with resistance together with chemotherapy may have a positive impact on outcome of therapy. The present study was initiated to investigate the factors those may be associated with the low success rate of cancer chemotherapy in HCC. In this direction we have developed a unique cellular model by using human hepatocellular carcinoma, Hep3B cells. Drug resistant cells of Hep3B (DRC) and inherently resistant single cell clone of Hep3B (SCC) were developed for resistance to paclitaxel. Studies conclude that molecules such as P-glycoprotein (P-gp), Caveolin-1 (Cav-1) and Fatty acid synthase (FASN) may play a crucial role in acquired drug resistant phenotype whereas FASN level associated with the development of inherent drug resistant phenotype in HCC.

Aims and objectives

1. The resistance of tumor cells to anticancer agents is a major cause for treatment failure. Alterations in proteins associated with the plasma membrane may contribute to the development of resistance and therefore explore such proteins.
2. An insight into the mechanistic involvement of these proteins will be explored while comparing parental and drug resistant cells.
3. Overcoming drug resistance is one facet for enhancing the potency of chemotherapy. Therefore, determining the cross resistance to other drugs is also utmost importance for achieving effective killing of cancer cells.

Work achieved

Development of drug resistant clones and drug response

Unique cellular model system to study the molecular mechanisms of acquired and inherent drug resistance was established as follows. For development of acquired drug resistant cells (DRC), Hep3B cells were exposed to increasing concentration of paclitaxel ranging from 0.3 to 1 μM in complete medium. After 24 h incubation, paclitaxel (300 nM) was added for another 48 h. Subsequently, medium was removed and fresh, drug free medium was added. The cells were incubated and allowed to grow. When confluency was reached, cells were trypsizined, replated and these cells were re-exposed to double the dose of drug. This process was repeated until clones developed resistance to 1 μM paclitaxel. After prolonged (>3 months) exposure to increasing concentration of

paclitaxel, surviving clones were pooled together and termed as drug resistant cells (DRC) and these were used for subsequent experiments. For development of single cell clone (SCC), $1x10^3$ Hep3B cells were seeded at a low density and allowed to form single cell clones. Individual clones were expanded and screened for resistant phenotype towards paclitaxel. The clone No.6 exhibiting resistance towards paclitaxel was used for further experiments and termed as single cell clone (SCC).

Characterization of paclitaxel-resistant cancer cells

The cell cycle kinetics and growth curve analysis of Hep3B cells, DRC and SCC was performed. The doubling time for DRC was calculated to be 24 h and that of Hep3B cells and SCC it was 36 h. No alterations in cell cycle distribution were detected. Paclitaxel IC_{50} value were calculated to be as 270 nM for Hep3B cells, 20 μM for DRC and 9.6 μM for SCC at 48 h. To compare the survival capabilities of Hep3B cells, DRC and SCC, all cells were treated with 1 μM paclitaxel for 48 h. Following treatment Hep3B cells exhibited membrane blebbing and they were rounded off. No noticeable morphological changes were observed in DRC and SCC. Initial characterization indicates that this cellular model mimics resistant phenotype and therefore will facilitate in further exploring the complexity to drug resistance phenomena.

Enhanced levels of P-gp protein was detected in DRC compared to Hep3B cells and SCC. Another membrane associated protein, Cav-1 which regulates signal transduction and protein translocation in cell membrane has also been implicated in the development of drug resistance. Additionally, Cav-1 is reported to be associated with a cytosolic protein FASN which is a multifunctional enzyme involved in the synthesis of palmitate from acetyl CoA / malonyl CoA and is reported to play a role in drug resistance in breast cancer cells. Yet another molecule of importance in drug resistance is Cytochrome 450 (CYP450), an enzyme involved in biotransformation of many drugs. Metabolism by CYP450 is a major determinant in reduction of drugs induced pharmacological effect. To unravel whether Cav-1, P-gp, FASN and CYP450 have a role to play in the development of resistance to paclitaxel in DRC and SCC, expression level of these molecules were examined. Cav-1 and P-gp levels are markedly increased in DRC whereas FASN and CYP450 levels increased in both DRC and SCC compared to Hep3B cells. Expression of Heat shock proteins (Hsps) has also been linked to the development of chemoresistance in cancer cells. For example, Hsp expression in gastric, breast and liver cancers has been correlated with the development of resistance to chemotherapy or radiation therapy. However, no significant alteration in the levels of Hsp70, Hsp40, Hsp 90, Hsp60 and Hsp27 between Hep3B, DRC and SCC were detected. Also, stem cell like property of cancer cells has been implicated in chemoresistance and we did not detect any alteration in the expression levels of stem cell markers vimentin, cytokeratin 8, cytokeratin 18. Cav-1 levels increased only in DRC suggesting its association with acquired drug resistance. Increased expression of FASN has been correlated with poor prognosis and chemo resistance in breast cancer cells and to our knowledge there is no report on the relevance of FASN levels in chemo sensitivity of HCC. Interestingly, FASN levels are increased in both DRC and SCC.

Role of P-gp and CYP450 in acquired and inherent drug resistance

P-gp level in DRC was elevated as compared to those in Hep3B cells or SCC. To verify the role of P-gp, verapamil an inhibitor/blocker, was used alone or combination with paclitaxel. Verapamil (40 μM) alone did not have any effect on the cell survival. Treatment of DRC with paclitaxel in the presence of verapamil promotes cell death and cell survival reduced by 20% in DRC while no difference in cell survival was observed in SCC. This result suggests that apart from P-gp, other molecules may also contribute to drug resistance phenotype in DRC and SCC. As CYP450 was upregulated in both DRC and SCC, we explored its involvement by siRNA mediated knockdown. Knockdown of CYP450 did not have significant impact on the survival of paclitaxel treated Hep3B cells or DRC or SCC.

Inhibiting FASN or Cav-1 in resistant cells enhances sensitivity to paclitaxel

It has been reported that FASN level correlates with tumor progression and it play an important role in tumor growth/survival as well as in the acquiring drug resistance. Combination treatment of potent inhibitor of FASN, cerulenin with paclitaxel significantly inhibited cell growth in DRC (38%) as well as SCC (43%) in comparison with paclitaxel treatment alone. Further, to confirm the specificity of involvement of FASN in drug sensitivity its knockdown was achieved by transfecting cells with FASN siRNA. Cell survival was inhibited by 23% and 29% in DRC and SCC, respectively in comparison to control siRNA transfected and paclitaxel alone treated cells. Thus, FASN appears to plays a central role in inherent as well as acquired resistance in HCC cells. Both FASN inhibitor cerulenin and FASN siRNA, had profound effect on the survival of paclitaxel treated DRC and SCC compared to paclitaxel treatment alone. Surprisingly, knocking down of Cav-1 by its specific siRNA or by treating cells with MCD, followed by paclitaxel treatment resulted in resensitization of DRC only. These results indicate that Cav-1 levels are associated with the maintenance of acquired drug resistance.

Acquired and inherent resistant cells exhibit cross resistance towards other anti-cancer agents

It has been reported that cells resistant to one drug also exhibit cross-resistance to other classes of drugs. To explore whether levels of these proteins also predicts cell fate following treatment with other chemotherapeutic drugs, sensitivity of cells assessed. DRC and SCC were resistant to vinblastine (Vin), methotrexate (MTX), doxorubicin (Dox) though they were equally sensitive to cisplatin (Figure 1B-E). The impact of downregulation of Cav-1, FASN, P-gp and CYP450 on the outcome of drug treatment was performed. Interestingly, knockdown of Cav-1 by siRNA followed by addition of drugs drastically decreases cell survival in DRC compared to drug alone treated cells. Downregulation of Cav-1 also affected survival of SCC treated with drugs. Knockdown of FASN by siRNA significantly reduces the survival of SCC and DRC. CYP450 downregulation had no effect on the survival of DRC and SCC with or without drug treatment. Interestingly, verapamil treatment diminishes cells survival to somewhat lesser extent then the levels achieved by knockdown of Cav-1 or FASN by siRNA. Interestingly, the resistant cells could be resensitized to these different classes of drugs by silencing the protein levels of P-gp, Cav-1 and FASN, while no change in the degree of resistance was

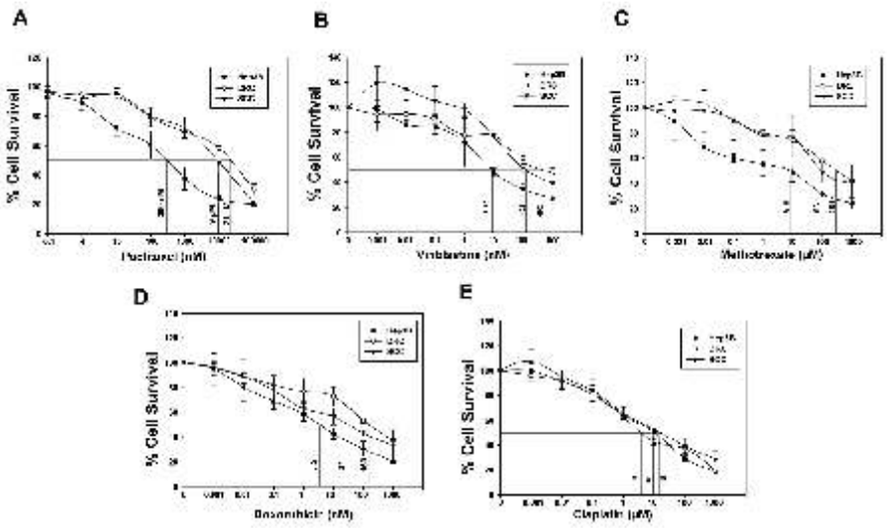

Fig. 1: DRC and SCC exhibit cross resistance to different classes of anti-cancer agents. (A-E) Hep3B cells, DRC and SCC were plated and treated with different concentration of paclitaxel, vinblastine, methotrexate, doxorubicin and cisplatin. After 48 h drug containing medium was replaced with fresh medium and cell survival was evaluated by MTT assay.

detected by abrogation of CYP450 levels. These results indicate that the factors responsible for chemo-resistance may vary depending on the class of drugs utilized.

Inter-relationship between Cav-1, FASN, P-gp and CYP450

It is evident from the results presented that resistant phenotype of the cancer cells is not due to alteration in only one cellular protein but other proteins may also play an important role. Therefore to better understand the inter-relationship between these molecules, we choose to look into the central role of FASN as its levels were enhanced in DRC as well as SCC. When FASN expression was knocked down by siRNA, it not only decreases FASN levels, but also diminished Cav-1 levels. Similarly, upon knock down of Cav-1 by its specific siRNA, FASN levels also decreased (Figure 2A). Knockdown of either

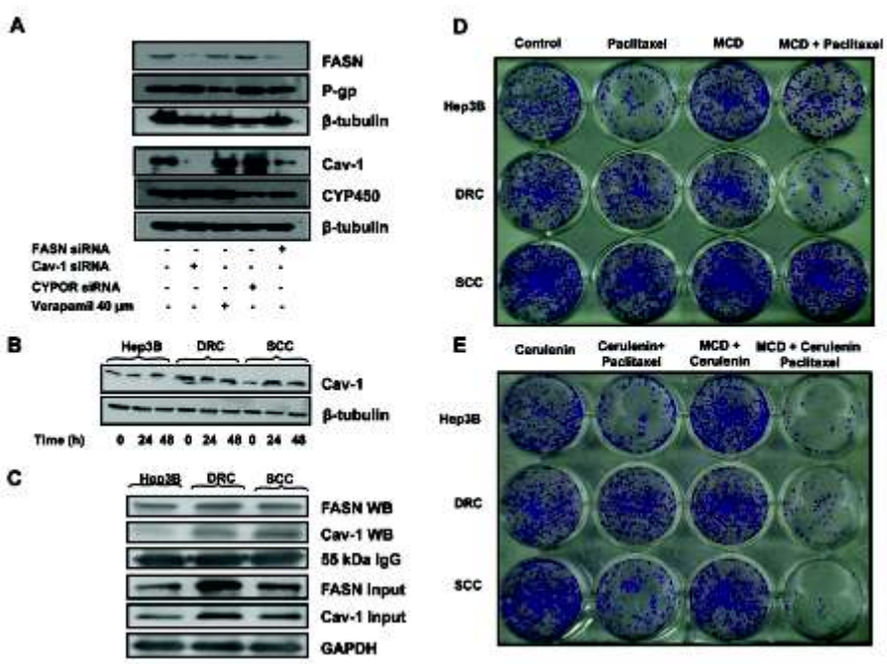

Fig. 2: Cav-1 knockdown inhibit the expression of FASN and vice versa. (A) DRC ($5x10^5$) were plated in 35mm petri plate. After 24 h, cells were transfected with siRNAs targeting Cav-1, FASN or CYPOR. Simultaneously, 40 µM verapamil was added for 24 h. Thirty-six hours posttransfection or 24 h verapamil treatment, cells were harvested and lysates were prepared. Fifty microgram whole cell lysate proteins were resolved on 8% or 10% SDS-PAGE and western blot was performed. (B) Hep3B cells, DRC and SCC were treated with 300 nM paclitaxel for 24 and 48 h respectively. Whole cell lysates were prepared and 30 µg was resolved on 10% SDS-PAGE and western blotting was performed. (C) Co-immunoprecipitation of Cav-1 and FASN in Hep3B cells, DRC and SCC. Immunoprecipitation was carried out using FASN specific antibody. Cav-1 and FASN were detected in the immune complex by immunoblotting. IgG heavy chain and GAPDH served as loading control.(D and E) Hep3B cells, DRC and SCC were plated and allowed to adhere for 24 h and cells were pretreated with MCD (4 h) or cerulenin (24 h). After inhibitor treatment, paclitaxel was added for additional 48 h. Cells were washed with PBS, fresh medium was added and cells allowed to form colonies for ~ 21 days. Colonies were stained with crystal violet and photographed.

CYP450 or P-gp did not have any impact on the expression levels of FASN or Cav-1 (Figure 2A). Interestingly, in paclitaxel treated Hep3B cells and SCC, Cav-1 level increased in a time dependent manner and in DRC no change in Cav-1 was detected (Figure 2B). To determine whether Cav-1 interacts with FASN, whole cell lysates from Hep3B cells, DRC and SCC were immunoprecipated with FASN specific antibody and the immunoprecipated complex was then subjected to western blot analysis and probed for Cav-1 and FASN. As shown in Figure 2C, we detected Cav-1 in the immunocomplex suggesting a functional interaction between Cav-1 and FASN in HCC cells. Recently, it has been reported that FASN and Cav-1 interact together and modulate each other in melanoma cells.

In summary, the present study highlights the fact that Cav-1 and P-gp plays a crucial role in acquired drug resistance caused by paclitaxel exposure. FASN appears to be a critical factor responsible for inherent and acquired resistance towards different classes of drugs. This study provides valuable information for the improvement of chemotherapies by targeting key molecules, such as Cav-1, FASN and P-gp in drug resistant HCCs. Importantly, strategies involving the use of FASN inhibitors prior to drug treatment may be preventive in transformation of inherently resistant cells to acquired drug resistance, which eventually culminates in development of multiple drug resistance phenotypes. Our finding suggests that FASN and Cav-1 interact is an indicative of similarities in the modulation of FASN by Cav-1 and vice versa between HCC cells and melanoma. Finally, it can be stated that the treatment with sub-optimal levels of paclitaxel enhances Cav-1 levels which further leads to stabilization of FASN and vice versa. In addition P-gp levels are also elevated. Using inhibitors of FASN, Cav-1 and P-gp together could resensitize

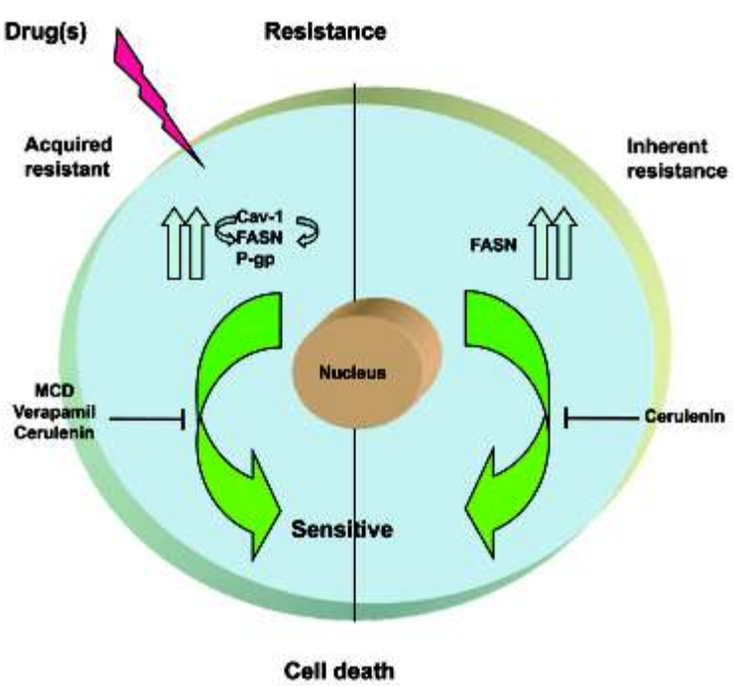

Fig. 3: Schematic representation of proposed mechanism of drug resistance in DRC and SCC.

resistant cells towards paclitaxel or use of FASN inhibitor alone would suffice to sensitize the cells to paclitaxel at early stages (Figure 3).

Future work

1.? Enhancement of effectiveness of cancer chemotherapy by exploration of combination therapies, bystander killing and molecules associated with drug resistance.
2. Metabolic malfunctioning and its impact on cancer cell growth and cancer chemotherapy.

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Understanding the F-box proteins functioning as tumor suppressors

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Background

Cell cycle progression is tightly controlled by sequential activation and inactivation of cell cycle regulatory proteins. Cells control the gene expression either through transcriptional or post-transcriptional regulation. Alteration of this regulation leads to genomic instability causing many diseases like cancer. In eukaryotes, most of the cell cycle regulatory proteins are activated and inactivated through proteasome mediated pathways. Proteasomal degradation mostly involves polyubiquitination of the specific substrates through specific E3 ubiquitin ligases. E3 ubiquitin ligases are classified into two groups based upon their structural domains: HECT type and Ring finger type domain containing E3 ubiquitin ligases. SCF (SKP1-Cul1-F-box) and APC/C complex are most widely studied RING finger E3 ubiquitin ligases. F-box proteins are the important component of SCF complex and determine the substrate specificity for the complex. Human genome comprise of 69 F-box genes out of which functions of only 9 are known till date. Therefore, functions of most of the F-box proteins are not known. Previous studies showed that F-box proteins may functions as tumor suppressor or oncogene or both. For example, FBXW7 functions as a tumor suppressor and SKP2 functions as an oncogene whereas ßTrCP functions as tumor suppressor as well as oncogene. Therefore, it is plausible that some of the F-box proteins might function as tumor suppressor. We are interested to identify F-box proteins functioning as tumor suppressor and elucidate their mechanism.

FBXO31, a member of F-box family, functions as tumor suppressor. Recent studied have shown that it is a novel DNA damage response checkpoint protein. It mediates the proteasomal degradation of cyclin D1 and arrests the cells at G1phase upon genotoxic stresses. It is mostly inactivated in many cancers like breast, ovarian, hepatocellular carcinoma and prostate cancers due to loss of heterozygosity. In addition, we have observed that expression of FBXO31 is very low under normal growth conditions and it stabilized upon inhibition of proteasome function. This led us to hypothesize that post

translational level of FBXO31 might be regulated through proteasome mediated degradation. Therefore, we performed an E3 ubiquitin ligase screen to identify the negative regulator(s) of FBXO31.

Aims and Objectives

1. Determination of how physiological level of FBXO31 is maintained under normal growth condition at post translational level
2. Identification of the molecular player(s) involved in FBXO31 post translational regulation
3. Elucidation of the molecular mechanism involved in post translation regulation of FBXO31
4. Identification of F-box proteins functioning as tumor suppressor

Research Findings

Aim 1: How physiological level of FBXO31is maintained under normal growth condition at post translational level

FBXO31 is regulated through proteasome mediated pathway

We have checked the transcriptional level of FBXO31 by real time RT-PCR. We did not find any alteration in transcriptional level of FBXO31 (Figure 1A). Previous results showed that protein level of FBXO31 varies throughout the cell cycle. These results encourage us to check the protein level of FBXO31 under inhibition of proteasome mediated degradation process. Interesting, FBXO31 is found to be stabilized in the presence of proteasome inhibitor (Figure 1, Panel B). However, lysosome inhibitor (chloroquine) does not have any effect on the protein level of FBXO31 (Figure 1B). These results conclude that FBXO31 is regulated via proteasome mediated pathway.

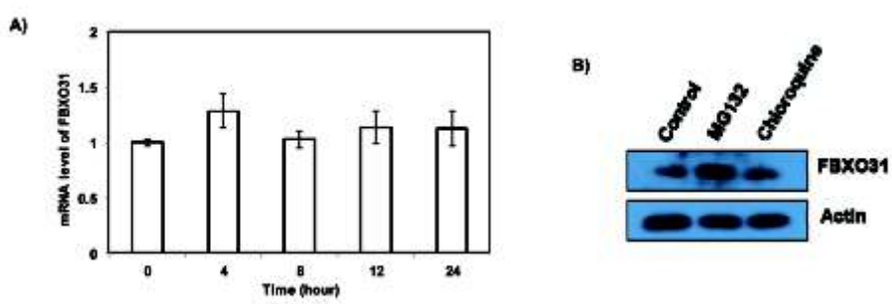

Fig. 1: Regulation of FBXO31 is at post translational level A) HEK 293T cells were synchronized with hydroxyurea and were collected, mRNA was isolated at different time points after released. Transcriptional level of FBXO31 were measured by real-time PCR. B) HEK 293T cells were treated with either proteasome inhibitor MG132 (10 μM) or lysosome inhibitor chloroquine (100 μM) for 12 hours. Whole cell lysates were separated in SDS-PAGE and FBXO31 level was checked by immunoblot.

Aim 2:Identify the molecular player(s) involved in FBXO31 post translational regulation

Identification of E3 ubiquitin ligase(s) involved in FBXO31 stabilization

FBXO31 levels oscillate during different phases of cell cycle. Sequential activation followed by inactivation of different cyclins, CDKs and CDK inhibitors is essential for normal cell cycle progression. Most of the cell cycle regulators are degraded through proteasome mediated pathway by ring finger E3 ubiquitin ligases. Our results have already shown that inhibition of proteasome leads to stabilization of FBXO31. Therefore, we thought that FBXO31 might also be regulated through ring finger E3 ubiquitin

ligase(s). To identify the ring finger E3 ubiquitin ligase(s) responsible for FBXO31 stability, E3 ubiquitin ligase screen was performed (Figure 2A). After performing three independent screens, we landed up 8 candidates that promote the degradation of FBXO31 significantly. The candidate obtained included Cdh1, Cdc20, FBXL8, FBXL12, FBXL13, FBXO21, FBXO44, and FBXO46. Further, we validated the candidate genes by checking the FBXO31 expression level under knockdown condition.

Aim 3: Elucidating the molecular mechanism involved in post translation regulation of FBXO31

Cdh1 and Cdc20 regulate FBXO31 during cell cycle progression

Cdh1 and Cdc20 are the adapter proteins of APC/C complex, which regulate G1 and G2/M phases of the cell cycle, respectively. Like F-box protein, Cdh1 and Cdc20 determine the substrate specificity for APC/C complex. They recognize the substrates having D-box motif in their amino acid sequence. Interestingly, FBXO31 has 8 D-box motifs and are well conserved among the mammals. Overexpression of Cdh1 and Cdc20 led to the degradation of FBXO31 and the same was inhibited upon addition of MG132 (Figure 2B & C).

FBXO31 is polyubiquitinated by Cdh1 and Cdc20

We observed that overexpression of Cdh1/Cdc20 promotes degradation of FBXO31. Therefore, we asked whether Cdh1 and Cdc20 interact with FBXO31. Through co-immunoprecipitation studies we found that Cdh1 and Cdc20 interact with FBXO31. Next we asked whether Cdh1 and Cdc20 promote polyubiquitination of FBXO31. To investigate the polyubiquitination, we performed in vivo ubiquitination assay and observed FBXO31 to be polyubiquitinated by Cdh1 as well as by Cdc20 (Figure 2D). These results established that FBXO31 is substrate for both Cdh1 and Cdc20.

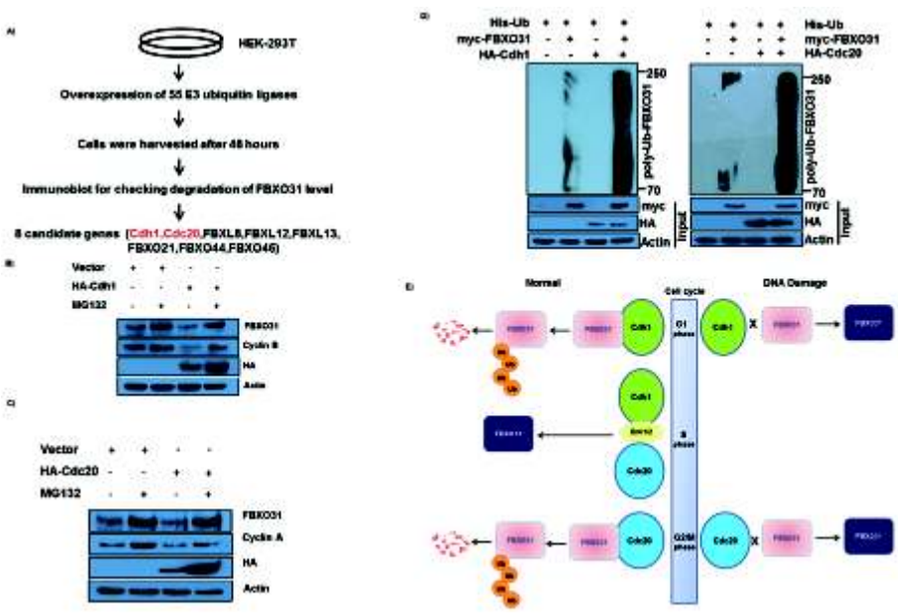

Fig. 2: Cdh1 and Cdc20 targets FBXO31 for its degradation. (Panel A) Schematic representation of the E3 ubiquitin ligase screen to identify candidate(s) involved in FBXO31 turnover. Exogenous expression of Cdh1 (Panel B) and Cdc20 (Panel C) led to the degradation of FBXO31 however, failed to do so in the presence of proteasome inhibitor MG132. *In vivo* ubiquitination assay for FBXO31 was performed (Panel D). Elucidating the molecular mechanism involved in post translation regulation of FBXO31 (Panel E).

How does FBXO31 function as a tumor suppressor?

Previous study established that tumor suppressor FBXO31 is a dedicated check point protein in DNA damage response. It stabilizes and arrests the cells at G1phase through proteasomal degradation of cyclin D1 upon genotoxic stress. We asked how FBXO31 is stabilized under genotoxic stress. Is it due to the loss of interaction between FBXO31 and Cdh1/Cdc20? To understand the underlying mechanism, we performed immunoprecipitation of FBXO31 with Cdh1 and Cdc20 under genotoxic stress. We found that Cdh1/Cdc20 fails to interact with FBXO31 under various genotoxic stresses. These results showed that Cdh1/Cdc20 cannot regulate FBXO31 under DNA damage conditions. Based on these observations we proposed a model (Figure 2E).

Future Work

In future, we will investigate why Cdh1/Cdc20 fails to interact with FBXO31 under DNA damage conditions, whether any post translational modification(s) involved in interactions.

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Role of mTOR signaling pathway in survival, proliferation and invasion of human gliomas

Background

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults. GBM is characterized by genetic instability, intra-tumoral histopathological variability and unpredictable clinical behavior with extremely poor prognosis. The clinical hallmarks of glioblastoma are its aggressive growth and inexorable recurrence due to invasive in?ltration into the surrounding brain tissue despite multimodal therapy with surgery followed by radiation and chemotherapy. Extensive aberrations in gene expression are reported among GBMs, particularly in genes involved in tumor invasion, angiogenesis, immune cell infiltration and extracellular matrix remodeling. Activation of various signaling pathways such as - Raf-MAPK-ERK, PI3K-PKB, PI3K-Rac-Rho, and PI3K-Akt- leading to NF-κB involvement play important role in survival and proliferation. These pathways also modulate migration, angiogenesis and invasion in tumors.

The mammalian Target of Rapamycin (mTOR) signaling network down-stream to PI3K/AKT pathway regulates cell growth, proliferation, and survival. The pathway functions with mTOR protein kinase forming two distinct macro molecular multi-protein complexes-mTORC1 and mTORC2. The mTOR Complex 1 (mTORC1) consists of mTOR, raptor (regulatory-associated protein of mTOR), Sin-1 and mLST8 (also known as GßL) and regulates cell growth translational machinery through effectors such as S6K1 and 4E-BP1. The mTOR complex 2 (mTORC2) comprises of mTOR, rictor (rapamycin-insensitive companion of mTOR), Sin-1 and mLST8, regulates the actin cytoskeletal functioning (RhoA, Rac1), PKCα and pro-survival kinase Akt/PKB by phosphorylating it on Ser-473 site. The targeted inhibition of up-regulated mTORC1 pathway is partially efficient but not sufficient to control the cancer growth and proliferation, so mTORC2-mediated pathway and their mutual interaction should also be explored as a target to control tumor growth and invasive potential.

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Aims and Objectives

1. To study the crosstalk between the m-TOR complexes (mTORC1 and mTORC2) pathways in human glioma cell lines and primary cultures of tumor samples using inhibitors such as Rapamycin and its analogs.
2. Interaction of mTORC2 and NFκB mediated signaling pathways in response to TNF-α.
3. To understand the mechanism(s) that contribute to invasiveness in gliomas with focus on the role of mTOR signaling.

Work Achieved

Recent work from our group shows that mTORC2 component -Rictor negatively regulates p65 activation and transcription of NF-κB target genes important in invasion by enhancing expression of IKKβ. The data also reveals the involvement of Raf-1/ERK pathway in the regulation of IKKβ and NF-κB activation. Rapamycin and its analogs are promising therapeutic agents with anti-tumor properties. Though initially, Rapamycin was thought to be specific inhibitor of mTORC1, it was later demonstrated that on prolonged treatment it also inhibits mTORC2 to suppress Akt/PKB phosphorylation at Ser-473 site essential for stability. In our studies, the effect of Rapamycin (RAP) and Temisirolimus (TEM), an analogue of rapamycin was analyzed in human glioma cell lines - LN-18 and LN-229 and primary cultures derived from tumors. The drugs had no significant effect on the cell survival and proliferation as assessed by MTT assay. To examine their effect on invasive potential of GBM, the expression of most commonly associated Matrix metalloproteases-MMP-2 and MMP-9 was determined at transcript and protein levels. As these proteases are secretory gelatinases, functional activity was measured by gelatinolytic zymography. We observed that LN-18 cells contain constitutively high level of MMP-2, while MMP-9 was induced by TNF-α. Our data revealed that RAP and TEM significantly reduced constitutive MMP-2 and induced MMP-9 at m-RNA (Fig.1A) and protein expression levels (Fig. 1B and 1C). The effect was more pronounced with TEM compared to RAP. The zymography results were in line with the

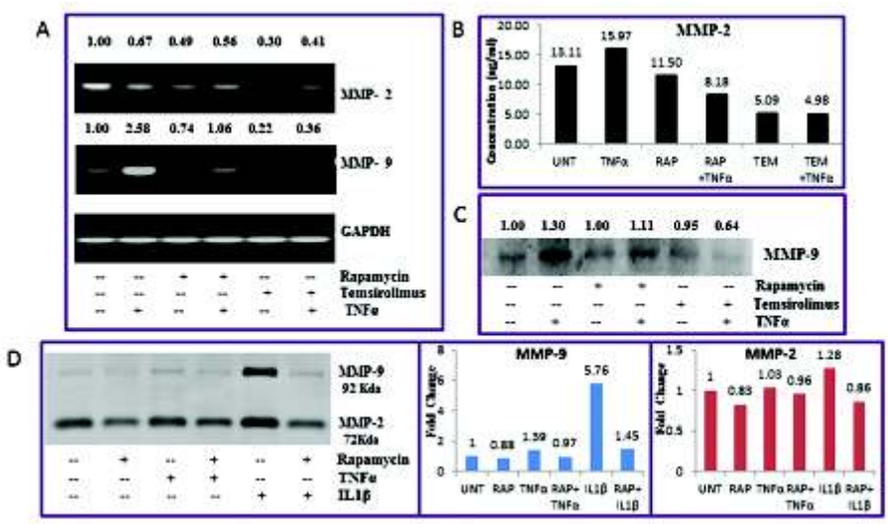
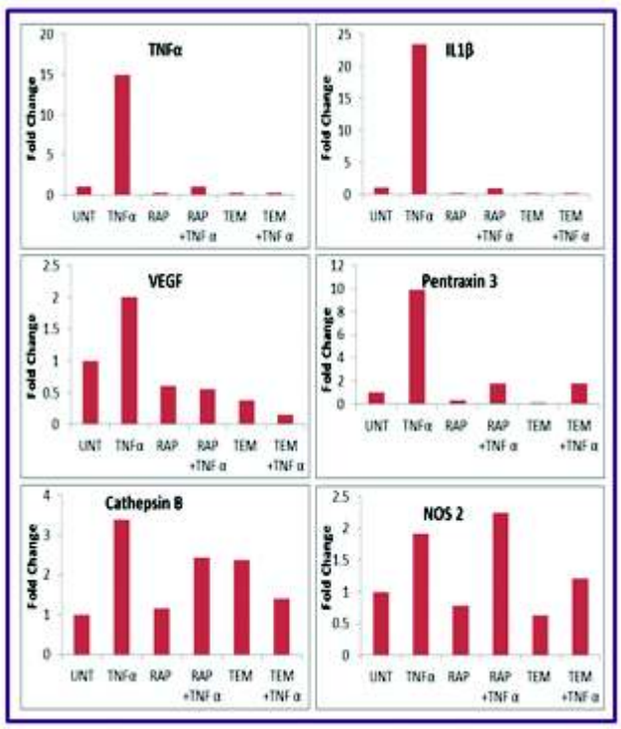


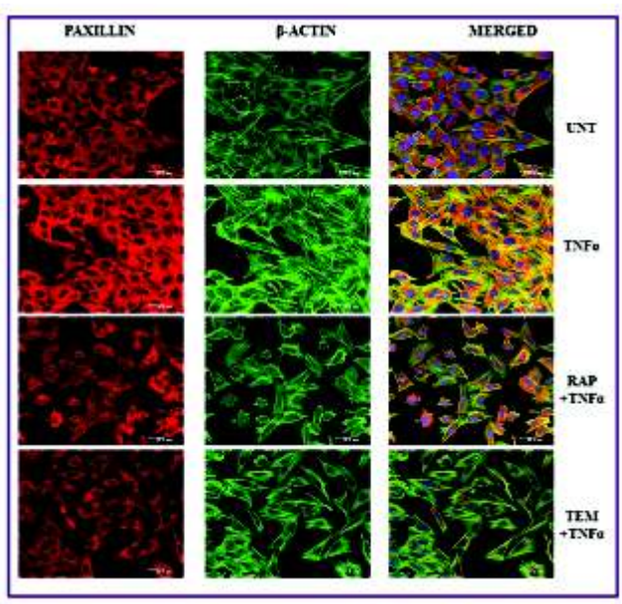
Fig. 1: Effect of Rapamycin (RAP) and Temisirolimus (TEM) on MMP-2 and MMP-9 at m-RNA, protein and enzymatic activity levels. **A.** LN-18 cells were treated with RAP (10μM), TEM (5μM) for 48h alone and in combination with TNFα (10ng/ml) for 12h before termination and semi-Quantitative PCR was performed. GAPDH was used as control. The figures indicate fold change estimated by densitometry compared to control. **B.** MMP-2 level was measured by ELISA in conditioned media of LN-18 cells treated using RAP (10μM), TEM (5μM) for 48h alone and in combination with TNFα (10ng/ml) for 12h before termination of time point. **C.** Immunoblotting for MMP-9 protein concentrations in conditioned media of LN-18 cells as processed for MMP-2 **D.** Gelatinolytic Zymography analysis for MMP-9 and MMP-2 was performed on conditioned media from LN-18 cells treated using RAP (10μM) alone and in combination with IL1β (10ng/ml) for 48h or TNFα (10ng/ml) for 12h before termination of time point. The fold change estimated by densitometric analysis is graphically represented.

Fig. 2: Effect of Rapamycin and Temisirolimus on NFκB targets induced by TNFα at m-RNA level. LN-18 cells were treated using RAP (10μM), TEM (5μM) for 48h alone and in combination with TNF-α (10ng/ml) for 12h before termination and Real-time PCR was performed with GAPDH used as internal control.



above observations (Fig. 1D).MMP-9 is a target gene for NFκB and is inducible in response to TNF α, so the effect of RAP and TEM was analyzed on expression of other NFκB target genes- TNFα, IL-1β, VEGF, Pentraxin-3, Cathepsin-B, and NOS-2 by quantitative Real Time-PCR. All the target genes except NOS-2 were effectively reduced by RAP and TEM (Fig.2). Studies done in primary cultures derived from GBM tumors, showed reduction of protein expression and enzymatic activity of TNFα or IL-1β-induced proteases by RAP and TEM. In contrast to the LN-18 cell line which showed MMP2 as

Fig. 3: Effect of Rapamycin and Temisirolimus on Paxillin and actin re-organistion. Immunostaining for Paxillin and β-actin was performed on LN-18 cells treated with RAP(10μM), TEM (5μM) for 48h in combination with TNFα (10ng/ml) for 12h before termination of time point.



single band, the conditioned medium of primary cultures showed 2 distinct bands representing pro- and active forms of MMP-2. Extending study to other factors contributing to invasion, Paxillin a docking site protein component of Focal adhesion complex and potent regulator of actin cytoskeleton was monitored. Reduction of TNF-α induced Paxillin protein expression and regulation of β-Actin organization were observed in LN-18 on treatment using RAP and TEM (Fig. 3). Our findings lead us to conclude that blocking of mTOR mediated signaling with inhibitors- rapamycin and Temisirolimus suppressed invasion in glioma cells treated with TNF-α and IL-β by suppressing NFκB activity, thus identifying the mechanism of action of these drugs in controlling invasion in tumor cells.

Future work

The study will be extended to investigate whether the rapalogs and other inhibitors of mTOR will be effective in controlling invasion of glioma cells influenced by extracellular matrix components and decipher the mechanisms involved.

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Understanding mechanisms of transformation elicited by 600bp non-coding RNA gene - Ginir

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Background

Long non-coding RNA's (lncRNA) act as key molecules in regulation of processes such as chromatin re-modeling, transcription, and post-transcriptional processing. They appear to form a new layer of gene regulation and contribute to the complexity of gene expression programs. We have identified a pair of long non-coding, over-lapping RNA transcripts- Ginir - (sense transcript) and Giniras (anti-sense transcript) which do not have protein coding potential. This pair of 612 nucleotides transcripts is localized to q arm of mouse X chromosome.Our studies show homeostasis to exist between this pair of cis-sense and anti-sense transcripts expressed in mouse fibroblasts. However, when one of the members - Ginir is over expressed, it leads to de-regulated cell cycle progression, genomic instability, cellular transformation in vitro followed by tumorigenicity and high metastatic potential *in vivo*. In contrast, anti-sense RNA counterpart – Giniras is non-oncogenic to the same cells. Over -expression of Giniras RNA antagonizes Ginir function to significantly restore order into cell cycle progression in Ginir-induced oncogenic cells and attenuates *in vivo* tumor growth from them. The potential of Ginir to form tumors is outcome of its key role in inducing genomic instability, the function from which it derives its name - "Ginir"- Genomic INstability Inducing RNA.

Aims and Objectives

1.? To investigate potential of ncRNA Ginir in inducing cellular transformation and metastasis in various mouse & human Ginir over-expressing cell systems.
2.? To specify targets through which Ginir mediates its effects on cellular growth and their relationship to various signalling pathways.
3.? To investigate the interaction partners of Ginir through which it mediates its functions associated with oncogenesis & embryo development.

Work Achieved

The oncogenic potential of Ginir is established for both mouse (NIH/3T3) & human (SKNMC) cells from the experimental data generated in our lab. We showed that the

potential of Ginir to form tumors is outcome of its key role in inducing genomic instability, the function from which it derives its name - "Ginir"- Genomic INstability Inducing RNA. In this study we aim to address role of transcripts - Ginir and Giniras in development, cell proliferation, transformation and metastasis.

To investigate potential of ncRNA Ginir in inducing cellular transformation and metastasis in various mouse & human Ginir over-expressing cell systems

Ginir plays a significant role during embryonic development wherein by LNA-FISH we show its prominent expression in the telencephalon region of 14.5 days mouse embryonic brain. A significant decrease in Ginir levels was found to occur as development proceeded from neonatal to adult stages with Giniras levels remaining constant. Similar pattern of Ginir expression was found in human brain wherein we found that Ginir and Giniras levels were very low in human adult brain but in tumors of CNS like glioblastomas (GBM) the levels of Ginir increased significantly in a graded manner indicating its function in tumorigenicity and suggesting its role as a RNA Bio-marker. Further, we investigated the potential of Ginir to cause invasion & *in vitro* migration by wound healing assays. Our data indicates that Ginir possessed the potential to induce migration and promoted invasion specifically in NIH/3T3-Ginir cells but not in NIH/3T3-Giniras & NIH/3T3-EV cells (Fig 1A, B). We also developed an *in vivo* experimental metastasis model to analyze potential of Ginir to induce metastasis (Fig 1C). The analyses of various organs in mice and

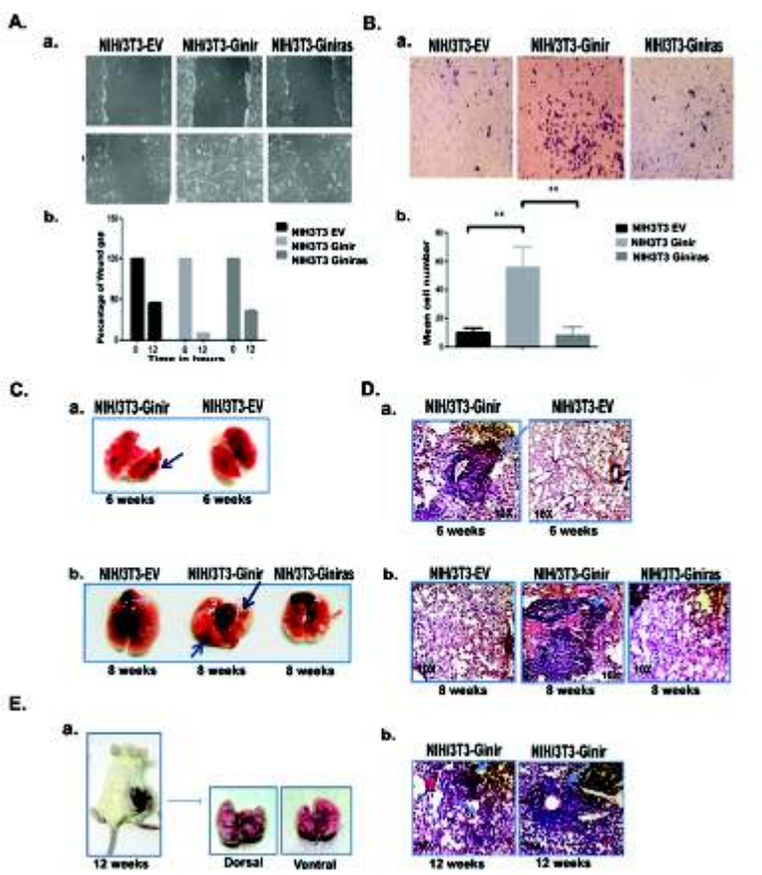


Fig. 1: Ginir cells show high invasive potential and induce metastasis in NIH cells. **A)** Wound healing assay with NIH/3T3, NIH/3T3-Ginir & NIH/3T3-Giniras cells (a) data represented as histogram using ImageJ (b). **B)** Matrigel invasion assay with NIH/3T3-EV, NIH/3T3-Ginir & NIH/3T3-Giniras cells followed by staining (a) & quantification by Image-J. Data represents Means ± SEM (**p≤0.01) (b). **C)** Tail Vein Metastasis assay in NOD/SCID mice injected with cells via tail vein (2 X 106 cells). - Lungs dissected after 6 weeks (a) & 8 weeks (b) of injection (n=3). **D)** Micrographs of H&E staining of lungs of SCID mice injected with cells. **E)** NOD/SCID mice injected subcutaneously with NIH/3T3-Ginir cells after 12 weeks of injection (a) Phase contrast H&E staining micrographs of lungs dissected out of subcutaneously injected mice with NIH/3T3-Ginir cells (b).

especially the lungs showed development of macroscopic foci that on H & E staining showed metastatic foci formation in case of mice injected with Ginir expressing cells whereas there were no such foci detected in case of mice injected with NIH/3T3-EV & NIH/3T3-Giniras cells (Fig 1D). Similar foci in lungs were also induced by Ginir on subcutaneous injections (Fig 1E). These studies exemplify role of Ginir in inducing metastasis in mouse and human cells. We have generated multiple stable Ginir or Giniras over-expressing mouse and human cell-lines and confirmed the transforming and metastatic nature of Ginir in these cells. Interestingly, the Ginir tumors were found to be enriched with a cancer stem cell population suggesting novel role for Ginir in stemness.

To specify targets through which Ginir mediates its effects on cellular growth and their relationship to various signalling pathways

Our studies indicated that Ginir over-expression caused de-repression of satellite repeat elements like LINE-1, MIN Sat and IAP-1, including dys-regulation of genes involved in DNA replication, chromosome segregation and cell-cycle checkpoints leading to tumorigenesis and cancer. The over-expression of Ginir in mouse and human cells caused activation of ATM/ATR DNA damage response pathways. We found that Ginir was capable of activating both ATM and NBS1. We demonstrated that Ginir induced ATM phosphorylation led to activation of a number of downstream substrates - NBS1, BRCA1 and SMC1 (structural maintenance of chromosome protein 1), suggesting a significant crosstalk between DNA damage & chromatin changes. Moreover many non-coding

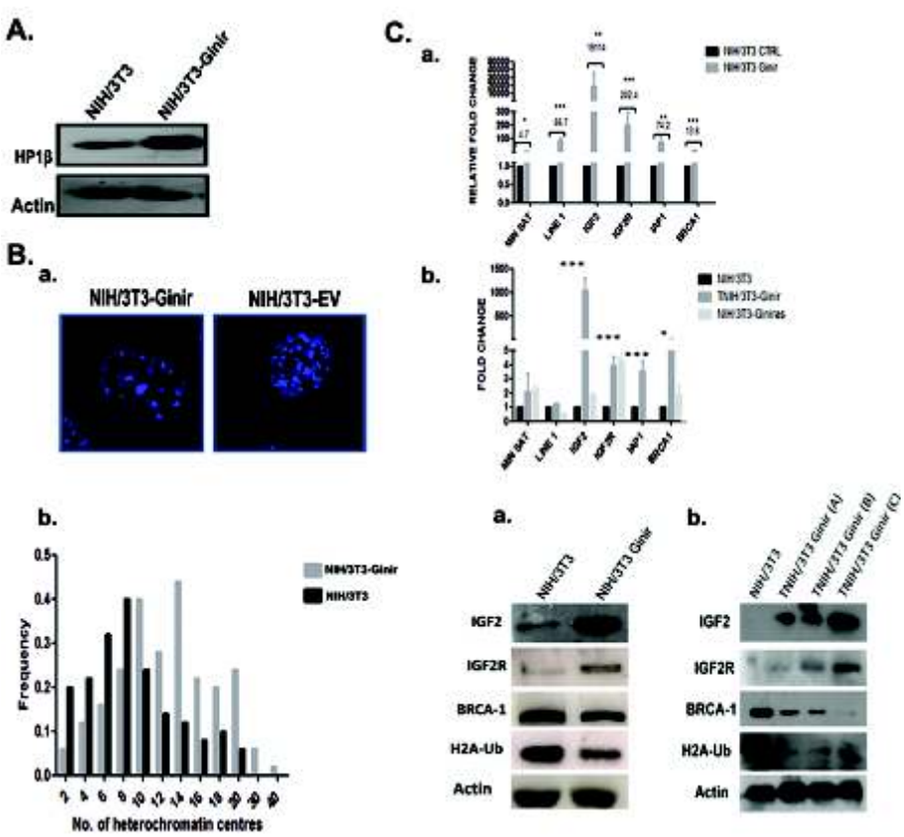


Fig. 2: Chromatin associated changes induced by Ginir; A) Immuno-blotting for HP1β protein in cells. B) DAPI staining of heterochromatin foci (a). quantification of number of heterochromatin centres and its representation as a histogram in NIH/3T3 & NIH/3T3-Ginir cells (b). C) Real time PCR for the expression of Min-Sat, Line1, IAP1, IGF2, IGF2R, BRCA1 & IGF1R in NIH/3T3 & NIH/3T3-Ginir cells (a) & in cells derived from *in-vivo* tumors inducedby Ginir (b). Graphs represent Average +/- SD (C). 18S rRNA used as internal control. Experiments were done in triplicates with independent sets of cDNAs & samples. Actin used as internal control for immuno-blotting D) Immuno-blotting for IGF-2, IGF2R, BRCA-1 & H2A-Ub with NIH/3T3 & NIH/3T3-Ginir cells (a) & cells derived from three independent in-vivo tumors (b).

RNAs have been reported to be involved in regulation at chromatin level. Interestingly, Ginir was involved in changing the chromatin state of the cell, wherein over-expression of Ginir caused change in heterochromatin evident in terms of increase in expression of HP1beta by Western blotting (Fig. 2A). This was validated by staining for heterochromatin foci & their quantification in Ginir overexpressing cells (Fig 2B). Heterochromatin associated changes caused de-repression of repeat elements like - Min-SAT, Line1, IAP1 and that was also associated with overexpression of imprinted genes like IGF2 & IGF2R by Ginir (Fig 2C, 2D). We believe that BRCA-1 may be involved in regulation of these changes by ubiquitination of H2A thus converting euchromatin to heterochromatin. There are reports that with BRCA-1 loss or mutation, there is no ubiquitination of H2A, loss of heterochromatin & increased expression of satellite repeats & imprinted genes. We found that Ginir caused loss of BRCA-1 at protein level (Fig 2D) but not at transcript level (Fig 2C). We have observed similar results in *in vitro* as well as in tumors *in vivo* (Fig 2C,b and 2D,b). Our studies indicated that Ginir caused down-regulation of BRCA-1 & thus loss in ubiquitination of H2A leading to loss of heterochromatin causing de-repression of satellite repeats & overexpression of imprinted genes. However, the binding partners of Ginir that mediate these changes are being investigated.

To investigate the interacting partners of Ginir through which it mediates its functions associated with oncogenesis & embryo development.

In an attempt to elucidate mechanisms through which Ginir is directly or indirectly mediating these effects, we performed RNA pull down assays with Ginir to investigate its binding partners. The interaction propensities of these proteins were analyzed by bioinformatics tools like CATRAPID (Data not shown). Based on our data we propose a model of action for Ginir action (Fig. 3).

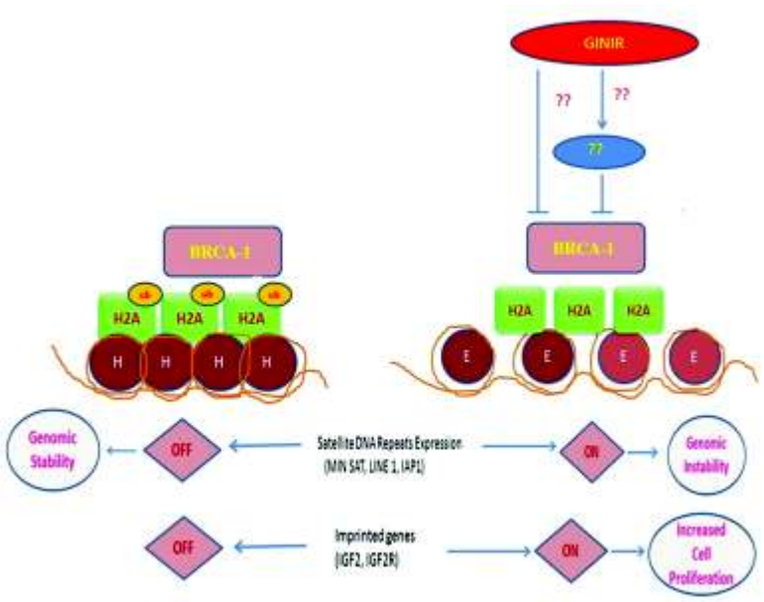


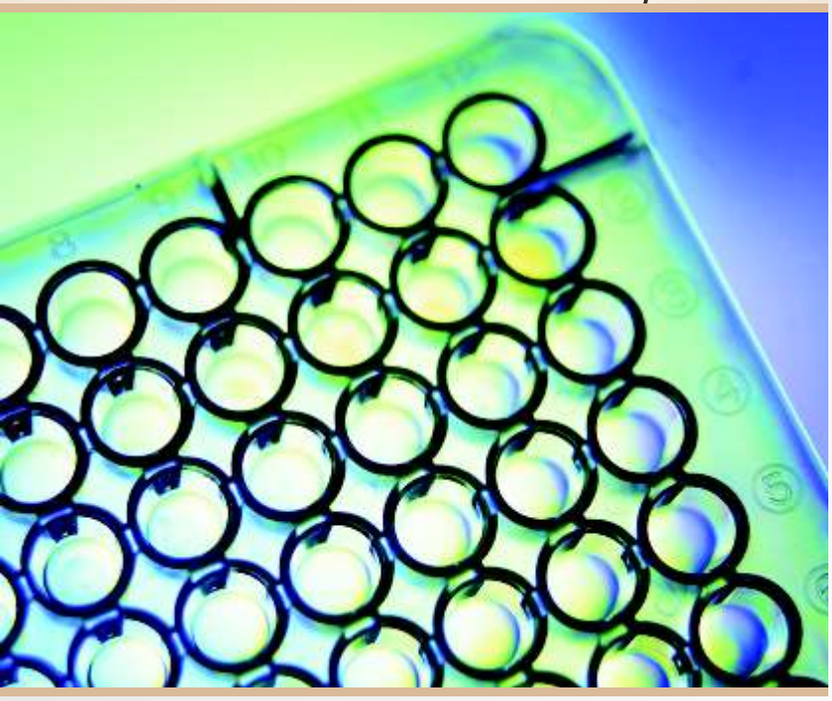
Fig. 3: Schematic representation of proposed mechanism of Ginir action.

In conclusion, our studies show that only Ginir but not Giniras causes defects in pathways associated with degradation of Cyclins D and E mediated through Culins 1 and 3 leading to faster cycling of cells through G1/S phase causing error-prone DNA synthesis leading to tumor development followed by metastasis. We propose that Ginir overexpression causes genome-wide changes that lead to cancer, whereas the Giniras RNA is required to maintain cellular homeostasis and also suppresses cancer *in vivo*. A similar effect is also manifested by loss of another nc-RNA - Xist localized on X-chromosome that results in X reactivation and consequent genome-wide changes that lead to cancer, thereby causally linking the X chromosome to cancer in mice (Yildirim et.al Cell, 2013). The identification of targets through which the oncogenic functions of Ginir are manifested is a focus of the present study as a detailed identification of targets will provide a mechanistic insight into role of Ginir in development, cell growth, differentiation, regeneration and cancer and help to answer fundamental differences that unify or diverge pathways related to transformation and stemness.

Future Work

1.? To study the involvement of Ginir in cell invasion & metastasis by *In-vivo* imaging & marker analysis and to deduce the mechanism involved.
2.? To investigate mechanisms of heterochromatin mediated changes like IGF-2 over expression & de-repression of satellites elements by Ginir.
3.? To identify and validate binding partners of Ginir by RNA pull downs & RNA CHIP assays

Research Report



Cell Biology

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Inter-cellular transport of Ran GTPase

Background

The well-structured nucleus helps the eukaryotic cells to achieve a fine-tuned regulation of gene expression, but demands the cell to have mechanisms in place to coordinate the transport of macromolecules across the nuclear membrane for effective nuclear-cytoplasmic communication and cell homeostasis. One of the major pathways regulating nuclear import and export involves the GTPase Ran. The asymmetric localization of Ran's regulators - the guanine nucleotide exchange factor RCC1 in the nucleus and the GTPase activating protein RanGAP1 in the cytoplasm - primarily generates a RanGTP gradient across the NE, which dictates the directionality of nuclear transport. One of the well-studied transport processes is mediated through the RanGTP-binding transport receptors called importins and exportins. The import complex, consisting of the cargo protein that possesses the nuclear localization signal (NLS) and the import receptors (importin α/β), is assembled in the cytoplasm, and is transported through the nuclear pore complex (NPC) to the nucleus. Binding of RanGTP to importin β displaces and releases the cargo inside the nucleus. Conversely, the export complex is formed in the nucleus by the trimolecular association between the cargo that possesses the nuclear export signal (NES), exportin1 (also called CRM1) and RanGTP, which upon reaching the cytoplasm through the NPC, is disassembled as a consequence of RanGAP1-mediated hydrolysis of GTP bound to Ran. Some transport receptors also help in localizing different RNA species/RNA protein complexes into the nucleus or to the cytoplasm. For example, Snurportin1 mediates nuclear import of spliceosomal UsnRNPs in an importin β-dependent manner and exportin-5 is an adapter for miRNA export from the nucleus to cytoplasm. Exportin-1 aids in the export of several UsnRNAs, a subset of mRNAs, and assembled ribosome subunits from the nucleus. Similarly, Exportin-t is an adapter used in the export of tRNAs from the nucleus to cytoplasm in a RanGTP-dependent manner. Apart from the well-defined function in nuclear transport, Ran GTPase also plays critical roles in mitosis, cell cycle progression and NE reformation, through a mechanism similar to that employed in nucleo-cytoplasmic transport.

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In addition to the intra-cellular signalling, multi-cellular organisms also had to evolve robust inter-cellular communication system to coordinate trivial processes during growth, development and adult homeostasis. One of the well appreciated forms of such communication is initiated by specific binding of a ligand secreted by one cell to the transmembrane receptor present on the recipient cell, and subsequent relay of signalling through defined protein-protein and protein-nucleic acid interactions. Recent studies have identified other modes of cell-cell communication to include distribution of molecules between cells through tunnelling nanotubes (TNTs) and microvesicles (exosomes and shedding vesicles). TNTs are inter-cellular actin-rich connections implicated in the inter-cellular transfer of molecules and organelles in cultured cells. However, the evidence for existence of TNTs in tissues is lacking. In addition to TNTs, inter-cellular macromolecule distribution also occurs through secreted vesicles, generally termed as microvesicles. Whereas exosomes are vesicles derived from multivesicular bodies, the shedding vesicles are generated by the direct budding from the plasma membrane. These microvesicles are shown to contain a plethora of proteins, mRNAs and miRNAs. Interestingly, TNTs and microvesicles are shown to function in immune cell signalling and cancer progression.

Preliminary results suggested that RanGTPse could exhibit inter-cellular movement.

Aims and Objectives

1. Confirm inter-cellular transport of Ran GTPase.
2. Understand the molecular mechanism of inter-cellular transport.
3. Study the functional relevance of the inter-cellular movement of RanGTPase.

Work Achieved

Intriguingly, we observed that transfection of HeLa cells with GFP-tagged version of RanQ69L (GTPase-deficient mutant, therefore predominantly GTP-bound), resulted in its expression in almost all cells (Fig 1A). RanQ69L showed very distinct accumulation on NE as reported earlier for RanG19V, a similar mutant resistant to GTP hydrolysis. On the contrary, under the same conditions, a lower number of cells expressed GFP control or a Ran mutant deficient in nucleotide binding (RanT24N). Transfected constructs expressed the proteins of expected molecular weight and their identities were confirmed by Western analysis with GFP and Ran specific antibodies (Fig. 1B). The presence of RanQ69L in most cells would indicate many possibilities, one of them being transfer of the Ran protein from one cell to the other.

To test the possibility that RanQ69L transfer occurs between cells, transfected HeLa (human) cells were co-cultured with untransfected NIH3T3 (murine) cells, which could be distinguished from HeLa cells due to the punctate staining of the murine nucleus by Hoechst dye (Fig 2, arrows). We could find that a significant number of NIH3T3 cells displayed GFP-RanQ69L, as compared to GFP control, suggesting that the protein has

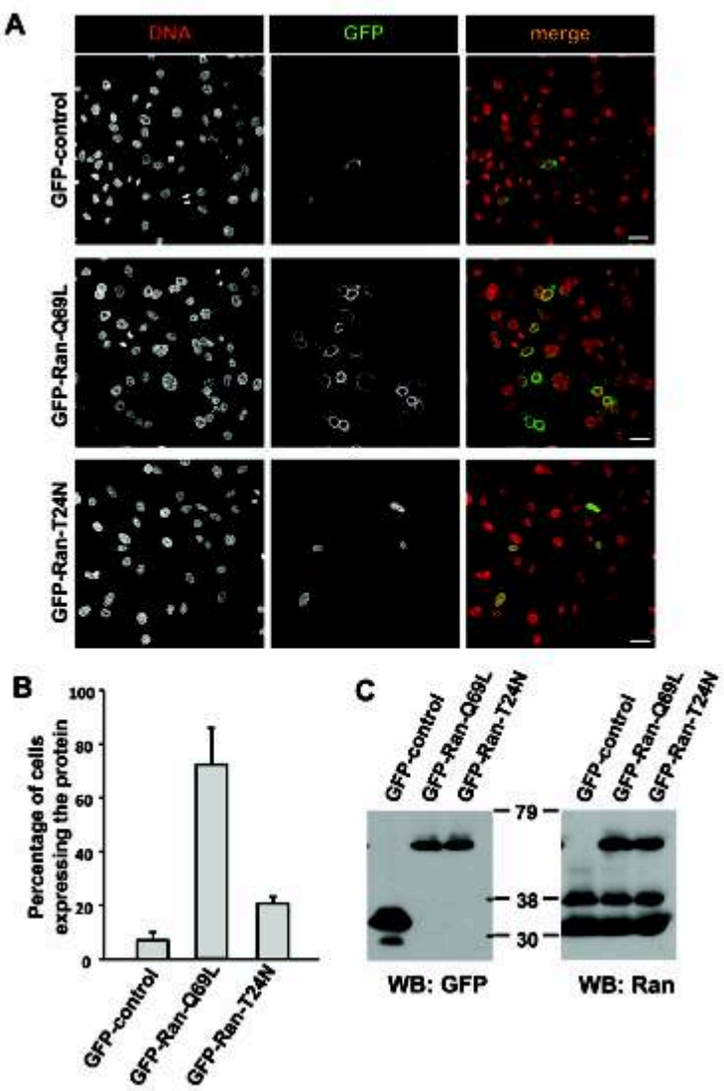


Fig. 1: Expression of Ran mutants. (A) HeLa cells were transfected with indicated constructs for 48 h and were processed for immunofluorescence microscopy. GFP is detected with a specific polyclonal antibody (green) and DNA was stained with Hoechst 33342 (pseudocoloured in red). Scale bar, 25µm. (B) Percentage of cells expressing the GFP proteins was derived from three independent experiments (in each experiment at least 100 cells were counted). Data are expressed as mean±SD. (C) Cells transfected with indicated constructs were lysed and analysed by western blotting (WB) with GFP and Ran specific antibodies. Molecular weights (in kDa) are in numbers.

been transferred from HeLa cells (donor) to NIH3T3 cells (recipient). Interestingly, GFP-RanT24N showed significantly less transfer as compared to RanQ69L (Fig. 2), indicating that the cell-cell distribution of Ran is GTP dependent.

To further study the details of inter-cellular transport of Ran GTPase, we developed a simple assay by transiently co-transfecting GFP-Ran with cherry-α-tubulin for 9h, and by monitoring the presence of fluorescently tagged proteins by microscopy. In GFP control and cherry-α-tubulin double transfected condition, we noticed that most of the transfected cells expressed both cherry-α-tubulin and GFP (95.25%, n=300, from three independent experiments). However, when GFP-RanQ69L was co-expressed with cherry-α-tubulin, we found many cells showing the presence of only GFP-RanQ69L. Moreover, we could identify the cell primarily transfected with RanQ69L (as judged by the presence of the transfection marker cherry-α-tubulin, referred to as donor cell) displaying

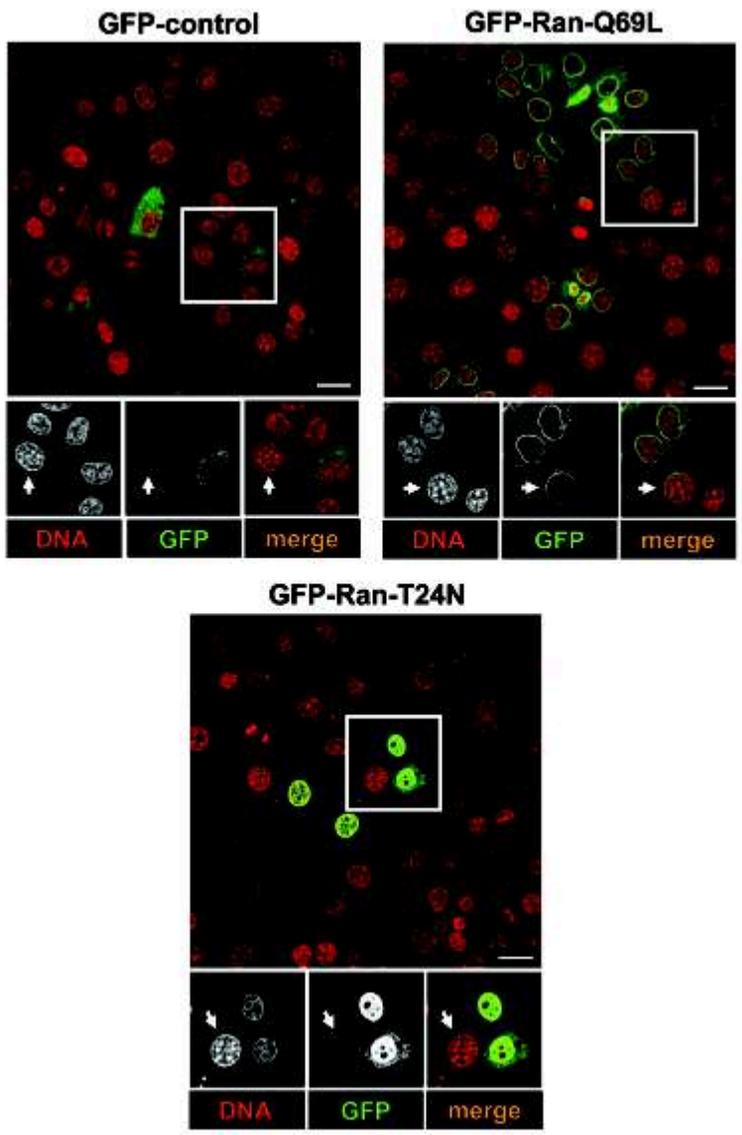


Fig. 2: Inter-cellular transfer of Ran. HeLa cells were transfected with indicated constructs for 9 h and were then co-cultured with untransfected NIH3T3 cells for 18 h. Cells were stained with GFP antibody (green) and the DNA dye Hoechst 33342 (pseudocoloured in red). Arrows indicate NIH3T3 cells as detected by the characteristic punctate staining of the nucleus. Scale bar, 25 µm

higher levels of GFP fluorescence (Fig. 3A). Strikingly, the recipient cells surrounding the Cherry-α-tubulin positive donor cell displayed a gradation of GFP fluorescence; the intensity being maximum in the cherry-α-tubulin positive donor cell and decreasing away from the donor cell as a function of distance. Intriguingly, even within the recipient cell, we could observe more accumulation of GFP on the part of the NE facing the donor cell as compared to the opposite side (arrowheads, Fig. 3A).

The above results show that the GTP-bound Ran specifically gets distributed from cell to cell. We further quantitated the extent of transfer of different Ran mutants, by counting the number of cells showing GFP fluorescence surrounding the donor cell. Within a period of 9-10 h post transfection, RanQ69L (GTP-Ran) showed maximum cell-to-cell transfer as compared to control GFP (Fig. 3B). RanG19V, another mutant deficient in GTP

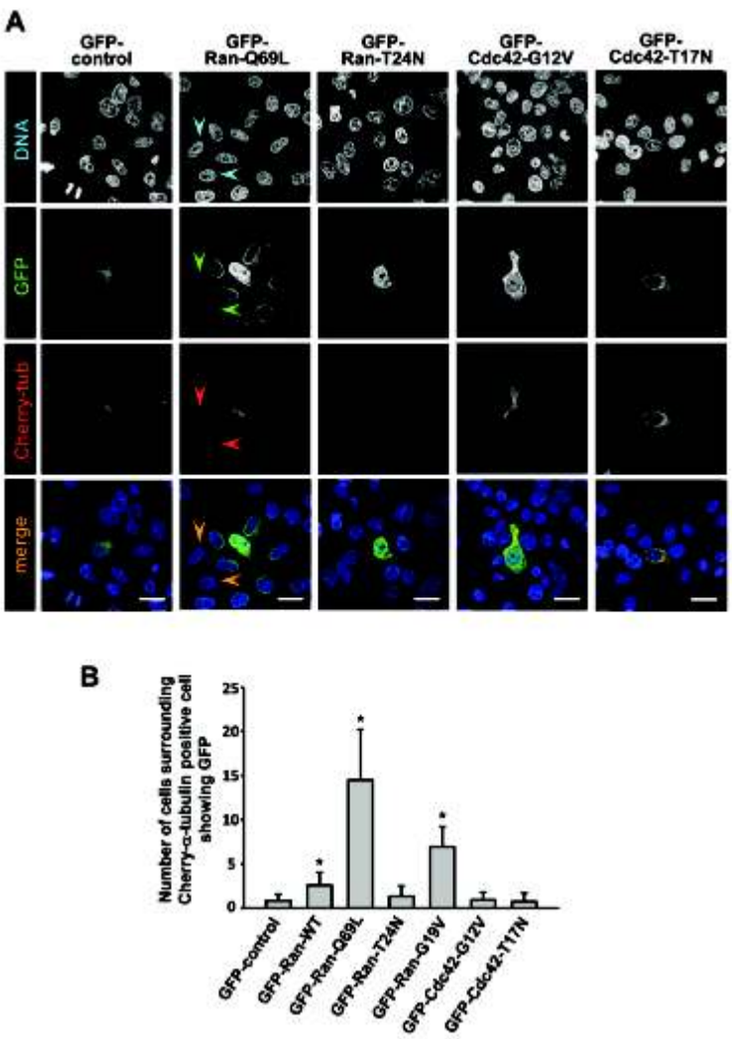
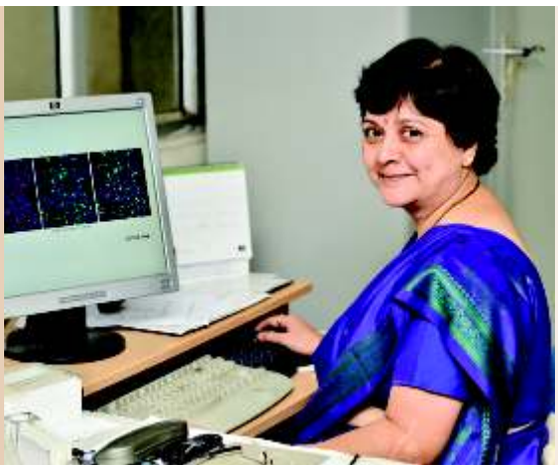


Fig. 3: Transcient transfection assay for inter-cellular transport of Ran. (A) HeLa cells were cotransfected with cherry-a-tubulin (transfection marker, Red) and indicated GFP constructs (Green). DNA was stained in blue. Arrow heads indicate receipient cells showing asymmetric localization of GFP-RanQ69L on the nuclear membrane Scale Bar, 25µm. (B)Quantitative data showing the number of recipient cells displaying GFP staining surrounding the cherry-α-tubulin positive donor cells. Cells were counted from 15 individual fields randomly across three independent experiments. Data are expressed as mean ± SD. *P < 0.001

hydrolysis and therefore GTP-bound, also showed enhanced transfer as compared to wild type or RanT24N mutant. The specificity of Ran GTPase transfer was also confirmed by the fact that mutants of Cdc42, another Ras-like GTPase, showed no such inter-cellular distribution (Fig. 3A, B). Collectively, these data demonstrate that Ran GTPase has the unique ability to move from cell-to-cell in a GTP-dependent manner.

Future Work

1. Understand the molecular mechanism of inter-cellular transport.
2. Study the functional relevance of the inter-cellular movement of RanGTPase.



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A study to determine the safety and efficacy of extra-cellular matrix (ECM) embedded bone marrow-derived endothelial progenitor cells (EPC) in healing of diabetic wounds using diabetic mouse model

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Background

Diabetes mellitus (DM) is a metabolic syndrome typically characterized by hyperglycemia and widespread endothelial dysfunction, among the other secondary complications. Endothelial Progenitor Cells (EPCs) contribute to neovascularization and homeostasis of the vasculature and effective migration of the same is imperative for efficient wound healing. An impaired migration ability of these cells is broadly termed as endothelial dysfunction. In DM, the endothelial dysfunction may arise due to several causative factors such as reduced nitric oxide (NO) bioavailability, insulin resistance, and hyperglycemia and may effectively lead to impaired wound healing.

These defective wound repair mechanisms can be counteracted by transfusion of EPCs from non-diabetic animals. Consequently, the delivery of normal EPCs to diabetic wound sites promises to be an effective mode of treatment. An effective and sustained delivery of normal EPCs to wound site is a challenge. Current systems allow a large bolus of cells to be delivered ectopically onto the wound bed. The efficacy of this system decreases as many cells are 'lost-in-transit', through nutrition deprivation-induced cell death or through 'flow-away' mechanisms. Various synthetic extra-cellular matrices fabricated from biocompatible polymers have been investigated for this purpose, each having its own advantages and limitations. Our objectives were to test for a matrix that allows growth and maintenance of EPCs and allows efficient and / or sustained delivery of these to wound site.

Aims and Objectives

1.? Isolation and standardization of long-term culture of EPCs from mouse bone marrow on standard (control) and novel (experimental) matrices.
2.? Choosing the most suitable matrix for study based on parameters such as EPC viability, functionality, and maintenance of stem cell niche as well as other parameters such as colony formation unit (CFU), colony adhesion assay (CAA), migration assay etc.
3.? Delivery of the cultured EPCs onto wounds created in diabetic animals and determination of safety and efficacy of the procedure.

Work Achieved

The first step was screening of suitable matrices for a sustained growth of EPCs in long term culture. We tested three substrates, namely, vitronectin (VN – non-matrix control), PLLA (poly-L-lactic-acid – matrix control) and PCG (polycarpolatone-gelatin) in detail for their EPC growth potential. The PCG matrix, fabricated by IIT, Bombay consisted of electrospun-nanofibres that were randomly oriented, uniform, smooth nano-fibres of 400-700nm diameters. A novel ratio (PC: G=1:3) of both the components was standardized by us for an optimal growth of EPCs and was used for fabrication of this matrix. This ratio is novel and offers a duel advantage of biocompatibility and ease of application. Scanning electron microscopy analyses showed that the matrix exhibited a random, non-woven smooth architecture with interconnected pores, which mimic the extra-cellular matrix (ECM) environment (data not shown).

The PCG matrix promotes growth and enrichment of EPCs from bone marrow-derived MNC population.

Under standard EPC growth conditions, it was observed that all three substrates viz. VN, PLLA as well as PCG supported MNC adherence and also promoted enrichment of true EPC population ($AcLDL^+$ / $UEA1^+$) over 14 d of culture. Further quantification revealed that all three substrates had different potential for EPC growth. At d 14, VN allowed only 185.75 ± 18.9 EPCs to be formed / 1000 seeded MNCs, PLLA allowed 373 ± 45.2 EPCs to be formed /1000 seeded MNCs while the PCG matrix proved to be the most efficient substrate for MNC attachment and EPC formation (529 ± 14.1 EPCs/1000 seeded MNCs) ($P<0.001$). These findings demonstrate that the PCG matrix has additional benefits for EPC growth as compared to VN and PLLA, both documented previously for allowing EPC growth.

PCG matrix does not affect stem cell status of the Endothelial Progenitor Cells (EPCs).

After having established that the PCG matrix promotes better EPC growth compared to VN and PLLA, it was imperative to compare the efficacy of these substrates in promoting EPC growth without compromising their stem cell status, especially since our initial seeding population was the entire 48 h non-adherent fraction of bone marrow MNCs, and no pre-selection process was employed. We found that 95.68 ± 0.63 % of the cells growing on the PCG matrix were Ac-$LDL^+UEA\text{-}1^+$ EPCs. This level of enrichment was

comparable to that found in control cells grown on VN (96.12 %) and better than that found in PLLA (90.66 ± 4.70 %; $p \leq 0.01$). These data show that PCG-matrix qualifies as a candidate for further research as a growth substrate for EPCs.

The PCG matrix promotes cell adhesion, colony formation and cellular proliferation along with maintaining cellular viability.

A primary concern for such application-oriented systems is the evaluation of the exact efficiency of cell growth and its numerical advantage over the control substrates. We further tested the efficacy of the PCG matrix by comparing the three groups for cellular parameters such as cell adhesion (CAA), colony formation (CFU), proliferation potential (MTT assay) and viability maintenance by the trypan blue dye exclusion test.

It was observed that VN, PLLA as well as PCG matrix allowed MNC adhesion and EPC formation. The PLLA and PCG matrices showed higher cellular adherence (2.16 and 4.42 folds higher; respectively; P<0.001), colony formation (1.10 and 1.33 fold respectively; P<0.001) as well as proliferation (1.25 and 1.17 fold respectively; P<0.001) compared to VN (Fig. 1 A, B and D respectively). The average colony size on the PCG matrix (24.16 ± 2.56 cells per colony) was also larger compared to that on VN (17.66 ± 1.63 cells / colony) and PLLA (20.27 ± 2.74) controls (P<0.010) Percent viability assessment revealed that loading onto PCG did not affect average cellular viability (Fig. 1C).

The PCG matrix allows sustained in vitro migration of EPCs.

Although we have now demonstrated that PCG matrix has the most number of live deliverable EPCs, it was necessary to ascertain whether the matrix actually delivers those cells onto the wound bed. Migration assay revealed that PCG and PLLA allowed a slower

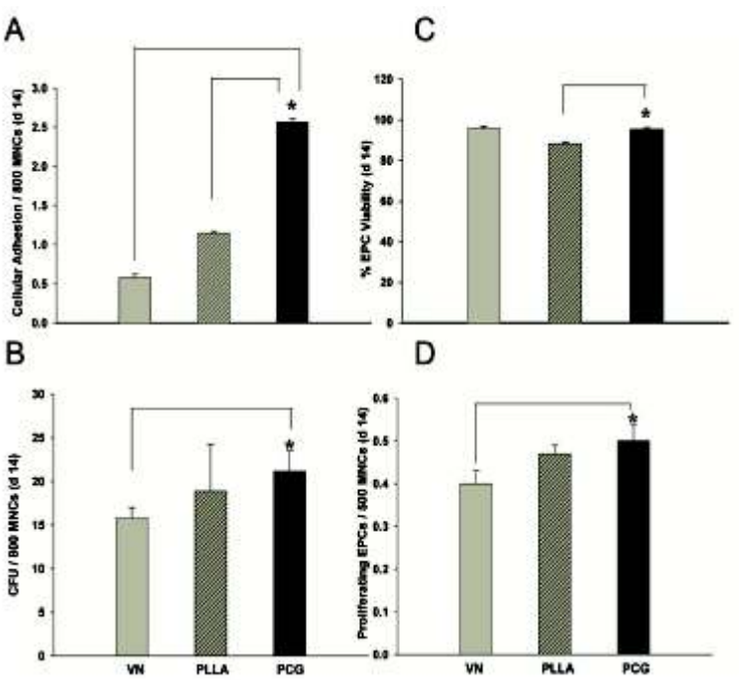

Fig. 1: Biocompatibility of the PCG matrix was assessed by estimation of cell adhesion **(A)**, colony forming cells /units **(B)**, % viability **(C)** and proliferation potential **(D)** on d 14, in comparison with the parallel VN- and PLLA-grown EPCs used as controls. Data are represented as mean of triplicate wells from 5 independent experiments (n=5) ± SD. * p <0.001

migration of cells towards VEGF and only 52.87 ± 7.24 and 50.36 ± 9.2 respectively % of total EPCs migrated by 72 h in comparison to VN, where 81.87 ± 6.17 % of cells had migrated by 72 h (Fig.5; P<0.001).

We further found that the migrated VN-EPCs showed a gradual reduction in % viability over the 24, 48 and 72 h of migration. PCG (95.36 ± 12.5, 90.37 ± 51.2 and 87.45 ± 37.2 at 24, 48 and 72h respectively) and PLLA (93.11± 12.8, 90.51 ± 13.6 and 84.84 ± 18.8) maintained a significantly higher level of viability of the migrated EPCs compared to VN (92.6 ± 20.0, 86.24 ± 16.5 and 76.58 ± 32.6 respectively) at the same time points. However, here too, comnia the PCG matrix showed a significantly higher cellular viability maintenance compared to PLLA (P<0.001).

Topical application of matrix-embedded EPCs onto diabetic wounds results in accelerated and scar-free wound healing.

We wished to further determine whether the potential evident in the in vitro studies translates into its in vivo applicability in diabetic wounds. Full thickness wounds were created on diabetic mice and wound healing was monitored by measuring % wound closure as a function of time (Fig. 2A). The data revealed that the EPC-treated, VN, PLLA or PCG, groups showed a significant improvement in wound healing from day 2 itself, as opposed to the untreated diabetic control groups (Fig. 2B; P<0.010). By day 10, the

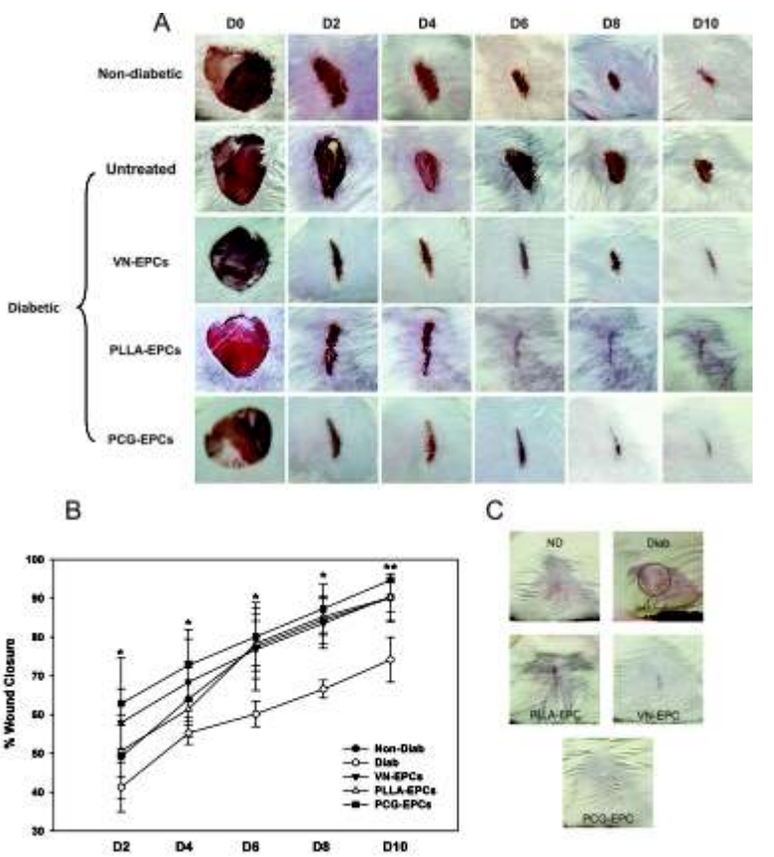

Fig. 2: (A).Photographic evidence of wound healing in non-diabetic animals in comparison to untreated diabetic, VN-EPC-treated, PLLA-EPC-treated and, PCG-EPC-treated diabetic animals, from d 0 to d 10. Photographs are from one representative experimental set from among 3. (B). Graphical representation of percent wound closure in various groups. Percent wound closure in PCG-EPCs is significantly higher compared to all other treatment groups on days 2 to 8 (*P<0.010) as well as on day 10 (** P<0.050).(C)Photographic evidence of scar tissue formation in non-diabetic (ND), untreated diabetic (Diab), VN-EPC-treated (VN-EPC), PLLA-EPC-treated and PCG-EPC-treated diabetic mice (PCG-EPCs) on day 22. Fibrotic tissue formed in untreated diabetic mouse is encircled in black.

master controls and the VN-EPC-treated animals showed comparable % wound closure (90.1 ± 6.24 and 90.47 ± 4.06 % respectively), while the PCG-EPC group consistently showed a higher wound closure from days 2 to 10 while achieving a significantly higher (94.77 ± 2.6, P<0.010), wound closure than the VN-EPC and PLLA control groups, including the diabetic control, which did not achieve complete wound closure (74.16 ± 5.8 %). The scar tissue formation studies (Fig. 2C) performed on d 22 showed an intense fibrotic tissue scar formation (encircled) in the healed untreated diabetic wounds. On the other hand, the diabetic animals treated with VN-EPCs showed only marginal scar formation and diabetic mice treated with PLLA-EPCs showed complete but irregular wound healing. On the other hand the diabetic mice treated with PCG-EPC showed no scar tissue formation and exhibited a complete healing of wound. These observations demonstrate that PCG-EPC system significantly improves the rate and quality of diabetic wound healing compared to master and VN-EPC controls.

PCG-EPCs show efficient incorporation into the diabetic wounds

EPCs were labeled with CMRL-Orange to investigate their incorporation into healing diabetic wounds. Cryosections of the wound biopsies taken after 48 h of application revealed that only a marginal extent of VN-EPCs got recruited into the healing wounds, while the recruited number of both PLLA- and PCG-EPCs into the wound bed was significantly higher (Fig. 3). It is probable that perhaps most of the bolus-delivered VN-EPCs got washed off post application. It is also probable that, the average viability count of the VN-EPCs may have dropped post-application due to the hostile in vivo conditions. The PLLA- and PCG-EPCs, on the other hand, were able to survive longer than the VN-EPCs as they were embedded in the matrix, which may have accorded some protection to these cells. Interestingly, though the PLLA-EPCs showed a better incorporation of EPCs in the wounds compared to the VN-EPCs, but they did not migrate as deep into the wound bed as the PCG-EPCs did. These data indicated that the PCG-EPCs were more active in this respect. It will be of interest to determine whether PCG-matrix activates specific signaling mechanism in the EPCs.

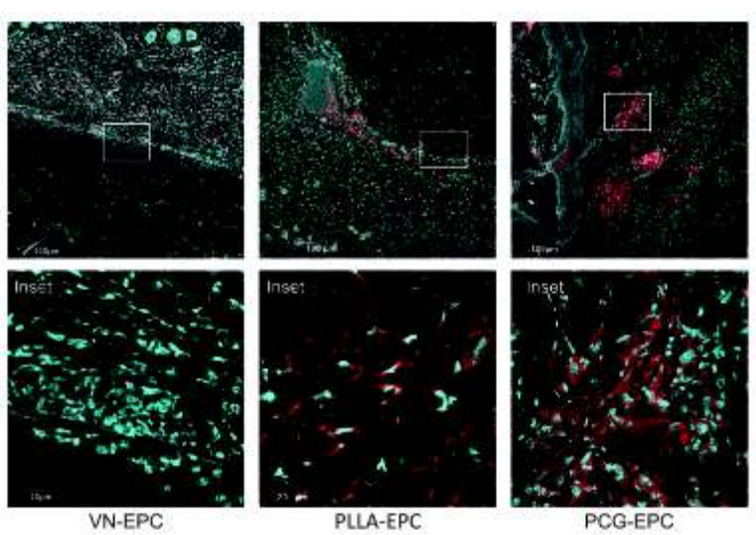

Fig. 3: PCG-EPCs migrate deep into the wound beds. VN-EPCs, PLLA-EPCs and PCG-EPCs, pre-stained with cell tracker-Orange, were delivered at d 0 onto diabetic wounds for 48h. Cryosections were counterstained with DAPI and analyzed for incorporation of pre-stained EPCs into wound beds of diabetic mice. Scale bars for images =100 µm; Scale bars for insets = 20 µm

Overall, the data show that application of PCG-EPCs results in an effective delivery of more number of live cells to the wound bed leading to their better recruitment.

Conclusions

This novel PCG-EPC system is a one-step "combined growth and delivery system" for direct application onto the dermal lesions, including the diabetic wounds. The dual advantage offered by this matrix in terms of its EPC growth-supporting property and its 'easy to handle' nature is of immense importance in clinics practicing cell therapy in human subjects as well as in the veterinary practice.

Future Work

Similar line of experimentation will be taken with human peripheral blood-derived EPCs grown on PCG-matrix and applied onto the wounds created in diabetic NOD/SCID mice. If found efficacious, this system can be used in a translational research program for its ultimate clinical application.

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Delineating Wnt mediated ES cells maintenance and pluripotency

Background

Embryonic stem (ES) cells are one of the most coveted sources of stem cells having immense implications in development and therapy. Maintenance of these cells in undifferentiated state during propagation is vital for the retention of their indefinite self-renewal and pluripotent characteristics and undoubtedly this is tightly regulated. The conventional means of maintaining ES cells in culture is either by culturing them on mitotically inactive fibroblast feeders or by supplementing the culture medium with cytokine LIF. LIF binding to its receptor activates JAK-STAT pathway that helps in the maintenance of ES cells in undifferentiated state by the expression of pluripotency associated genes and the suppression of differentiation inducing factors. In recent years, several other pathways have also been identified to be operational during ES cells maintenance. Our earlier findings have indicated the activation of canonical Wnt signaling in help maintaining the unlimited self-renewal and pluripotency in ES cells. While BMP acts in concert with LIF during ES cells maintenance, and we have seen co-modulatory effect of BMP and Wnt during various developmental events, it remains to be seen whether there exists any synergy between LIF-Stat3 and Wnt pathways during the maintenance of ES cells. Hence, we extended our investigation to delineate the mechanistic basis of Wnt mediated ES cells maintenance, whether it would work independent of or in concert with LIF-Stat3 pathway.

Aims and Objectives

1. The maintenance of ES cells under Wnt activation conditions and assessing their stemness and pluripotent characteristics both *in vitro* and *in vivo*.
2. Investigation of the mechanistic basis of Wnt mediated ES cells maintenance.

Work Achieved

Our earlier findings indicate the canonical Wnt/β-catenin signaling but not the non-canonical one was adequate for indefinite self-renewal of embryonic stem (ES) cells under the LIF deprived condition. Although Wnt signaling seemed dispensable under LIF supplemented condition, it helped in ES cells maintenance in the absence of LIF and

Participants

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retained pluripotent characteristics. Interestingly, a marked decrease in Stat3 was evident in ES cells maintained under the Wnt activation conditions, compared to the LIF maintained ones, thereby providing direct evidence of its LIF-Stat3 independent action. Hence, it is was presumed that, parallel LIF-Stat3 and Wnt signaling might be operational during ES cells maintenance. To further authenticate this, Stat3 knock down was carried out in ES cells. The Stat3 deficient ES cells exhibited an overall growth impairment compared to control irrespective of their maintenance in presence of either LIF or Wnt activator in the medium, and more drastically in the latter case. However, these ES cells retained the expression of pluripotent associated ES cell signature genes and were capable of generating teratoma upon injection into SCID mice. Strikingly though, there was significant variation in the teratoma inception and their respective growth characteristics among different samples that were injected. In fact, the Stat3 deficient cells maintained in presence of Wnt activator yielded comparatively smaller teratoma upon injection into SCID mice and with a longer inception period, while in the LIF maintained ones it was comparable to the control.

Further attempt was made to determine the *in vitro* differentiation potential of Stat3 deficient and sufficient cells to determine the role of Stat3 during the same. Since the IL6 members; LIF and CNTF are known to have critical influence during both neuronogenesis especially during the generation of dopaminergic (DA) neuronal subtypes, and gliogenesis, we established stable Stat3 knockdown clones using Th-EGFP ES cells. The

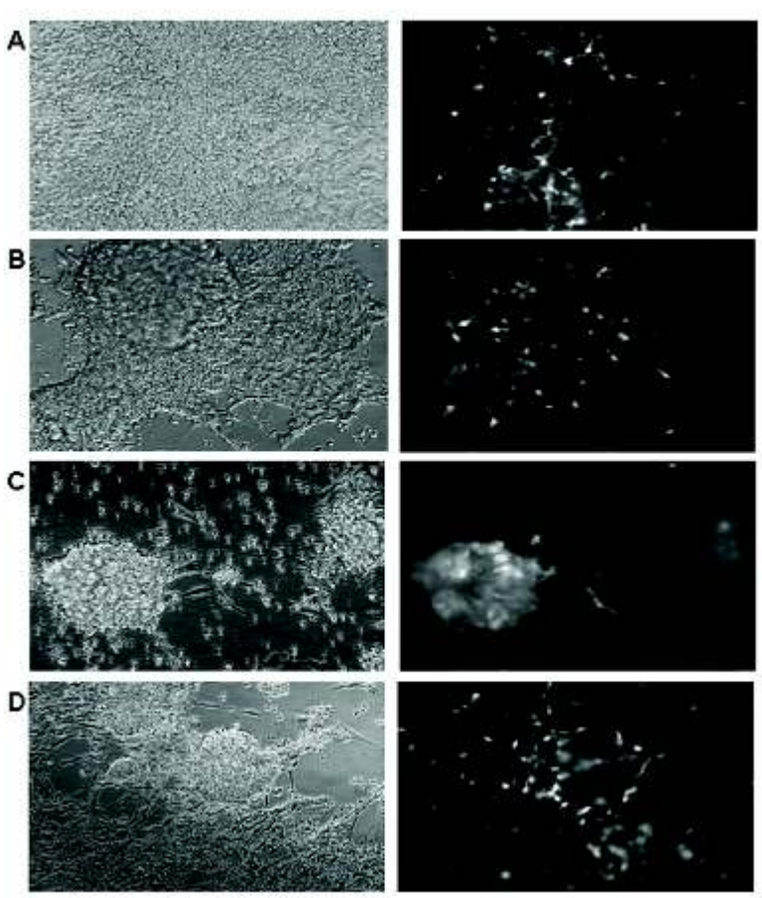

Fig. 1: Differentiation of Control (A,B) and stat3 knock down ES cells maintained either under LIF (A,C) or Wnt activation (B,D) conditions into DA neurons.

Th-EGFP ES cells are the stable clones established earlier in the laboratory where EGFP is under the regulatory control of the DA neuron specific promoter, the Tyrosine Hydroxylase. These Stat3 knockdown clones were maintained either in the presence of LIF or the Wnt activator and further subjected to monoculture based neural differentiation. Irrespective of the maintenance conditions used such as LIF or Wnt activation, under which the cells were being maintained in undifferentiated state and taken further for differentiation, all of them exhibited EGFP expression during neural differentiation reflecting to DA neuron generation. However, the neurite extension was severely impaired in case of Stat3 knockdown compared to control (Fig. 1). Interestingly, the same was reversed in case of Stat3 knockdown clones maintained in presence of Wnt activator and hence suggesting that, Wnt might be overcoming the Stat deficiency and exerting its pro-neural influence. This was authenticated further by immunostaining with neuronal specific marker, Map2. Further investigations are underway to determine the mechanistic basis of Wnt mediated ES cells maintenance and also the role of Stats during neurogenesis and neurite extension.

Future Work

Further investigation will be undertaken to ascertain the mechanistic basis underlying Wnt mediated ES cells maintenance and also the hither-to-unknown pivotal role of Stat3 during neuronal arborization.

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Studies on expansion, cryopreservation and differentiation of hematopoietic stem cells

Background

A. Ex vivo Expansion of HSCs

Ex vivo expansion is attempted to overcome the problem of limited cell dose of Umbilical Cord Blood for transplantation and thus widening its effectiveness for adult patients. Though ex vivo expansion is an attractive strategy the deteriorated quality of the expanded cells limits its utility. The involvement of apoptosis during the expansion has been accounted for the inferior quality of the expanded cells. Earlier, we have demonstrated the beneficial effect of the apoptotic inhibitors on expansion of HSCs in suspension cultures. However, cell-to-cell contact seems to be indispensible to maintain potential for self-renewal of HSCs. The supportive effect of co-cultures with stromal / feeder layer has been recognized. Mesenchymal stem cells (MSCs), one of the constituents of the HSC niche *in vivo* have shown to enhance expansion of HSCs *in vitro*. Thus, in the present work we have established the HSC MSCs co-culture system to elucidate apoptotic signaling operative between the stromal cells and HSCs.

B. Studying the effect of oral administration of nutraceuticals on hematopoiesis and thrombopoiesis in mice

Previous studies from our laboratory have shown that poly unsaturated fatty acids (PUFAs) enhance megakaryopoiesis and platelet formation from umbilical cord blood derived CD34+ cells *in vitro*. Since PUFAs are classified as well known nutraceuticals, as an offshoot of this work, we decided to study the effect of oral feeding of these nutraceuticals on hematopoiesis and megakaryopoiesis of mice. Initial study showed that oral administration of docosahexaenoic acid (DHA, a N3-PUFA) enhances *in vitro* hematopoietic and thrombopoietic potential in mice. *In vitro* hematopoietic potential was checked by side population assay and multi-potent HSCs were detected by KTLS analysis using Flow Cytometry. Colony forming unit (CFU) assay has been used to detect Hematopoietic Stem and Progenitor Cells (HSPC) representing multipotential

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progenitors and late committed progenitors. Colony Forming Unit-Megakaryocyte (CFU-MK) assay which is similar to CFU assay, was carried out to detect megakaryocyte progenitors.

C. Identification of cellular and molecular events involved in Platelet biogenesis *in vitro* in the presence of Poly Unsaturated fatty acids.

Megakaryopoiesis and thrombopoiesis involve the commitment of hematopoietic stem cells towards Megakaryocyte (MK) lineage and platelet production. A better understanding of the mechanism of platelet generation and identifying the agents that can enhance the platelet production will help in improving *in vitro* platelet generation. Earlier studies in our laboratory show that Poly-unsaturated fatty acids enhance megakaryocytes and platelets generated from cord blood derived CD34+ cells. But the exact molecular mechanism is unknown. In the present project proposal we are trying to understand this mechanism by using the Megakaryoblast cell line MEG-01 and megakaryocytes obtained from cord blood derived CD34+ cells as models. These cells will be differentiated to MKs and platelets using different growth factors and also in presence of nutraceuticals to study the cellular and molecular events occurring during their generation.

Aims and Objectives

1. To elucidate apoptotic signaling operative during the expansion of HSCs in HSCs MSCs co-culture system.
2. Evaluation of the effect of oral feeding of nutraceuticals belonging to the class of poly unsaturated fatty acids on the haematopoiesis and thrombopoiesis of mice.
3. To understand the Cellular and Molecular events involved in megakaryocyte and platelet formation *in vitro* in the presence of PolyUnsaturated fatty acids.

Work Achieved

Ex vivo expansion of HSCs

Earlier we have demonstrated the beneficial effect of the apoptotic inhibitors on expansion of HSCs in suspension cultures. In the present work we have established the HSC MSCs co-culture system with MSCs isolated from Cord tissue (CMSCs) and placental tissue (PMSCs) to elucidate apoptotic signaling operative between the stromal cells and HSCs. The cells expanded in the co-cultures in the presence of apoptotic inhibitors displayed higher proliferation with significantly more retention of the primitive $CD34^{+}CD38^{-}$ and $CD\ 133^{+}$ cells and improved *in vitro* functionality as compared to control co-cultures (Fig 1 a). Amongst the feeders we observed a difference in the ability of CMSCs vs. PMSCs for the expansion of HSCs. We discovered that co-culture with the PMSCs in presence of apoptotic inhibitors harbored significantly more primitive HSCs (Fig 1 b) with improved *in vitro* functionality (Fig 1 c). Thus we conclude that the synergy between MSCs and apoptotic inhibitors facilitate superior *ex vivo* expansion of HSCs. The placental MSCs in presence of apoptotic inhibitors pose a better system for expansion of

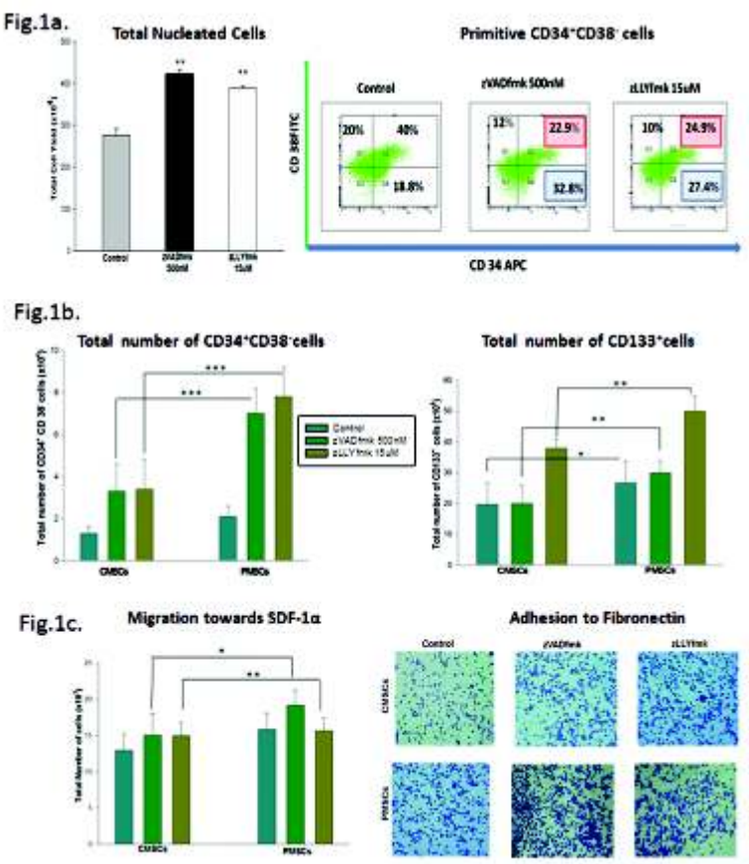

Fig. 1: UCB CD 34^{+} cells were co-cultured with MSCs (CMSCs / PMSCs) in the presence of growth factors and with or without apoptotic inhibitors for 10 days **(a)** Total Nucleated Cell yield, FACS profile of representative sample depicting primitive CD34^{+}CD38^{-}cells. **(b)** Differential ability of the CMSCs and PMSCs for the expansion of CD34^{+}cells: Preservation of primitive HSCs upon co culture with PMSCs in presence of apoptotic inhibitors. **(c)** Superior in vitro migratory and adhesive properties of the CD34^{+} cells expanded with PMSCs with apoptotic inhibitors.

UCB CD 34+ cells. Therefore our expansion strategy which results in superior quality graft may find direct application in UCB transplantation settings in the clinics.

Nutraceuticals feeding

In continuation with previous work, in the present study, we have found that DHA also significantly increases multipotent stem cells *in vivo* as detected by CFUS assay. (Fig.2a)

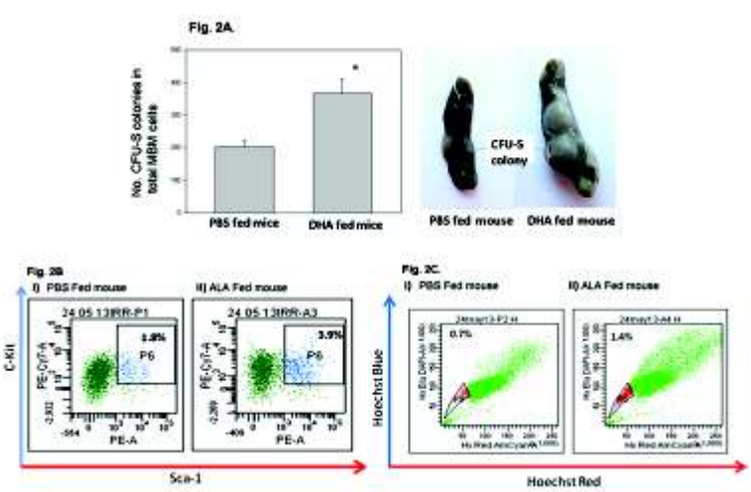

Fig. 2: Oral administration of DHA/ ALA shows significantly enhanced *in vivo* hematopoietic potential (Fig. 2A); increased *in vitro* hematopoietic potential (Fig. 2B.) and enriched primitive progenitor cell population

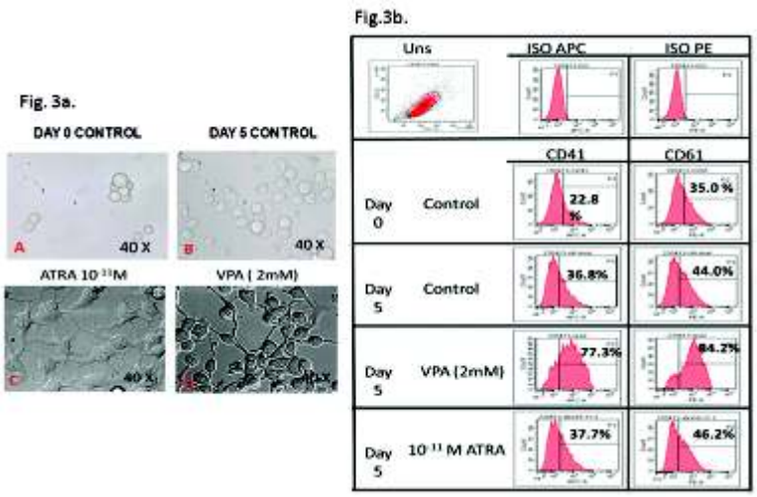

Fig. 3: Differentiation of MEG-01 cells in presence of 2mM Valproic acid and 10^{-11}M ATRA shown by **(a)** Changes in morphology observed from day 0 to day 5 where round cells (A) become semi-adherent (B) in control, and show extensions in the presence of ATRA (C) and VPA (D). **(b)** Flow cytometry analysis done on day 0 and day 5 showing increase in the expression of CD41 and CD61 in cells treated with VPA and ATRA as compared to control.

The assay estimates the stem cells having homing and engraftment potential. In the further study, we orally administered mice with the parent fatty acid of DHA i.e. ALA (alpha linolenic acid). Its effect on hematopoiesis was evaluated using side population assay and LSK analysis. Oral feeding of ALA also enhanced the LSK cell population compared to control (Fig. 2b). ALA fed mice showed an increase in side population cells as compared with PBS fed mice (control) (Fig. 2c).

Platelet biogenesis

Differentiation studies of MEG-01 cells were carried out using Valproic acid and all-trans retinoic acid. Cells were grown in the presence of VPA and ATRA for 5 days and change in morphology was observed (Fig.3 a).Untreated cells were used as control. The cells were analyzed on day 0 and day 5, for megakaryocyte specific markers CD41 and CD61 by Flow Cytometry (Fig.3 b). Analysis of VPA and ATRA treated cells show an increase in expression of megakaryocyte specific markers which shows the beneficial effect of these additives. Now we propose to add nutraceuticals to the media and see their effect on differentiation.

Future Work

1. Elucidation of the differential ability of the MSCs derived from Cord and Placenta for the *ex vivo* expansion of the CD34+ cells.
2. To determine the individual effect of oral administration of DHA and ALA on the early primitive progenitor cell population using Long Term Culture (LTC) assays and Extended-LTC assay. To check the effect of Arachidonic acid (n-6 PUFA) and Linoleic acid (precursor of AA) on haematopoiesis and megakaryopoiesis.
3. MEG-01 cells and Cord Blood derived CD34+ cells will be treated with various combinations of ATRA, VPA ,TPO and nutraceuticals, their concentration and incubation period will be standardized.

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Translational Regulation of Insulin mRNA

Background

Insulin is a small peptide hormone secreted from the pancreatic beta cells and is important for glucose homeostasis in mammals. The deregulation of insulin biosynthesis leads to diabetes and subsequent abnormalities. Insulin expression begins at embryonic (e)9.5 day in the gut endoderm. Insulin expression in β cells is regulated by many nutrients and glucose is the pre-eminent one controlling almost all cellular processes like transcription, splicing, translation, processing and secretion of insulin. Interestingly, insulin secretion is immediately followed by several fold specific increase in translation upon glucose stimulation. A number of studies have focused on the mechanism of glucose mediated insulin translation regulation and revealed the role of the 5' and 3'un-translated regions (UTRs) and their trans-acting factors on insulin mRNA. In mouse, two non-allelic genes encode for insulin and specific splice variants from these genes have also been reported. Some splice variants have the altered 5'UTR and have differential translation efficiency and hence implicated to play some role in diabetes.

We have previously reported the expression of a novel splice variant with a 12 base deletion for the mouse insulin2 gene. This specific alternate splicing of insulin2 mRNA results in significant changes to the 5'UTR sequence and structure. . We have also shown that this 12 base deletion in the 5'UTR of mouse insulin2 mRNA (mIns2) results in a shorter 5'UTR splice variant named mIns2-S, which contributes three fourth of the insulin2 mRNA pool. This deletion resulted in the differential binding of the factors to the 5'UTR and differential translation efficiency. We believe that the differential translation of these splice variants is due to differential binding of the trans-acting factors to the insulin mRNA. We have also shown that specific proteins such as PDI and PABP bind to the 5'UTR of the insulin and activate its translation upon glucose stimulation. In the present study we show that PABP is one of the factors that preferentially associate with insulin 2L splice variant. The exact role of such association is still under investigation.

Participants

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Aims and Objectives

1. Isolation and characterization of the insulin mRNA UTR binding factors and their partners.
2. To understand the basic mechanism of translational regulation of insulin mRNA and the role of trans acting factors in this regulation.

Work Achieved

Factors in Btc6 extract associates differentially to both isoforms

We analysed the sequence specific binding of extract by competitive RNA EMSA. The lysate was incubated in presence of labeled probe and different molar excess of unlabeled RNAs. The specific complex that is with the long 5'UTR probe was competed out efficiently by long unlabeled RNA while the short RNA probe was much less efficient (Fig 1). This result indicates that mIns2L 5'UTR RNA associates with higher affinity/stability with cytoplasmic factors when compared to the short insulin 5'UTR, suggesting that the absence of 12 bases causes alteration to the sequence and structure of the 5'UTR and that may result in loss of certain binding sites for the cytoplasmic factors in βtc6 cells.

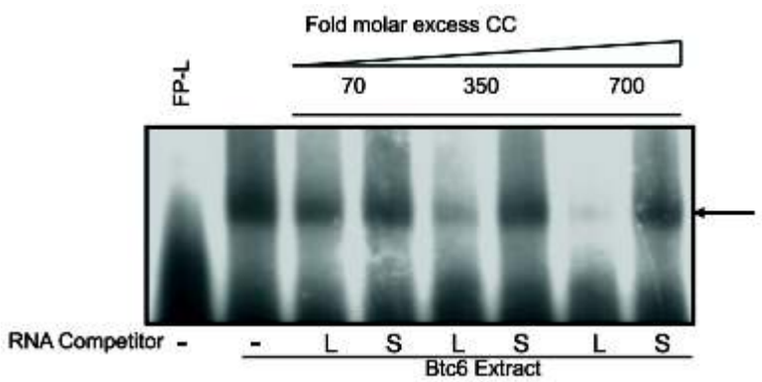

Fig. 1: Differential binding of factors from βtc6 cell to the long and short insulin 5'UTR RNA. The long isoform of insulin2 5'UTR was radiolabeled and was used as RNA EMSA probe. The EMSA reactions were performed by incubating the probe with cytoplasmic extract in the presence of varying amount of unlabeled long and short insulin 5'UTR RNA as competitors. The lanes are as indicated.

Recominant His-PABP binds more efficiently to long as compared to short splice variant

His-PABP was expressed in bacterial system and the purity of protein was assessed on 10% SDS-PAGE (Fig 2A). In order to assess the differential binding ability of PABP to mIns2 splice isoforms, we performed a competitive EMSA with purified recombinant PABP and using radiolabeled mIns2 long RNA as probe (Fig.2B). A specific complex was formed with mIns2 long RNA and His-PABP (as indicated by an arrow). The RNA-protein complex was competed out efiiciently by addition of unlabeled long RNA, but similar fold molar excess of short unlabeled RNA was noticeably less effective. This prompted us to conclude that PABP binds to mIns2 long RNA with better efficiency as compared to mIns short RNA. We are hypothesizing that the PABP is one of the factor in the binding complex.

Functional assessment of Recombinant His-PABP

PABP is known to be a translation activator but its actual role in insulin translation was assessed by in vitro translation assay. We performed in vitro translation of luciferase reporter gene containing the two different insulin 5'UTR in presence and absence of PABP.

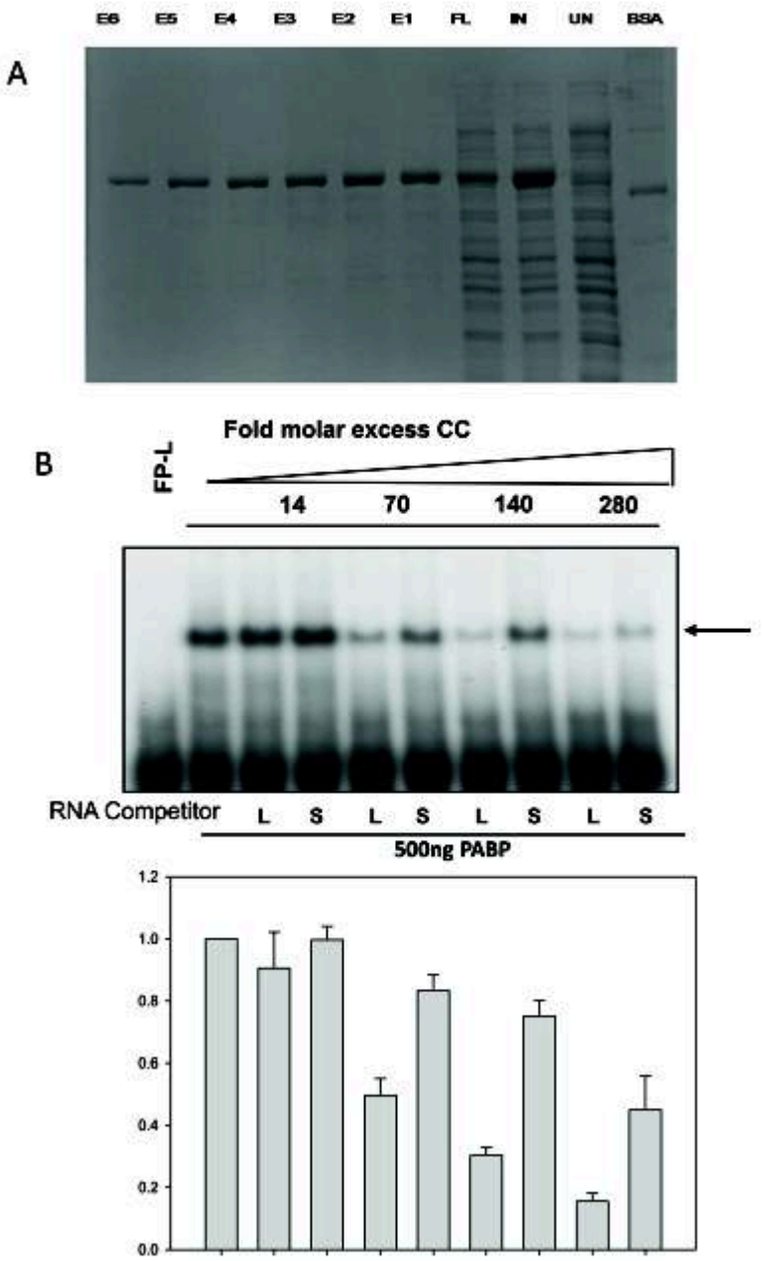

Fig. 2: Recombinant PABP associates with insulin 5'UTR splice variants in a differential manner. His tagged recombinant PABP was expressed in E. coli and purified using Nickel NTA coloumn (A). 500 ng of the purified PABP was incubated with long insulin UTR probe in the presence of varying amount of the long and short competitor RNA the molar fold excess of the competitor is as indicated (B top panel). The amount of the labeled RNA protein complex was quantitated by densito metric scan and represented in a graphical form as a fraction of the complex observed in the absence of the coompetetors (B lower panel).

As expected, addition of PABP resulted in 2 fold induction of translation of long isoform, while the addition of GFP (non specific protein control) does not affect the translation (Fig 3A). In case of short RNA, PABP inhibit the translation activity but this repression is not specific since even the control GFP inhibited the translation of short isoform (Fig 3B). We also analysed the specificity of the translation activation of the long isoform by performing the translation reactions in the presence of the RNA competitors (Fig 3C). The presence of either long or short insulin UTR RNA competitors did not affect the increase in translation observed with long 5'UTR suggesting that the increase in translation observed is likely due to the general translation activity of PABP and not due to specific interaction

with insulin 5'UTR. These results suggest that although PABP can specifically associate with insulin 5'UTR splice variants in a preferential manner but the binding alone is not sufficient to result in differential translation of the two isoforms. We believe that additional factores may interact with insulin 5'UTR that may regulate the differential translation of insulin splice isoforms.

UV crosslinking experiments were performed to identify the molecular weight of the trans acting factors binding differentially to the 5'UTR of mouse insulin2, the proteins were cross linked to the labeled insulin 5'UTR by UV and the unbound RNA region were RNase digested. The indirectly labelled proteins were resolved on 10% SDS-PAGE (Figure 3D). UV-cross linking data show that predominant band is present at about ~45kDa and additional minor band is at ~75kDa. We believe that the 45kDa protein is HuD while 75kDa corresponds to PABP.

Our earlier research had shown that both the insulin splice variants are translated differentially so we hypothesized that this difference is due to differential binding of trans acting factors. Here we are showing that one of the factor is PABP which is binding differentially but in vitro translation studies suggest that binding of PABP alone does not cause differential translation. U.V crosslinking experiments suggest that the insulin 5'UTR complex contains atleast two factors that get cross-linked (mol wt of about 45kDa and 80kDa). Recently it was reported that HuD a 45kDa protein is capable of interacting with insulin 5'UTR. We hypothesize that the 45 KDa band that we detect in UV crosslinking experiments may correspond to HuD while the 80 kDa band may correspond to PABP.

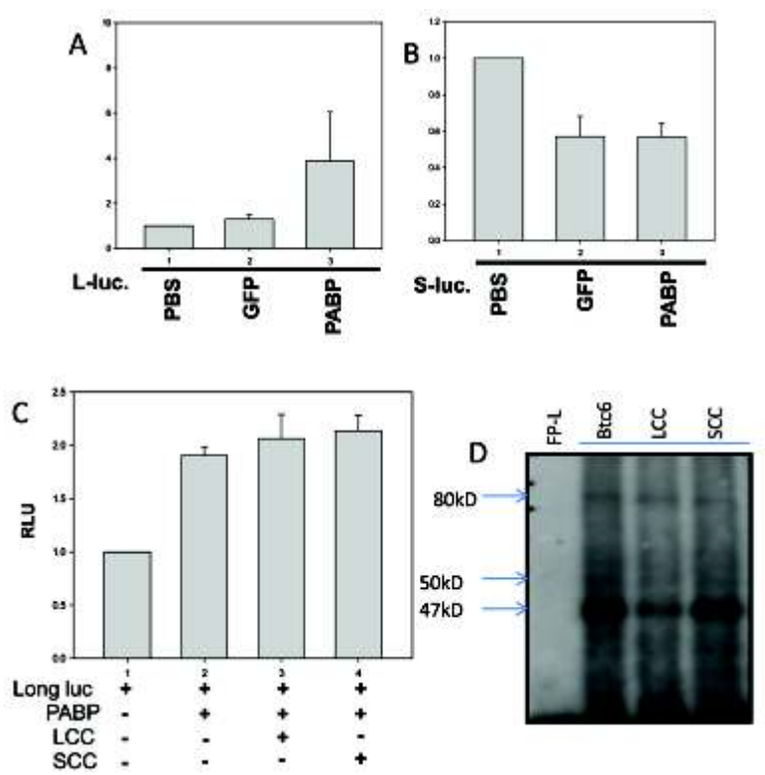

Fig. 3: PABP activates general translation. Invitro translation assay was performed using rabbit reticulocyte lysate. RNA coding for luciferase gene and containing long (A) or short (B) insulin 5'UTR was translated. Specificity of the PABP was assessed by the addition of similar amounts of His-GFP in the translation reactions. Unlabeled cold long and short insulin 5'UTR were added to the translation reaction to assess the specificity of PABP translation activation (C). The translation levels were normalized using renilla RNA with non specific UTR and the translation rates were represented as fractions of translation in the absence of any protein. UV crosslinking experiments were performed with βtc6 cell exract and long insulin 5'UTR RNA probe. The samples are as indicated and the position of crosslinked protein bands are indicated by arrows.

Thus our results indicate that specific factores associate with the two splice isoforms of insulin mRNA in preferential manner and they may be responsible for the differential translation regulation of the splice isoforms.

Future Research Plans

1.? To identify the mechanism of differential translation regulation of insulin mRNA splice variants differentially binding proteins those bind to mIns 5'UTR by RNA-pull down and Mass spectroscopy

2.? Functional characterization of Hud and PABP and their interaction with insulin mRNA

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Investigation of anti-angiogenic efficacy/ potency and molecular mechanism of the novel anti-cancer compound AECHL-1

Background

Recently, we reported the isolation and characterization of a new compound, a triterpenoid *Ailanthus excelsa* chloroform extract-1 (AECHL-1) ($C_{29}H_{36}O_{10}$; molecular weight 543.8) from the root bark of *Ailanthus excelsa* Roxb that possesses anti-cancer activity against a variety of cancer cell lines of different origin. (Lavhale et. al. PLoS One. 2009;4(4):e5365).

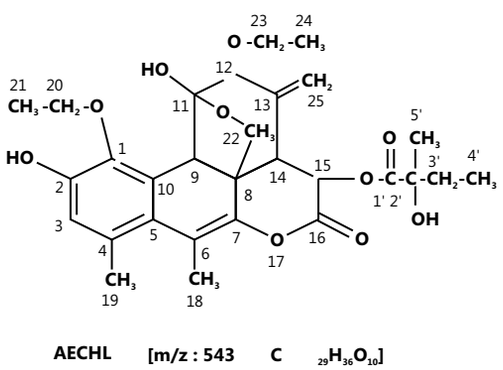

Figure : Structure of AECHL-1 with its mass fragments by NMR spectroscopy.

Further we are trying to understand its molecular mechanism and anti-angiogenic activity. Understanding the mechanism of action of AECHL-1 governing its bioactivity is essential for the development of further effective compounds.

A growing body of evidence indicates mitochondria as a novel target of anticancer chemotherapy. Studies also indicate an increasing interest in the discovery of compounds that directly affect mitochondria especially the triterpenes like Betulinic acid and avicins (Valsala). All of these agents induce apoptosis by either disrupting the membrane potential or releasing ROS, suggesting that the inner mitochondrial membrane is the primary target.

Participants

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Also invasion and metastasis, hallmarks of malignant tumor, are the main reason for the clinical death of most cancer patients. Tumor invasion and metastasis are complex, multi-step biochemical processes, which involve cell detachment, invasion, migration, intravasation and circulation, implantation, angiogenesis and proliferation. Therefore, prevention of tumor metastasis has been the biggest challenge in cancer chemotherapy. In recent years, many natural products have been found to posses anti-invasive and anti-metastatic activities. Triterpenoids are highly multifunctional and the antitumor activity of these compounds is measured by their ability to block nuclear factor-κB activation, induce apoptosis, inhibit signal transduction, and activate transcription and angiogenesis. Several triterpenoids, including ursolic and oleanolic acid, betulinic acid, celastrol, pristimerin, lupeol, and avicins possess antitumor and anti-angiogenic properties. AECHL-1 is also a tri-terpenoid and its anti-angiogenic activity has not been investigated.

Since AECHL-1 can be a futuristic anti-cancer compound, its mechanism of action and anti-angiogenic property needs to be studied and further, its therapeutic potential needs to be widely explored for chemotherapy against cancer.

Aims and Objectives

1. To investigate the mechanism underlying the anticancer activity of AECHL-1 on MCF-7 cells *in vitro* and *in vivo*, especially the role of mitochondria and redox regulation
2. To evaluate the anti-angiogenic activity of AECHL-1 *in vitro* and *in vivo*.

Work achieved

Our previous work showed induction of apoptosis by AECHL-1 in MCF-7 cells. The molecular mechanism of apoptosis indicated increased mitochondrial calcium which was inhibited by the cell-permeable calcium chelator 1, 2-bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid tetrakis acetoxymethyl ester (BAPTA-AM), increase in ROS and RNS and depletion of GSH which could not be inhibited by the extracellular addition of NAC and GSH, which indicated that not oxidative stress, but an increase of mitochondrial calcium may be a critical event in mediating AECHL-1-induced apoptosis. AECHL-1 treatment also resulted in loss of mitochondrial membrane potential but did not induce the activation of caspases8 and 9 indicating that the anticancer activity of AECHL-1, is caspase independent, and so we further studied the caspase independent pathway.

Since, a major checkpoint of the cell death pathway is the ratio of anti-apoptotic (BCL-2) to pro-apoptotic (BAX) members. A decline in this ratio leads to two major execution programs: the caspase pathway and mitochondria dysfunction. Mitochondrial dysfunction includes a change in the mitochondrial membrane potential (? Ψm), production of reactive oxygen species (ROS), opening of the permeability transition pore (PTP), and the release of the intermembrane space protein, cytochrome *c* (Cyt c) and AIF ultimately leading to apoptosis.

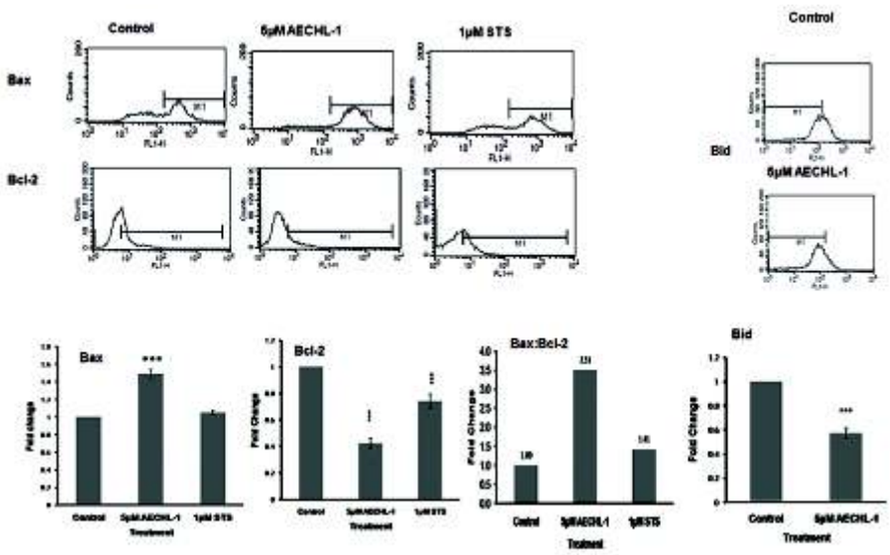

Fig. 1: Effect of AECHL-1 on Bax, Bcl-2 and Bid levels in MCF-7 cells by flow cytometry. The histograms indicate an increase in Bax, decrease in Bcl-2 and Bid levels in MCF-7 cells after treatment with 5μM AECHL-1. An increase in the Bax: Bcl-2 ratio, indicate an increase in the pro-apoptotic proteins and a decrease in the anti-apoptotic proteins. The graph plots indicate fold change for the same. 1μM staurosporin was used as a control.

Thus in order to detect whether AECHL-1 induces any change in the Bcl-2:Bax ratio, a flow cytometric analysis was carried out using appropriate antibodies and it was observed that after 24 hrs of 5μM AECHL-1 treatment, the level of anti-apoptotic protein Bcl-2 decreased while that of the pro-apoptotic protein Bax increased, indicating the involvement of mitochondria in the cell death pathway (Fig. 1).

In order to study the involvement of a caspase- independent pathway, first we investigated the role of AIF, a ?avoprotein, synthesized in the cytosol as 67 kda protein and stored in the mitochondrial intermembrane space as 57 kDa protein. It has dual functions in the cell. In normal mitochondria, it functions as an antiapoptotic factor via its oxidoreductase activity. During apoptosis, it translocates from the mitochondria to the nucleus to function as a proapoptotic factor in a caspase-independent pathway and brings about DNA fragmentation and chromatin condensation.

It was observed that AECHL-1 treatment at 10μM and 20μM concentrations cleaved the AIF fragment (57KDa) that was found to translocate into the nucleus, thus proving that AECHL-1 induced apoptosis is carried out through a caspase independent pathway (Fig. 2).

Survivin (BIRC5) is a member of the inhibitor of apoptosis (IAP) family protein. It negatively regulates apoptosis by inhibiting caspases 3 and 7, Bax and prevents Fas activation. Survivin expression is highly regulated by cell cycle and is only expressed in the G2-M phase. Survivin localizes to the mitotic spindle by interacting with tubulin during mitosis, thus contributing in regulating mitosis. Wild type p53 inhibits survivin expression. Survivin is expressed highly in most human tumours (lung and breast), but is completely absent in terminally differentiated cells. This fact therefore makes survivin an ideal target for cancer

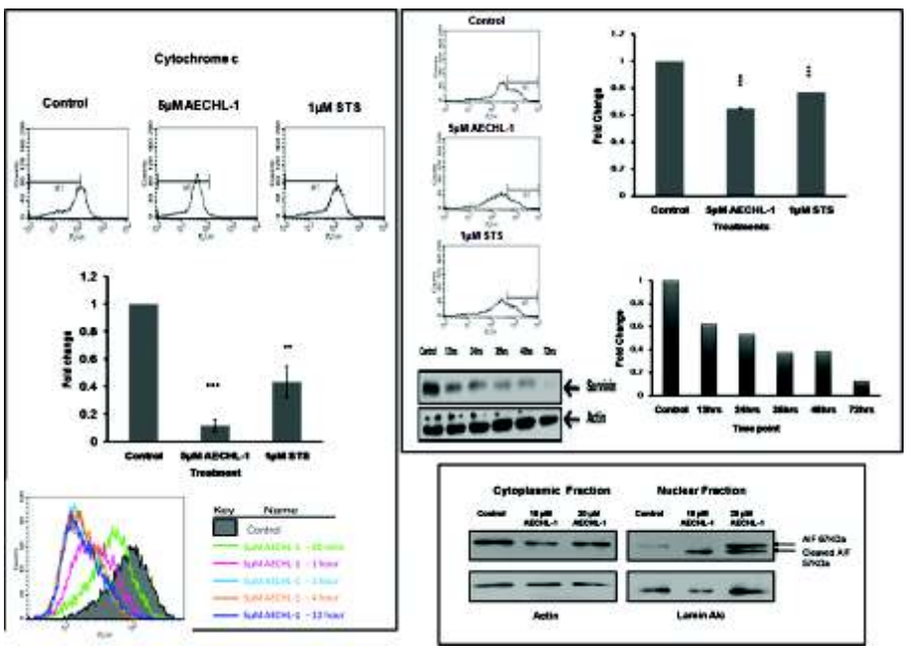

Fig. 2: AECHL-1 decreased the level of cytochrome c, the anti-apoptotic protein Survivin and a translocation of AIF into the nucleus as a 57KDa cleaved fragment in MCF-7 cells after treatment with 5μM AECHL-1 indicating a caspase independent mechanism of apoptosis. The histograms indicate the levels of cytochrome c in MCF-7 cells after treatment with 5μM AECHL-1 and the graph plots indicate fold change for the same. 1μM staurosporin was used as a control.

therapy as cancer cells will be targeted while normal cells are left alone. It was observed that, treatment of MCF-7 cells with 5μM of AECHL-1 decreased the level of the anti-apoptotic protein Survivin. These studies confirmed that AECHL-1 works through AIF cleavage and inhibition of the anti-apoptotic protein Survivin.

In the second part, we had shown the anti-angiogenic activity of AECHL-1 by MTT assay, (3^{H}) thymidine incorporation, Annexin V-PI staining and cell cycle analysis. The classical in

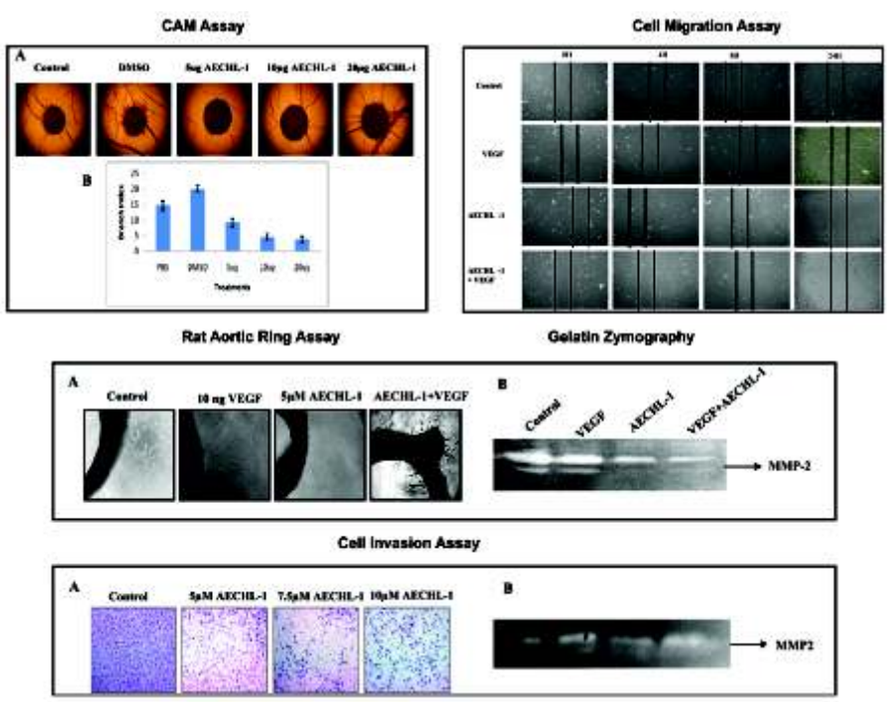

Fig. 3: CAM assay:(A) Effect of AECHL-1 on CAM angiogenesis. CAM were treated with different concentrations of AECHL-1 for 48 h and photographed. (B) Vessel Branch Index was calculated as mean of tertiary and quaternary branches, n=6.
Cell migration assay:Cell migration was carried out using scratch wound assay. Images were taken after 4, 8 and 24hrs.
Rat aortic ring assay (A) Decrease in sprouting and vessel branching was observed in the treated samples as compared to control and VEGF. (B)Supernatants were collected and gelatin zymography was carried out. Decrease in MMP-2 activity was seen in the treated samples.
Cell invasion assay (A)Endothelial cell invasion was studied using BD Matrigel invasion kit. Images of invasive cells were taken after 22Hrs. (B) Gelatin zymography of MMP-2 in conditioned media of HUVECs after treatment. Decrease in MMP-2 activity was seen in the treated samples.

vitro tube formation assay on matrigel was carried out on MAECs (Mouse aortic Endothelial Cells) and HUVECs. Further, we have shown the anti-angiogenic activity of AECHL-1 by CAM assay, rat aortic ring assay, cell migration, cell invasiveness and gelatin zymography for metallo matrix protein-2 (MMP-2).

Future work

We will continue to investigate the anti-angiogenic efficacy/potency and the mitochondria dependent molecular mechanism of AECHL-1 using *in vivo* models.

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Studies on role of IL-3 in regulation of bone remodeling

Background

Bone is a highly dynamic tissue that is continuously remodeled by bone resorbing osteoclasts and bone forming osteoblasts. Osteoclasts differentiate from hemopoietic precursors of monocyte/macrophage lineage whereas osteoblasts differentiate from mesenchymal/stromal stem cells. Osteoblasts regulate osteoclast differentiation through secretion of receptor activator of NF-κB ligand (RANKL), which binds to its receptor RANK on osteoclast precursors and stimulates the formation of multinuclear osteoclasts. Osteoblasts also secrete osteoprotegerin (OPG), a decoy receptor for RANKL that prevents the binding of RANKL to RANK and inhibits osteoclast formation. Thus, RANKL/RANK/OPG pathway regulates osteoclast differentiation. Osteoclast differentiation is also regulated by activated T lymphocytes through secretion of RANKL and other cytokines. Thus, in bone microenvironment, interactions between T cells, osteoclasts, and osteoblasts are crucial in maintaining the bone homeostasis.

IL-3, a cytokine secreted by activated T lymphocytes is known to regulate proliferation, differentiation, and survival of hemopoietic stem cells. Previously, we have demonstrated that IL-3 regulates bone remodeling by inhibiting osteoclast differentiation induced by RANKL. The inhibitory action of IL-3 on osteoclast differentiation was seen even in the presence of many other proinflammatory cytokines such as TNFα-, IL-1, TGFβ- and IL-6. These results suggested that IL-3 is a potent inhibitor of osteoclast differentiation. We have also observed that IL-3 enhances osteoblast differentiation and bone formation from mesenchymal stem cells derived from bone marrow. Thus, IL-3 has a potential to inhibit bone loss and to increase bone formation. Interestingly, IL-3, prevents the development of inflammatory arthritis, and protects cartilage and bone destruction in mice. However, the molecular mechanism(s) of IL-3 action on osteoclast and osteoblast differentiation, and its *in vivo* role in bone remodeling is not fully delineated.

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Aims and Objectives

1. To investigate the *in vivo* role of IL-3 in bone remodeling using animal model of human osteoporosis.
2. To investigate the effect of IL-3 on RANKL and OPG expression.
3. To investigate the molecular mechanism(s) of IL-3 action in bone remodeling.

Work Achieved

Role of IL-3 in regulation of osteoclast differentiation in rat

IL-3 inhibits osteoclast differentiation and bone resorption *in vitro* from both mice and human osteoclast precursors. To investigate the *in vivo* role of IL-3 in bone remodeling in a rat model of human osteoporosis, we first investigated the role of IL-3 on RANKL-induced osteoclast differentiation in rat bone marrow-derived osteoclast precursors. Cells were incubated with M-CSF and RANKL in the absence or presence of different concentrations of IL-3 for 6 days and cells were examined for formation of multinuclear osteoclasts. We found that IL-3 significantly inhibited rat osteoclast differentiation (Fig. 1) and also inhibited tartrate-resistant acid phosphatase (TRAP) activity. These results suggest that the inhibitory action of IL-3 on osteoclast differentiation is conserved across three different species. TNF-α is known to induce osteoclast differentiation and it also inhibits osteoblast differentiation. Previously we have shown that IL-3 inhibits TNF-α-induced osteoclast differentiation *in vitro* by down regulating its receptors TNFR1 and TNFR2. To assess the role of IL-3 on TNF-α-induced osteoblast differentiation, we incubated rat primary calvarial osteoblasts in osteogenic medium with TNF-α in the absence or presence of IL-3. In preliminary studies we observed that IL-3 protects TNF-α-induced inhibition of osteoblast differentiation.

To study the *in vivo* role of IL-3 on bone remodeling we developed an animal model of human osteoporosis. Wistar rats of 3 months age were ovariectomized bilaterally and bone loss was assessed in distal femur metaphysis and proximal tibial metaphysis by micro-computed tomography. Three months after ovariectomy we observed that cancellous bone indices such as bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th.), trabecular separation (Tb.Sp.), trabecular number (Tb.No.), trabecular pattern factor(Tb.Pf.), structure model index (SMI), and connectivity density (Conn.Den.) were affected. The clinical symptoms of bone loss observed in rats were very close to that of post-menopausal osteoporosis in humans. We will use this animal model to study the *in vivo* role of IL-3 in bone remodeling.

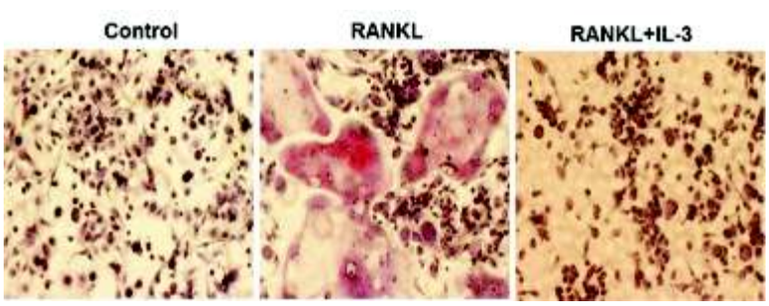


Fig. 1: IL-3 inhibits RANKL-induced osteoclastogenesis in rat osteoclast precursors. Bone marrow-derived osteoclast precursors were cultured in 96-well plates with M-CSF (30 ng/ml) and RANKL (60 ng/ml) in the absence or presence of IL-3 (10 ng/ml). After 6 days cells were fixed and stained for TRAP to detect osteoclasts.

Effect of IL-3 on RANKL expression in osteoblasts

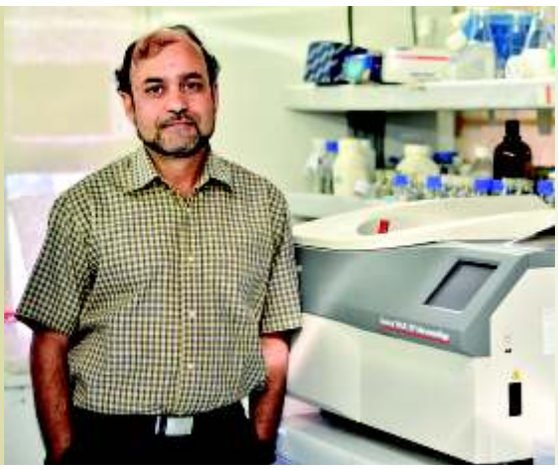
The functional equilibrium between osteoclasts and osteoblasts is regulated by two major proteins RANKL and OPG. RANKL/OPG system is a critical regulator of bone metabolism and highly essential for maintaining the structural integrity of bone. High RANKL/OPG ratio causes bone loss in osteoporosis while a low RANKL/OPG ratio cause abnormally increased bone formation that leads to osteopetrosis. To investigate the role of IL-3 in regulation of RANKL expression, we incubated mouse primary calvarial osteoblasts for 24 and 48 hours with different concentrations of IL-3 and expression of RANKL was examined at gene and protein level. It was observed that IL-3 increases RANKL expression at 24 hours and showed no effect at 48 hours. Using co-culture model of osteoblasts and osteoclasts we next observed that RANKL induced by IL-3 is not sufficient to induce multinuclear osteoclast formation. In further studies we are investigating the role of IL-3 in regulation of RANKL/OPG ratio under both normal and pathological conditions *in vivo*.

Future Work

1. To investigate the effect of IL-3 on bone metastasis of breast and prostate cancer.
2. To investigate the role of IL-3 transduced mesenchymal stem cells in the amelioration of rheumatoid arthritis in mice.
3. To investigate the role of IL-3 in regulation of chondrocyte development.

Research Report

Chromatin Architecture & Gene Regulation



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Global gene regulation by tumor suppressor SMAR1

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Background

Scaffold/Matrix attachment region 1 binding protein (SMAR1) is a MAR binding transcription regulator which tethers chromatin to the nuclear matrix and modulates the architecture of the chromatin by forming inactive loops. SMAR1 was first identified in mouse double positive thymocytes. It was found attached to the 400 bp region upstream of Eβ enhancer in the T cell receptor locus (TCR) which was characterized to be a nuclear matrix/ scaffold-associated region, referred to as MAR. This region was also reported to associate with the known MAR binding proteins SATB1 and Cux. SMAR1 shares 21.4 % identity and 64.3% similarity with the MAR binding domain of human and mouse SATB1, 23.9% identity and 56.5% similarity with a region of Bright that contains the tetramer domain (Chattopadhyay et al., 1998). SMAR1 acts as a docking site for chromatin modifiers like HDAC1 and modifies histones over the range of 5 kb on the Cyclin D1 promoter (Rampalli et al., 2005). SMAR1 transgenic mice show abnormal V(D)J recombination at specific Vβs (Kaul-Ghanekar et al., 2004). Further studies revealed that SMAR1 is a potent tumor suppressor protein, interacts with p53 and delays tumor progression in mouse melanoma model by imposing cell cycle arrest (Kaul-Ganekhar et al., 2003). Serine-arginine rich domain of SMAR1 specifically interacts and stabilizes P53 (Jalota et al., 2005). SMAR1 was also found to inhibit the expression of various cell cycle regulatory genes like Cyclin D1 (Rampalli et al., 2005), IkB (Singh et al., 2009), Bax, PUMA etc. (Sinha et al., 2011). In the higher grades of cancer, SMAR1 levels were found to be drastically downregulated. The stress responsive nature of SMAR1 is also characterized as its levels were found to be elevated upon DNA Damage (Singh et al., 2007).

Aims and Objectives

1.? Regulation of CD44 gene splicing and its Implication in cancer
2.? DNA damage repair by SMAR1 through Ku70 deacetylation
3.? Control of cytokine genes for T_H1-T_H2- T_H17 and T-reg differentiation

Work Achieved

SMAR1 mediated alternative splicing of CD44

During the multistep process of cell cycle modulation, a minor alteration in gene expression which is regulated at different stages like transcription, pre mRNA processing and translation can cause cellular transformation. Transcription and pre-mRNA splicing have emerged as highly coordinated processes. Alternative pre-mRNA splicing is indispensable for post transcriptional gene regulation. The spatial and temporal generation of splice variants of pre-mRNA demands that alternative splicing is subjected to stringent regulation. It has also been proposed that chromatin could play a role in the chaperoning of the neosynthesized pre-mRNA with consequences on splicing. However, it remained highly unclear how the chromatin modulators and especially nuclear matrix proteins regulate transcription coupled constitutive and alternative pre-mRNA splicing.

The role of alternative splicing in the production of oncogenes and tumor suppressors has attracted considerable interest, as variants (v) of same set of genes are often found specifically and distinctly in tumors from diverse tissues. For example, the CD44 family of receptors includes multiple variant isoforms, several of which have been linked to malignant properties including migration, invasion and metastasis. A large variety of alternatively spliced CD44 variants (CD44v) are expressed in different tumors with possible implication for tumor progression, formation of metastasis and survival. The signal transduction activators of RNA (STAR) family proteins, like Sam68, ASD-2 are reported to regulate the incorporation of the variable exons by directly binding to the RNA elements.

We have found that SMAR1 interacts with Sam68 and the ERK-MAPK activation leads to the abrogation of SMAR1 and Sam68 interaction. This has led us to believe that SMAR1 maintains Sam68 in a repressive state. As SMAR1 has the tendency to maintain its interacting proteins in a repressive state by interacting with deacetylases, we checked the interaction of SMAR1 with different histone deacetylases. We discovered that both SMAR1 and Sam68 interact with HDAC6, thus Sam68 is maintained in a deacetylated state by forming a triple complex with HDAC6. Knockdown of SMAR1 and/or ERK-mediated phosphorylation of SMAR1, favours the acetylation of Sam68, enhancing its affinity for pre-mRNA and thus enhances the incorporation of the variable exons. Using RNA immunoprecipitation and chromatin IP, we found that SMAR1 is accumulated endogenously into the coding region along the variable exons of CD44, which inversely correlates with the RNA Pol II Ser 5 phosho-CTD upon ERK activation, favoring the inclusion of variable exons. These results conclude that the low levels of SMAR1 in the higher grades of tumors, confers the metastatic potential through favorable inclusion of the variable exons. Our data for the first time suggests the ERK-MAPK-dependent phosphorylation and translocation of SMAR1 from the nucleus to the cytoplasm that ultimately leads to the acetylation of Sam68. Our work also highlights the ERK-MAPK activation-mediated phosphorylation of SMAR1 that favors the acetylation of RNA binding protein Sam68, which successively regulates the alternative splicing events (Figure 1).

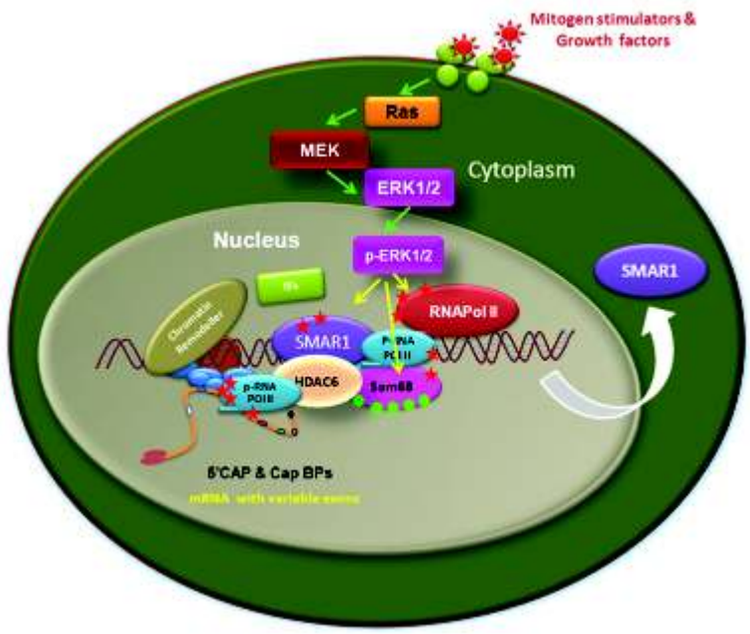

Fig. 1: A schematic representation of CD44 gene alternative splicing through ERK-MAPK pathway. Phosphorylation of SMAR1 abrogates SMAR1-Sam68-HDAC6 complex and acetylates Sam68. Acetylated Sam68 enhances CD44 gene alternative splicing. Knockdown of SMAR1 follows an identical non-canonical pathway of Sam68 acetylation and CD44 gene alternative splicing.

SMAR1 mediates DNA damage repair through deacetylation of Ku70

Matrix attachment region binding proteins are very crucial for cell survival as they are important in many cell processes such as cell cycle arrest, apoptosis etc. Previous studies have established the importance of SMAR1 in helping DSB repair. Further we extended our study that how SMAR1 regulates DNA repair and we found that SMAR1 gets modified post IR. Phosphorylation of SMAR1 at Ser 370 residue increases upon IR in an ATM-dependent manner and such post translational modification increases the activity of SMAR1. Recruitment of SMAR1 on chromatin was also studied as chromatin-bound fraction contains all the repair associated proteins. SMAR1 gets recruited to chromatin upon DNA damage and this recruitment is ATM dependent as found by decreased recruitment when cells were pretreated with ATM inhibitor KU55933 and PI3K inhibitor caffeine. Acetylation status of Ku70 decides the cell's fate and it was found that SMAR1 modulates the acetylation of SMAR1 by favoring the deacetylation of Ku70 through its interaction with HDAC6. Deacetylated Ku70 interacts with pro-apoptotic protein Bax and inhibits the translocation of Bax from cytoplasm to mitochondria. Interaction studies between Bax and Ku70 were done and it was discovered that SMAR1 inhibits the release of Bax from Ku70. Knockdown of SMAR1 causes weak interaction between Bax and Ku70. Localization of Bax was also studied upon SMAR1 over expression and knockdown. SMAR1 favors the Bax localization in the cytoplasm and thus inhibits apoptosis. By inhibiting apoptosis, SMAR1 regulates the cell survival also. It was found that SMAR1 causes better cell survival, both endogenously and post IR. All such results strongly suggest the crucial role of SMAR1 in DNA damage repair and cell's fate decision making.

Control of cytokine genes for T_H1-T_H2- T_H17 and T-reg differentiation

Scaffold/matrix attachment region proteins play important role in the orchestration of chromatin architecture, crucial for the regulation of transcription. S/MAR proteins have

been implicated in transcriptional regulation, either activating or repressing the gene function. We have deciphered the role of SMAR1 in the differentiation of T helper cells. SMAR1 is selectively expressed in Th2 cells under the control of lineage specific master regulator GATA-3. Expression of SMAR1 in Th2 cell lineage elicits transcriptional repression of both Th1 and Th17 lineage differentiation. We thereby propose the relevance of chromatin associated protein SMAR1 for the functional establishment of Th2 cells by GATA-3. Exogenous induction of SMAR1 causes reduced Th1 and Th17 cell formation, with a concomitant Th2 biasness of naïve T cell differentiation. SMAR1 regulates the Th1 differentiation by repressing the Notch mediated transactivation of T-bet and formation of repressive complex with HDAC1, SMRTe etc. It can also inhibit the Th17 differentiation by binding to the IL17 gene and mediating the epigenetic modifications of the region. SMAR1 deficiency in T cells caused compromised Th2 cell differentiation both *in vitro* and *in vivo*. We also show that T cell specific conditional knockout mice of SMAR1 were resistant to airway inflammation and asthma disease with low IgE production and Th1 biasness. These mice were further found to be susceptible for Th17 mediated encephalitis with MOG administration and increased Th17 production. Thus, taken together, we propose SMAR1 to be a critical regulator of T cell differentiation and investigate novel targets of immuno-modulation through nuclear matrix proteins.

Future work

1. Recently from fish database we found that SMAR1 is expressed in Zebra fish. We shall knock down SMAR1 in fish embryos and look at its role in development.
2. We have identified new compounds that induce SMAR1. We are testing anticancer properties using these synthetic compounds.
3. One of major targets of SMAR1 is miR 371-373 cluster where it acts as a repressor. Further, we are looking into the anti-metastatic properties of SMAR1 through regulation of miRNA 373. Since, miR 371-373 cluster is also involved in neuronal differentiation; we are looking more closely if such observation has any relevance taking ES cell differentiation.

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Mechanisms of global gene regulation by SATB1

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Background

CD4+ T cells that develop in the thymus migrate to the periphery where they differentiate into multiple lineages of effector cells, which help in clearance of the antigen. The differentiation of CD4+ T cells to T helper cells is orchestrated by master transcription factors specific to each lineage, along with and NF-κB. NFAT was shown to be important during the T helper differentiation by transcriptional regulation of lineage-specific transcription factors such as T-bet (Th1), Gata-3 (Th2), RORγt (Th17) and Foxp3 (iTregs). In this study we explored the role of chromatin organizer SATB1 during T helper cell differentiation using an in vitro culture system wherein CD4+ T cells are differentiated into distinct helper T lineages. Previous studies from our laboratory and others have shown the role of SATB1 in Th2 cell differentiation and its regulation of Th2 cytokine locus. SATB1 is upregulated during Th2 differentiation. A recent report using Th17 polarized cells demonstrated that knockdown of SATB1 resulted in downregulation of IL-17 showing the importance of SATB1 during Th17 differentiation. Tregs also exhibit downregulated expression of SATB1. These results suggested that SATB1 might be important during the differentiation of CD4+ T cells to various helper phenotypes. We therefore wished to establish if SATB1 is necessary for the differentiation of CD4+ T cells into T helper lineages.

Aims and Objectives

1. To study the role of SATB1 in diffrentiation of CD4 T cells into T helper lineages
2. To dissect the role of SATB1 in regulation of cytokine genes
3. To elucidate the molecular mechanism(s) of regulation of SATB1

Work Achieved

1. SATB1 is essential for Th2 differentiation

Since SATB1 is preferentially upregulated in Th2 cells compared to Th1, we wished to examine whether SATB1 is necessary for Th2 cell determination. Earlier studies in D10.G4.1 (Th2) T cells demonstrated that SATB1 binds across the Th2 cytokine locus and regulate the coordinated expression of IL-4, IL-5 and IL-13 (Cai et al., 2006). It was also shown that knockdown of SATB1 in D10.G4.1 cells led to downregulation of IL-4

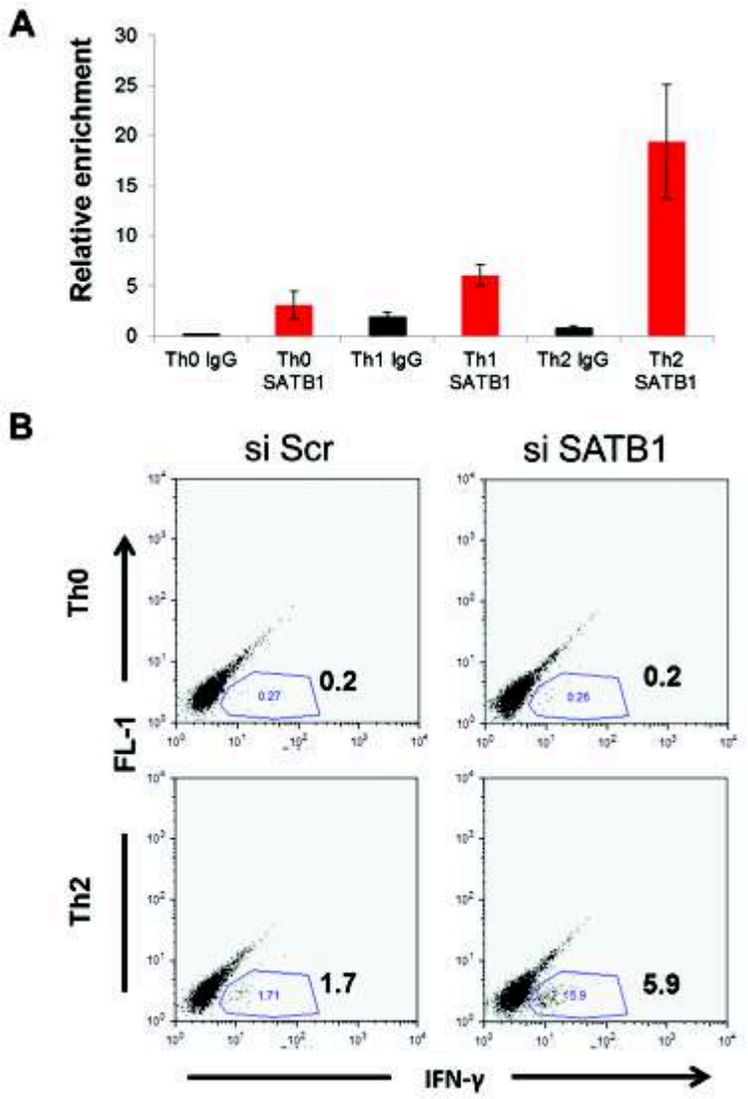

Fig. 1: SATB1 represses Interferon-γ expression in Th2 cells. **(A)** Mouse $CD4^+$ T cells were polarized under Th1 and Th2 conditions. Th0 cells served as control. Post polarization for 3 days cells were harvested and occupancy of SATB1 on *Ifn*-γ promoter was determined by ChIP analysis. Bar graph represents relative enrichment by SATB1 antibody i.e. SATB1 occupancy on the *Ifn*-γ promoter locus. IPs performed using IgG serve as negative control. Error bar depicts the standard deviation calculated from triplicates. **(B)** $CD4^+$ T cells were transfected with Scr or siSATB1 and cells were polarized in Th0 or Th2 conditions. After 48 h cells were harvested and stained for nterferon-γ and analyzed by flow cytometry. Gated populations in the FACS plots represent the percentage of IFN-γ positive cells.

expression. To extend these studies in primary cells we transfected CD4+ T cells with scrambled and siSATB1 RNA duplexes using nucleofection and then polarized the cells to Th2 phenotype. After confirming effective knockdown of SATB1 we monitored the transcript levels of IL4 in scr and siSATB1 transfected cells. The expression of IL-4 in scr or siSATB1 transfected Th0 polarized control cells did not exhibit any relative change. In contrast, knockdown of SATB1 in Th2 cells significantly downregulated IL-4 expression in comparison with scr transfected Th2 cells (Fig. 1A). To further confirm whether expression of IL-4 cytokine in Th2 cells is regulated by SATB1 we quantified the expression of IL-4 by intracellular cytokine staining. Towards this CD4+ T cells were transfected with scrambled siRNA and siSATB1 and then were polarized under Th2 conditions for 2 days. The cells were activated with PMA and Ionomycin 6 h prior to harvesting time and later treated with Brefeldin-A which prevents the secretion of recently synthesized proteins and blocks them at Golgi bodies. Harvested cells were stained with fluorescently-tagged IL-4 antibody and analyzed via flow cytometry. We observed that upon knockdown of SATB1

there is complete loss of IL-4 positive cells. However, scr transfected cells exhibit IL-4 expressing cells (Fig. 1). These results suggested that SATB1 expression is necessary for the differentiation of CD4+ T cells to Th2 phenotype by regulating the expression of effector cytokine IL-4.

2. SATB1 negatively regulates IFN-γ expression

Signals that induce Th2 differentiation in CD4+ T cells inhibit the expression of interferon-γ (IFN-γ). Since we observed that SATB1 is specifically upregulated in Th2 cells as compared to Th1, we wanted to address if SATB1 has any effect on IFN-γ expression in Th2 cells. Towards this we performed ChIP analysis in Th1 and Th2 cells polarized for 3 days, and assessed the occupancy of SATB1 on *Ifn*-γ promoter. We performed quantitative PCR of ChIP products and observed that SATB1 is highly enriched on *Ifn*-γ promoter in Th2 cells as compared to Th1 and Th0 cells (compare lanes 2, 4 with lane 6) (Fig. 2A). Rabbit IgG represents negative control for the ChIP experiment and did not reveal any enrichment. To further validate the effect of SATB1 on the expression of IFN-γ in Th2 cells, we knocked down SATB1 expression in CD4+ T cells and polarized them to Th2 phenotype. We observed that ablation of SATB1 in Th2 cells has increased the percentage of IFN-γ positive cells in comparison to scr transfected CD4+ T cells (Fig. 2B).

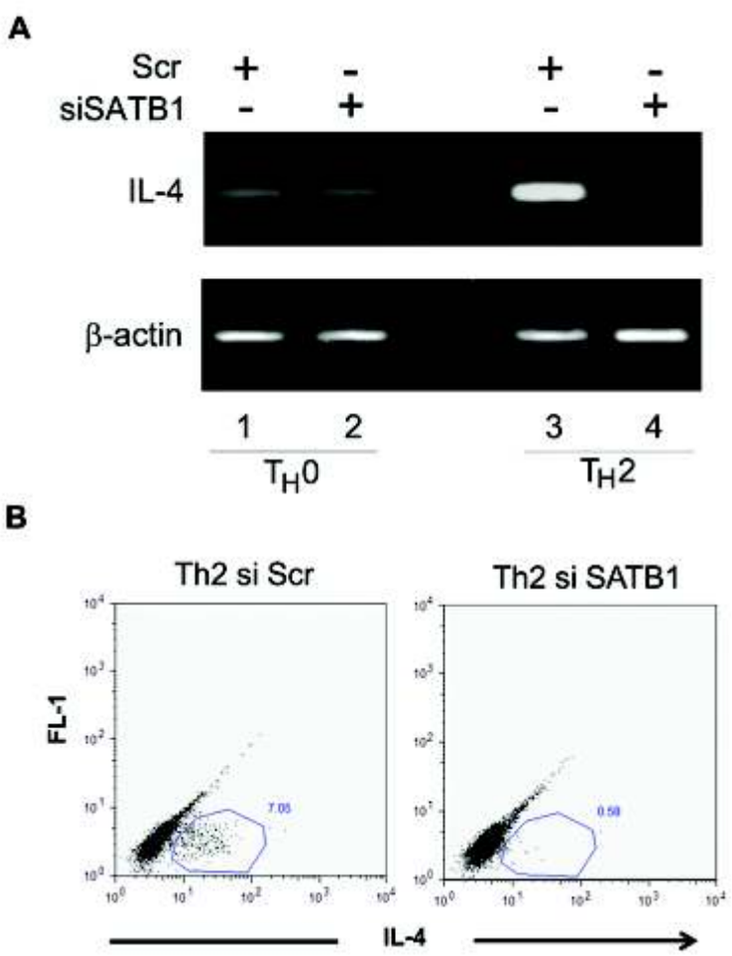

Fig. 2: SATB1 regulates IL-4 expression in CD4+ T helper cells. **(A)** $CD4^+$ T cells were transfected with Scrambled (Scr) siRNA or siSATB1 and then polarized to Th2 phenotype. The cells were harvested 48 h post transfection and used for monitoring the expression of IL-4. IL-4 expression was quantified by RT-PCRs. Actin transcript was used as loading control. **(B)** Scr and siSATB1 transfected cells were analyzed for intracellular IL-4 cytokine expression. Gated populations represent the cells expressing cells positive for IL-4 cytokine expression.

Whereas in CD4+ T cells transfected with either siSATB1 or scr and polarized under neutral conditions (Th0) i.e. with only TCR stimulation and no polarizing cytokines, the IFN-γ positive cells were unchanged. These results suggested that SATB1 is not only required for coordinated expression of the IL-4 effector cytokine locus in Th2 cells, but is also necessary for inhibition of IFN-γ gene expression.

3. STAT6 signaling regulates SATB1 expression in Th2 cells

Since SATB1 levels are specifically upregulated in Th2 and we observed that it is necessary for Th2 differentiation, we wished to understand the regulation of SATB1. Studies from our laboratory have shown that IL-4 dependent regulation of SATB1 is mediated by STAT6. It was also shown that RNAi induced silencing of STAT6 in mouse CD4+ T cells and later polarization under Th2 conditions downregulated SATB1 expression. We therefore wished to test if STAT6 directly binds to SATB1 and regulates its gene expression. Towards this we performed gel mobility shift assays using radioactively labeled SATB1 promoter probes of which two probes 644-741 and 644-643 harbor consensus STAT6 binding sites. As control, probe 741-748 does not harbor the consensus STAT6 binding site (Fig. 3A). If STAT6 protein complex from the lysate binds to the consensus STAT6 binding site on

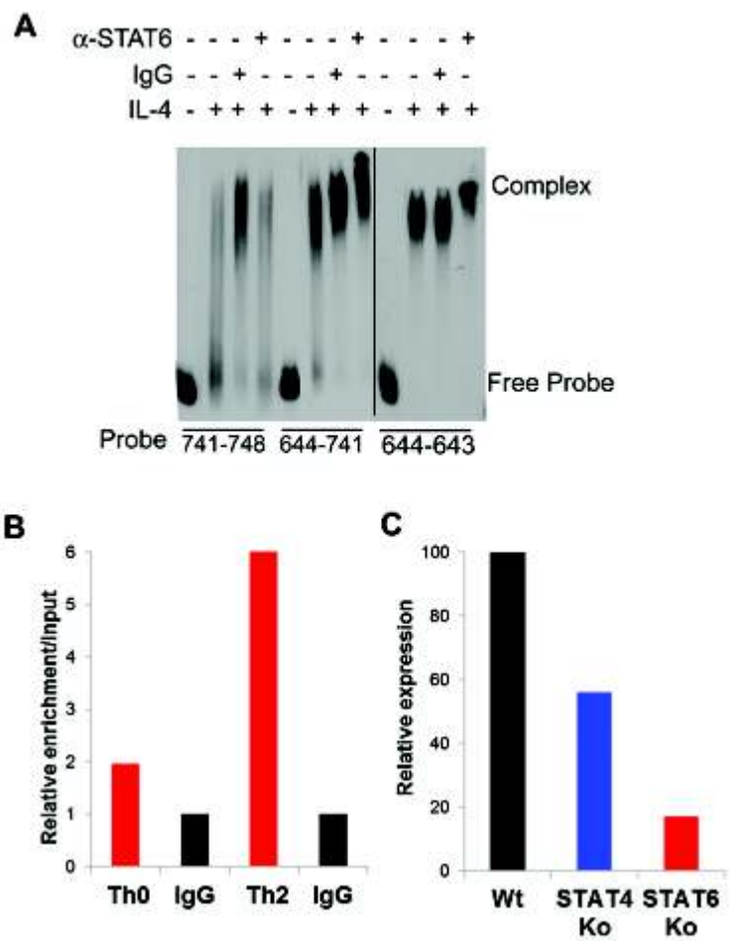

Fig. 3: STAT6 signaling regulates SATB1 expression in Th2 polarized cells. (A) Increasing amounts of $CD4^+$ T cell lysate were incubated with three different radioactively labeled DNA probes. Probe 741-748 of SATB1 promoter has no STAT6 binding site and served as negative control. Probe 644-741 and 644-643 are twodifferent probes with the STAT6 consensus binding sites. Probe along with lysates were incubated for 1 h at room temperature and later with STAT6 antibody or rabbit IgG. The complexes are resolved on native polyacrylamide gel and analyzed by autoradiography. (B) ChIP analysis was performed in $CD4^+$ T cells polarized to Th0 and Th2 phenotype. IPs were performed using either STAT6 antibody or rabbit IgG. The bar graph represents the relative occupancy of STAT6 on SATB1 promoter. (C) Bar graph represents the relative expression of SATB1 in $CD4^+$ T cells in Wt, STAT4 and STAT6 knockout mice. The data is analyzed using the ChIP- seq data from Durant et al., 2010

SATB1 promoter we should observe a greater shift in the complex when incubated with STAT6 antibody. We observed that probe 644-643 and 644-741 have retarded mobility of the complex and observed a greater shift when the whole complex is incubated with anti-STAT6 antibody compared to control R-IgG. We observed no further retardation of the complex with probe 741-748 in comparison to R-IgG or with anti-STAT6 antibody, which does not have a consensus STAT6 binding site. We then performed ChIP analysis to verify the in vivo binding of STAT6 on SATB1 promoter. As observed in gel mobility shift studies we found significant enrichment of STAT6 on SATB1 promoter using quantitative PCR analysis (Fig. 3B). A recent study has elucidated the role of STAT proteins in dictating T helper differentiation by modulating the epigenetic changes and regulating gene expression. We further analyzed the data pertaining to the expression pattern of SATB1 from Wei et al., and observed that STAT6 deletion had profound effect on SATB1 expression compared to STAT4, although deletion of either STAT proteins led to downregulation of SATB1 (Fig. 3C). These results indicated that SATB1 expression during Th2 differentiation is positively regulated by STAT6 signaling.

Taken together, we have shown that SATB1 integrates multiple signaling pathways to regulate T helper cell differentiation (Figure 4).

Future Work

We would now like to study the regulation of SATB1 during T cell development and differentiation.

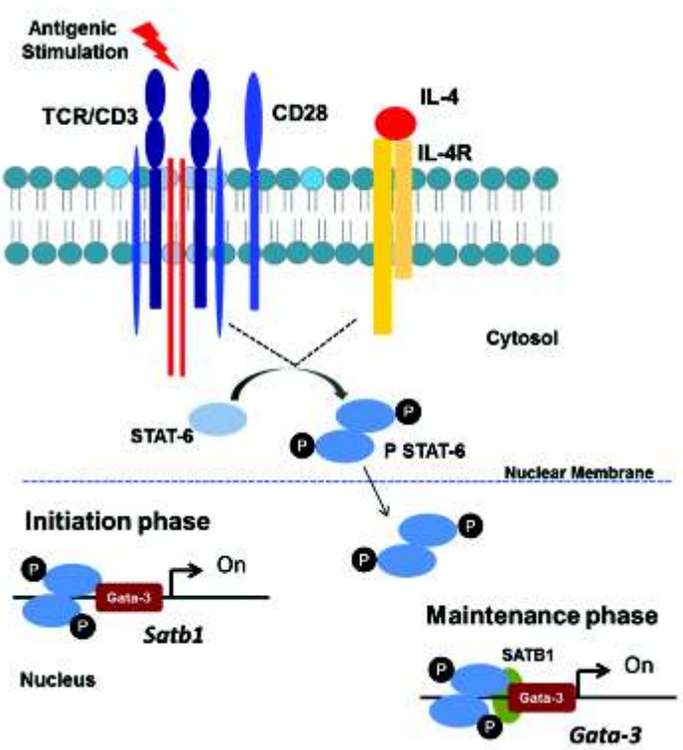

Fig. 4: Model depicting the role of SATB1 during Th2 differentiation. Upon antigen stimulation and appropriate cytokine signaling naïve $CD4^+$ T cells activate STAT-6, which then translocates into the nucleus. Inside the nucleus, STAT-6 along with Gata-3 transactivates SATB1 expression, which is required for the expression of IL-4 and suppression of IFN-γ and thus initiates Th2 differentiation. Once the cells are committed to Th2 lineage SATB1 binds to *Gata-3* promoter along with Gata-3 itself and is required for the maintenance of Gata-3 expression.

Research Report

Infection & Immunity

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Cellular and molecular mechanism of chemokine receptor signaling during inflammation and tolerance

Background

Inflammation is the complex set of reactions involve a set of cytokines, chemokines and adhesion molecules. There are several secreted chemokines, cytokines and its specific ligands expressed into the inflamed microenvironment, and a joint venture of pro- and anti-inflammatory functions are initiated together by infiltrating leukocytes, tissue fibroblasts, epithelial and endothelial cells. Chemokine receptors and cell adhesion molecules present on the cell surface are known to be involved in the migration of immune cells into the inflamed tissue. These chemokines and cell adhesion molecules are well studied in the migration of cells. However, do intrinsic signaling from these receptors perturbs the cell differentiation and function is not well characterized.

Most of the chemokines and some of the adhesion molecules are G-protein coupled receptors (GPCRs). G-proteins are heterotrimer consist of α-, β-, and γ-subunits and transduce signals from surface receptors to intracellular effectors. Upon receptor activation, G-protein complex dissociate into α and βγ-subunits which in turn recruit various signaling components at the inner surface of the plasma membrane followed by production of array of intracellular second messengers such as IP3, DAG, Ca^{2+}, cAMP and IP3. G-protein signaling regulates number of cellular events such as induction of cell polarity, actin polymerization, reversible cell adhesion, myosin dynamics, transmigration, cell activation, differentiation and functions.

CCR6 is a GPCR, expresses on various immune cells and interacts with its specific chemokine CCL20. CCR6 play an important role in various diseases such as experimental autoimmune encephalitis (EAE), inflammatory bowel disease, psoriasis, chronic hepatitis, rheumatoid arthritis, chronic pulmonary sarcoidosis, cancer metastasis and graft-versus-host disease. How does CCR6 signaling affect differentiation and function of the CD4 T cells is not known?

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Aims and Objectives

1. How does chemokine activation together with co-stimulatory molecules affect the differentiation and function of CD4 T cells?
2. How does chemokine receptor signaling perturbs the epigenetic marker in regulatory elements of the genes?

Work achieved

Acute autoimmune colitis was induced in C57BL/6 mice by giving dextran sodium sulfate (DSS; 2% w/v) in drinking water. DSS induced bloody diarrhea leading to 25% body weight-loss within 7 days (Figure 1A). DSS treatment showed increased expression of CCR6, RORγt, Tbet and down-regulated Foxp3 into CD4 T cells in spleen, mesenteric lymph nodes and Peyer's patches compared to untreated mice (Figure 1B; only RORγτ in spleen shown). These results suggest that DSS induced colitis is characterized by strong Th1 and Th17 response in the mice. Mouse CCR6 gene was cloned into pIRES-gfp vector as bi-cistronic construct. Stably expressing T cell line (BW5147.3) were transfected and eGFP$^+$ cells were sorted and maintained in presence of G418 medium (Figure 2). eGFP$^+$

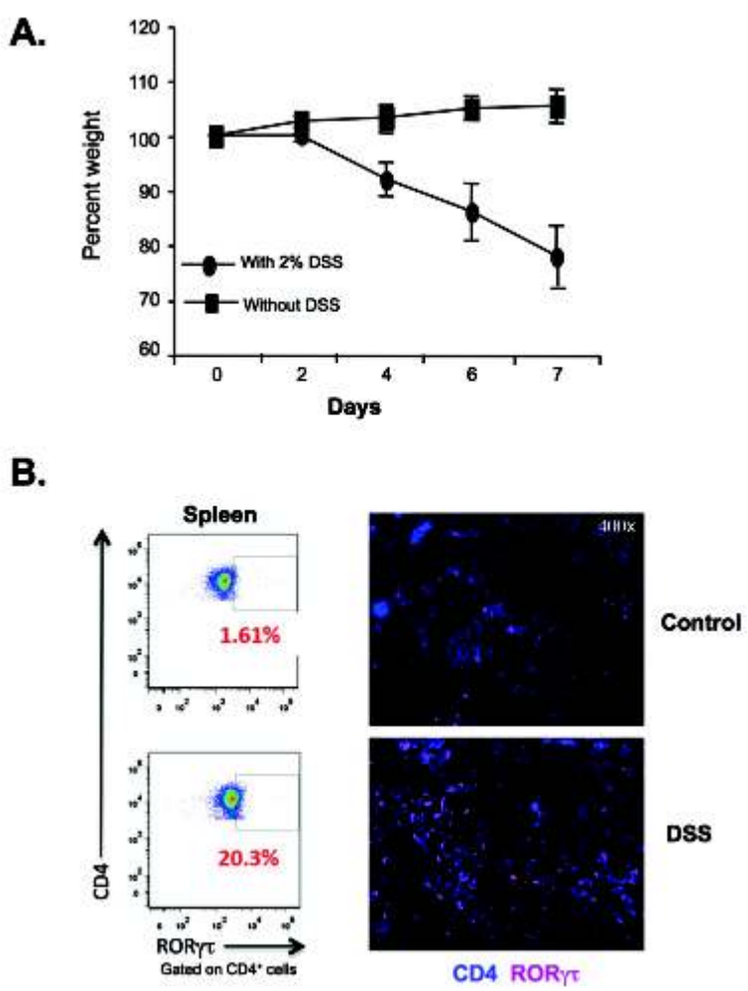

Fig. 1: DSS treatment induced colitis and increased the differentiation of CD4$^+$RORγτ$^+$ cells in spleen. **(A)** C57BL/6 mice were given DSS in the drinking water. Weights of mice were recorded and plotted as percentage weight loss from the day 0. N = 5 mice/group. **(B)** Mice were sacrificed at day 7. Percentage of RORγτ$^+$ cells in spleen was analyzed using flow cytometry (left) and immunoistological staining (right).

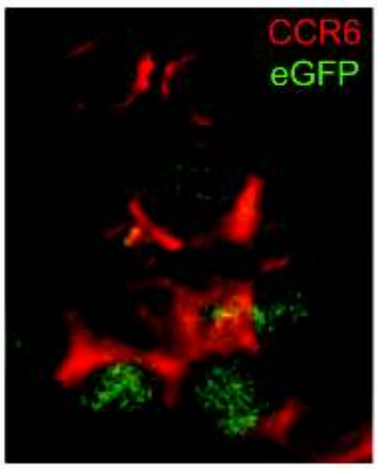

Fig. 2: Bw5147.3 cells (T cell lymphoma cell line) were tranfected with pIRES-eGFP-CCR6 construct. Stable clones were selected in presence of G418. Expression of CCR6 and eGFP were analyzed using fluorescent microscope.

cells were further used for the cell signaling studies. We have also cloned and expressed mouse CCL20 as Ig-fusion protein, which will be used for *in vitro* and *in vivo* studies.

We are currently investigating the molecular mechanism of CCR6 signaling and its role on Th17 and Treg cell differentiation and function. Understanding the CCR6 signaling in CD4 T cells will help us to develop novel therapeutic strategies to control the autoimmune diseases.

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Host cell factors in HIV pathogenesis and identification of new anti-viral lead molecules

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Background

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS). The hallmark of the disease is gradual depletion in the number of CD4+ T cells leading to the onset of opportunistic infections. The incidence of HIV infection has reached pandemic levels worldwide including India. The therapeutic regimen being used at present can reduce the viral load remarkably but are not the ultimate answer to AIDS patients. Our group has been working on different aspects of HIV pathogenesis, related to host-virus interactions, immune response and drug discovery. The primary objective is to gain more understanding of the virus and its interaction with the host cell, which may lead to new antiviral strategies.

Aims and Objectives

1. Role of viral regulatory proteins Tat and Nef in HIV pathogenesis.
2. Differential gene expression studies to identify molecular mechanism of HIV-1 induced T-cell apoptosis.
3. Identification of novel molecules with anti-HIV activity from plant source and their potential for use as microbicides.

Work Achieved

Role of viral regulatory proteins Tat and Nef in HIV pathogenesis

HIV-1 Tat protein is one of the most important regulatory proteins for inducing viral gene expression in the host cell. It functions primarily by binding to initial short transcript of HIV genome named transactivation responsive region, which results in recruitment of positive transcription elongation factor B (P-TEFb) complex to the LTR promoter. The P-TEFb complex then hyper-phosphorylates the C-terminal domain of RNA polymerase II increasing the processivity of polymerase, which leads to elongation of transcription. Tat also functions to recruit histone acetyl transferases (HAT) to the integrated viral genome and thereby activating its transcription. There are convincing evidences that Tat also

functions independently of TAR element to activate the LTR promoter. The NFκB and SP1 sites of the LTR have been found to be important for this mechanism. Earlier we have shown that direct binding of Tat to the NFB enhancer sequences on the LTR promoter as one of the mechanisms underlying TAR independent transactivation. Thus, DNA binding activity of Tat could be also one of the potential mechanisms of TAR independent Tat mediated regulation of cellular gene expression. We have also studied the genome wide occupancy of Tat protein on host cell chromatin by ChIP-on-chip analysis in HIV-1 infected T cells to look for a potential role of Tat on cellular gene expression. We have identified a number of genes, which show a significantly high localization of Tat protein in HIV-1 infected T-cells, a majority of the recruitment being observed on gene promoters. Recruitment of Tat on these promoters tend to modulate their gene expression in HIV-1 infected T cells as was observed for about twenty selected genes (Fig-1). Based on these results, Tat was identified as a repressor of c-Rel in the present study as it down regulates expression of *c-Rel* in HIV-1 infected cells. We have also shown that Tat down regulates c-Rel promoter activity by interacting with its specific NFκB sites. Thus, we have not only analyzed the genome wide recruitment of Tat protein in HIV-1 infected cells but also identified *c-Rel* promoter as a case study, which is down regulated by Tat using specific NFB enhancer sites in the promoter.

The Human Immunodeficiency Virus Type 1 encodes a 27 KDa protein, Nef, which has come a long way from being termed as a negative factor to being one of the most important proteins of HIV-1. However, its role in HIV-1 replication and gene expression remains to be clearly understood. Although involvement of heat shock proteins in viral pathogenesis has been reported earlier, a clear understanding of their role remains to be elucidated. We have earlier shown that Hsp40 and Hsp70 reciprocally regulate HIV-1 gene expression and replication, using Nef and Vpr proteins of the virus respectively. We have also shown that HSF-1 positively regulates HIV-1 gene expression and replication by

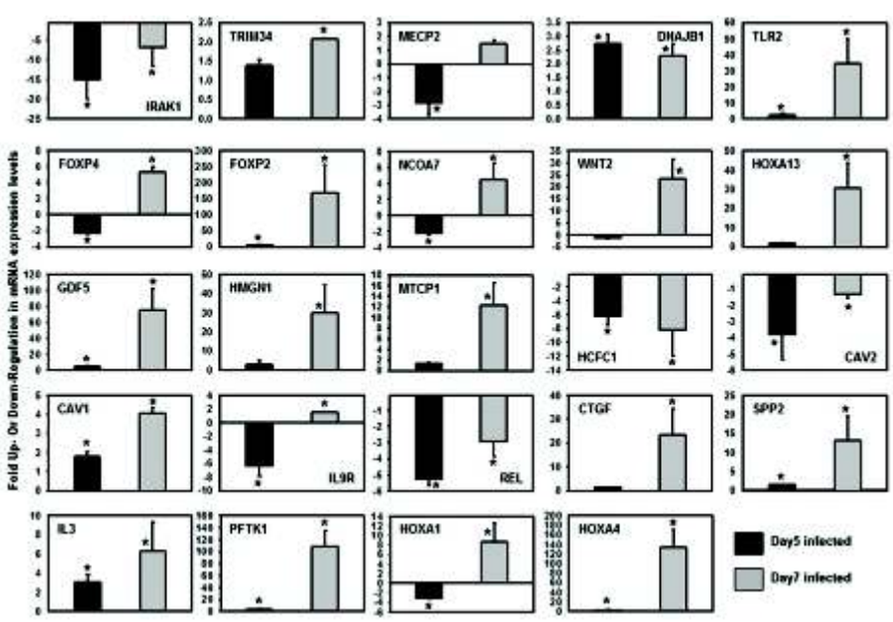

Fig. 1: Expression profile of the selected Tat target genes in HIV-1NL4-3 infected CEM-GFP cells. Quantitative real time PCR analysis was performed using RNA extracted from uninfected, day 5 and day 7 infected CEM-GFP cells. The fold up- or down-regulation values in the bar graphs depict the changes in the gene expression over the expression levels in uninfected cells normalized to GAPDH. The data is representative of at least three biological replicates and represents mean ± SEM.

two distinct mechanisms. Firstly, along with Nef it increases HSP40 expression that promotes viral gene expression and replication. Secondly, HSF1 directly interacts with a novel HSF-1 binding sequence in the HIV-1 long terminal repeat (LTR) promoter and induces viral gene expression and replication. We have now initiated a comprehensive study of all the HSP protein family members during HIV infection.Due to significant overlap in the function of HSPs, they were originally classified by their molecular weight into different families but recently they have been renamed as HSPA, HSPB, HSPC, HSPD, HSPH, and DNAJ family. Each family is represented by different HSP members and their isoforms, encoded by different genes. In order to identify all the HSP genes that are modulated during HIV-1 infection we have infected CEM-GFP cells with HIV-1 virus. RNA from these cells was used to study the modulation of different members of the HSP family. Our results indicate that isoforms of different HSP family members are differentially regulated during HIV-1 infection. Validation of these results and further characterization of the role individual isoforms are currently in progress. We have also initiated studies on an HSP70 binding protein, HspBP1. It is a member of HSP70 co-chaperone family. HspBP1 negatively affects the binding of substrate to HSP70 by accelerating nucleotide exchange of ATP domain. It has also been reported that HspBP1 levels increase in the serum of HIV-1 infected individuals. HSP70 is associated with various phases of HIV-1 life cycle and HspBP1 can regulate various HSP70 activities; so it's worth studying the role of HspBP1 during HIV-1 infection, if any. Expression of HspBP1 gets down-modulated during HIV-1 infection in T-cells. Further studies are in progress to elucidate the mechanism.

The lack or delay of disease progression in Nef deleted HIV -1 infected individual indicate Nef to be a pathogenic factor. However, the major problem in defining Nef function has been its possible involvement in multiple pathways and its pleiotropic role in HIV-1 life cycle. Nef directed regulation of transcription can be best visualized when HIV infection was allowed to progress in presence or absence of Nef. Definite role of Nef in enhancing viral replication is evident from our results where ? nef HIV infected cells produced less virus as compared to WT infected cells. When we compared the gene expression profile of WT and Nef deleted virus infected cells using microarray, we got a large number of genes differentially modulated in these cells. Gene expression profiling comparison shows differential regulation of genes belonging to many of the biological processes including receptor activity and cell signaling, TCR pathway, targets of miRNA etc (Fig-2). Nef induces all these changes to favor HIV-1 infection progression. However what all mechanisms Nef utilizes to bring about all these changes remains to be clearly elucidated.

Differential gene expression studies to identify molecular mechanism of HIV-1 induced T-cell apoptosis

Studies done till date to elucidate the pathways involved in HIV-1 induced T cell depletion has revealed that apoptosis underlie the etiology; however, a clear molecular understanding of HIV-1 induced apoptosis has remained elusive. Although evidences pointing towards the importance of mitochondrial energy generating system in apoptosis exist, its exact role remains to be clearly understood. The OXPHOS system

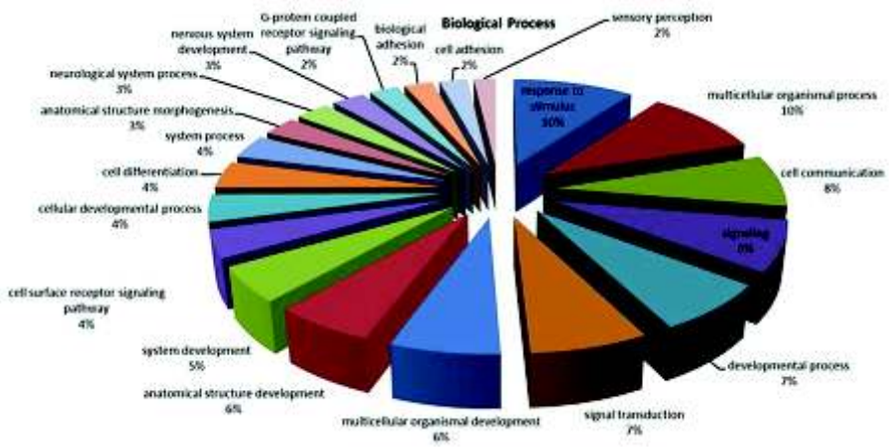

Fig. 2: Pie chart depicting array of genes participating in a particular biological process being modulated due to the presence of Nef in course of HIV-1 infection progression. The results were analyzed by Genespring GX Software version 12 using gene expression profiling data of wild type and Nef deleted HIV-1 infected CEM-GFP cells.

comprises of five enzyme complexes, subunits of which have been implicated in various functions in addition to their primary role in energy generating process. Our results also show that the activities of enzyme complexes I, II and III are decreased while those of Complex IV and V are increased at the time of acute infection and apoptosis. This differential regulation in activities of OXPHOS system complexes indicate a complex modulation of host cell energy generating system during HIV infection that ultimately leads to T cell apoptosis. We have also initiated studies on the role of autophagy in HIV-1 infection with specific focus on the role of Nef protein.

Furthermore, HIV-1 infection is known to be associated with the hijacking of a number of cellular factors including the cell cycle associated molecules. The subversion of the host cell cycle during HIV-1 infection progression includes arresting of the normal cell cycle at mitosis, specifically at the boundary of the G2-M phase. Although many studies validate that Vpr and Vif is involved in causing the G2-M arrest associated with HIV-1 infection, not much information is available related to other viral and cellular protein interactions that might be crucial in the background. Also, the nucleolar proteins which play a significant role in the cell cycle regulation might also be targeted by the virus to establish a favorable system for its replication. We have now initiated studies intending to look into such aspects of HIV-1 pathogenesis.

Identification of novel molecules with anti-HIV activity from plant source

The current therapeutic strategy involving the use of highly active anti-retroviral therapy (HAART) has proven to be useful in controlling the virus but is not sufficient to eradicate the virus from the patients. Extensive work is being done throughout the globe to identify new ant-HIV therapeutic strategies. Our studies with NIPER have resulted in identification of a number of novel molecules showing potent anti-HIV activity. Several of these molecules are now being further studied for potential development of a microbicide formulation. We are also working with IICB Kolkata for analyzing the potential of Accaciaside B as an anti-HIV microbicide. Accaciaside B seems to inhibit viral entry with IC50 values in nano-grams and a high safety index. Evidences obtained from a number of experiments indicate it to be a lead molecule for microbicide development. We have also

initiated structure based development of new Integrase inhibitors in collaboration with Birla Institute of Technology, Mesra. Finally, we have started screening of a library of pharmacologically active bio-molecules for identification of novel anti-HIV molecules, with ultimate objective to identify novel targets for inhibition of HIV-1

Future Work

Our results till date indicate that heat shock proteins 40 and 70 play an important role during HIV-1 infection. We are now trying to elucidate the role of other heat shock proteins in HIV replication and pathogenesis, which will provide us a comprehensive knowledge about the role of HSPs during HIV infection. We are also trying to identify the specific role of different HSP isoforms during HIV-1 infection. We are continuing characterization of several new Nef interacting host cell proteins identified previously for their functional relevance in HIV lifecycle. We are also involved in the study of mechanism of Tat mediated regulation of both viral and cellular gene expression. Identification of differentially expressed genes and their relevance to HIV induced cell death is being continued, with a focus on proteins involved in cell cycle and autophagy. Finally, studies to identify new anti-HIV molecules will be continued with the objective to identify novel lead molecules with potential for use as anti-HIV microbicides.

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Host Pathogen Interaction in Leishmanial Infection

Background

Leishmania donovani causes Leishmaniasis referred to as visceral leishmaniasis or kala-azar that is widespread in the Indian subcontinent, Asia and East Africa. It is transmitted by the bite of female phlebotomine sand fly and newly acquired infections of *L. donovani* remain mostly subclinical and are asymptomatic. During disease progression, it tends to become oligosymptomatic and if untreated, it becomes fully established with hepatosplenomegaly leading to death. *Leishmania* is a digenic parasite which exists as motile flagellated slender promastigotes found in sand fly gut and non-motile oval amastigote form within macrophage phagolysosome. These parasites need to adapt to the environmental changes when it shifts from sand fly to vertebrate host which has higher body temperature and reducing environment within the macrophage. The leishmanial parasite is well suited for intracellular survival at low pH inside the phagolysosome and higher body temperature of the host by expressing a different set of genes including several stress response genes. Therefore, it was necessary to ensure and assess the proteins expressed in these two forms of the parasite.

Aims and Objectives

The aim of this specific project was to perform total protemic analysis if promastigotes stage and in vitro diffentiated axenic amastigotes and to map the proteome of *Leishmania* based proteogenomic approach.

Work achieved

Until recently there was no genome sequence information available for *L. donovani* and proteomic analysis was mostly carried out using homology based database dependent searches using related *Leishmania* species (*L. infantum*, *L. major and L. braziliensis*) whose genomes were sequenced. In a recent study by our group we used a similar comparative homology based proteogenomic approach to map the proteome of *L. donovani*. This approach resulted in identification of >3,700 protein coding genes in *L. donovani* based

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on homology with *L. infantum* which is taxonomically the closest to *L. donovani*. Recently *L. donovani* genome sequencing was completed and this data is now publicly available. We used a proteogenomic approach to map the proteome of two different life stages of *L. donovani* i.e. promastigote (insect) and amastigote (human) stages. This resulted in identification of 17,455 unique peptides upon database dependent search against *L. donovani* proteins. These peptides were assigned to 3,999 unique proteins in *L. donovani*. Of these 3,999 proteins identified in *L. donovani* the expression of 2,258 proteins was detectable in both the life stages of *L. donovani*, while 635 and 1,116 proteins were identified only in the amastigote and promastigote life stages, respectively. The proteomic data was also searched against the six-frame translated *L. donovani* genome and this resulted in identification of 214 genome search specific peptides (GSSPs) which in turn resulted in identification of 12 novel genes and correction of 24 existing gene models (N- and C-terminal extension) in *L. donovani*.

In a similar study, we have searched *L. major* promastigote proteomic data against *L. major* protein database. This resulted in identification of genome search specific peptides (GSSPs) which maps uniquely to the genome which are not part of the protein database. We have identified 3,613 proteins in *L. major* promastigotes and covered 43% of its proteome. We also identified 28 GSSPs which resulted in the identification of 4 novel genes previously not identified in *L. major* and we also corrected assignment of 15 annotated genes resulting in N-terminal extension of the protein product. This study shows the utility of proteomic approaches in mapping proteome of given pathogen and annotating its genome.

Future Work

To ascertain the differentially expressed proteins in *L. donovani* amastigotes as compared to promastigotes by quantitative proteomic approach using iTRAQ labeling.

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Plasmodium falciparum merozoite invasion inhibition by malaria patients' antibodies and merozoites invasion profiles in field isolates

Background

Plasmodium falciparum infections may be influenced by the concurrent presence of another *Plasmodium* species. It has been reported that the severity of malaria is reduced due to the presence of another parasite. This may be due to presence of cross-reactive antibodies. Bioinformatics analyses revealed similarity between *P. falciparum* Pfclag9 and the *P. vivax* ortholog, Pvclag7, and cross-species recognition of *Plasmodium falciparum* and *P. berghei* sporozoites by anti *P. vivax* CSP serum samples. Previous parasitic infection may influence parasite merozoite invasion and its immunobiology. Host cell invasion is a prerequisite for the malarial parasite survival. The clinical manifestations of Pf malaria are directly linked to the blood stage of the parasite life cycle. Circulating merozoites in the blood invade erythrocytes via specific invasion pathways, often identified as sialic acid-dependent and -independent. The invasion process involves multiple receptor-ligand interactions that mediate a complex series of events leading to invasion by PF merozoites, the molecular nature of which is currently not fully understood. It has been reported that Indian field isolates of *P. falciparum* use multiple RBC invasion pathways that are independent of sialic acid residues. Erythrocyte entry by the malaria merozoite is known and requires different parasite proteases. Organellar components of the merozoites (micronemes, rhopteries etc.) undergo specific photolytic processing during merozoite invasion.

Variations in the parasites' proteins alter the pattern of receptors usage for invasion of human erythrocytes. This provides a unique strategy for parasite invasion in the face of erythrocyte receptor polymorphism and host immune response. The molecules on the erythrocytes surface are highly polymorphic in human population. We are investigating the role of cross-reactive antibodies in merozoite invasion inhibition and merozoite invasion pathway (receptor-ligand interaction) in cultured field parasite isolates.

Participants

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Aims and Objectives

1. Characterization of *P. falciparum* and *P. vivx* parasite-specific antibodies in malaria patients' plasma/ sera samples.
2. Determination of the efficacy of malaria patients' sera antibodies in in vitro merozoite invasion inhibition & intraerythrocytic parasite growth inhibition.
3. Expression of merozoites invasion molecules (EBAmolecules and Pf Rh group) in local *P. falciparum* strains.

Worked achieved

In earlier studies, we have estimated IgG, IgM and IgA cross-reactive antibodies to *P. falciparum* and *P .vivax* parasite antigens. We have tested the effect of plasma samples from malaria patients infected with *Plasmodium falciparum* (15) and *P. vivax* (20) on inhibition of invasion by merozoites of 3D7 and two cultured field parasite isolates, *in vitro*. The sera samples from *P. falciparum*-infected patients showed 5.45 to 9.25 percent invasion inhibition by merozoites of the 3D7 parasites. Six plasma samples showed more than 15 percent invasion inhibition in the two cultured field parasite isolates. The inhibition was less than 8.5 percent in 9 of the plasma samples. The plasma samples from *P. falciparum*-infected patients were more active against intracellular parasite growth inhibition, with 12 samples showing 15.50 to 21.72 percent inhibition of 3D7. Eleven samples showed more than 11 percent inhibition of the field parasite strains. We have also studied the invasion pathway in *P. falciparum* 3D7 and two local field parasite isolates. To understand host-parasite receptor-ligand interaction, we exposed the schizonts (with merozoites) to RBCs treated with the enzymes, neuraminidase and trypsin. These enzymes specifically remove RBC receptors which are important for merozoite-ligand protein interaction during invasion into host cells. The merozoite inhibition observed was 5.55, 6.75 and 9.42 percent with the 3D7, F1 and F2 parasites, respectively, when trypsin-treated O+ RBCs were used. The invasion by merozoites and parasite growth in enzyme-treated RBCs will be studied further in eleven *P. falciparum* local field isolates which are adapted to lab culture conditions.

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Role of non-T cells and T cells in anti-leishmanial Immunity

Background

Toll-like receptors recognize the pathogen-associated molecular patterns and trigger the production of pro-inflammatory cytokines, which are required for elimination of most pathogens. Likewise, TLRs are reported to play important roles in immune responses against *Leishmania major*, a protozoan parasite that survives in TLR expressing macrophages. The survival of *L. major* in macrophages therefore implies an unknown role of TLRs in anti-leishmanial immune responses. Here, we have examined the involvement of TLR2 in the modulation of anti-leishmanial immune response.

Aims and objectives

The aim of the project is to analyze the role of TLR2 in the regulation of immune response. Specifically, we had three aims:

1. To examine the effect of blocking TLR2 on *L. major* infection in BALB/c mice;
2. To assess the effects of three different TLR2 ligands on anti-leishmanial immune responses;
3. To assess the nature of CD40-TLR cross-talk in macrophages, as CD40 is also expressed on macrophages and plays crucial roles in anti-leishmanial immune responses.

Work Achieved

Role of TLR2 in *L. major* infection

As TLR2 interacts with *Leishmania* expressed lipophosphoglycan (LPG), a virulence factor, and as TLR9 has been implicated in host-protection, we studied whether shown to promote a host-protective response. However, whether there is a relationship between TLR2 and TLR9 that modulates *L. major* infection. We observed that the levels of LPG expression were associated with decreased TLR9 expression, accompanied by TGF-beta and IL-10 production, in macrophages. Addition of anti-LPG or anti-TLR2 antibodies

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prevented the reduction in TLR9 expression. Co-treatment of mice with anti-TLR2 antibodies and CpG reduced parasite load in the draining lymph nodes, accompanied by an interferon (IFN)-g predominant T cell response. We thus showed for the first time how LPG-TLR2 interaction turns pro-leishmanial via cytokine-mediated decrease of TLR9 expression.

Effect of TLR2 ligands on *L. major* infection

TLR2 can combine with TLR1 or TLR6 to form heterodimers, recognizing triacylated or diacylated lipopeptides, respectively. However, whether such recognition of different ligands by these two different receptors leads to different functions is not known. Therefore, we examined the effects of three TLR2 ligands- Pam_3CSK_4 (TLR1/2 ligand), PGN and BPPcysMPEG (TLR2/6 ligand)- on the modulation of *L. major* infection. We observed that BPPcysMPEG elicited host-protective anti-leishmanial immune response and worked as an efficient adjuvant, providing significant protection to *L. major* challenge infection.

TLR-CD40 cross-talk

TLRs were shown to activate adaptive immune responses mediated by T cells. In T cell responses, CD40-CD40L interaction plays important roles. Therefore, we examined whether CD40 and TLRs modulate each others expressions. We observed that all TLRs enhanced the expression of CD40 but only TLR9 expression was increased by CD40. However, when BALB/c mice were co-treated with anti-CD40 and CpG, no additional protection was observed over and above either agent alone.

Future work

1. To analyze the TLR2-modulation of anti-leishmanial T cell response
2. To compare the signaling from TLR2/TLR1 and TLR2/TLR6
3. To investigate if CD40 signaling is affected by TLR ligands

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Understanding viral complement regulation at the molecular level

Background

Viruses are amongst the most successful pathogens to have co-existed with the hosts by maintaining a precarious balance between the two for their successful existence. Being predatory in nature, viruses are constantly in the pursuit of survival and thus, there exists a constant struggle for endurance between the viruses and their hosts: viruses pursue host for their propagation and the hosts on the other hand defy the viral intrusions owing to their well-developed and interconnected network of innate and adaptive immune defense mechanisms.

The complement system is one of the essential modules of this host immune network and is known to neutralize diverse DNA as well as RNA viruses. It is therefore not surprising that complement became a viral target for immune evasion. For example, viruses in particular pox and herpesviruses are known to encode functional homologs of complement regulators. These homologs belong to RCA. Our laboratory focuses on the molecular characterization of viral homologs belonging to the RCA family with the expectation that it would not only provide a new insight into host-virus interplay, but would also exemplify novel features of complement regulation that are central to the biology of complement.

Aims and Objectives

1. Which are the functional determinants of the viral complement regulators?
2. What role viral complement regulators play in viral pathogenesis?

Work Achieved

Although both pox as well as herpes viruses encode RCA homologs, poxviral complement regulators are more similar in sequence (>91%) compared to the herpesviral regulators (43%-89%). It is therefore conceivable that molecular

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characterization of various pox and herpesviral complement regulators would help in determining the structural as well as functional diversity in these regulators. Our laboratory therefore is engaged in characterization of complement regulators encoded by both these viral families. Interestingly, our earlier efforts revealed that though pox viral regulators differ only marginally (<5%), these differences are sufficient to alter their species specificities. In particular, charged residues in the central domains participate in dictating the species specificity.

Because viral RCA homologs are structurally as well as functionally similar to human RCA proteins and regulate complement by employing common mechanisms, we asked: Are functional sites similar and spatially conserved in viral and human RCA proteins? To answer this question, we utilized HVS CCPH as a model protein and mutated it's predicted "functional site residues" based on earlier identified human RCA functional site residues. In addition, we also examined whether incorporation of "functional site residues" in CCPH leads to gain-of-function.

Amino acids R118 and F144 play critical role in factor I cofactor activity of sCCPH

Earlier we have shown that CCPH regulates complement by serving as a cofactor in protease (factor I)-mediated inactivation of complement proteins C3b and C4b (cofactor activity), which are the subunits of C3-convertases. Further, we also showed that domains 1 and 2 play critical role in imparting the cofactor activities and domains 3 and 4 contribute to its optimal activity. We therefore selected residues for mutation that belonged to all the four domains of sCCPH. In addition, we also selected residues that are located in the linker regions between domains 1-2 and 2-3, as charged residues in these linkers have been shown to be crucial in mediating the cofactor activities in human RCA proteins. Altogether, eight residues (R35, K40, N92, S100, R118, K128, F144 and K191) that are located in the different domains of sCCPH, and three residues (K61, K62 and K126) that are part of the linkers, were chosen for mutation to Ala. These mutants were then examined for C3b and C4b cofactor activities using a fluid-phase assay wherein C3b or C4b was incubated with sCCPH or the mutant of interest and factor I, and inactivation of C3b/C4b was assessed by measuring the cleavage of their α-chains. Of the above eleven mutants, only R118A and F144A (Fig. 1) showed significant decrease in the C3b and C4b cofactor activity compared to the wild-type protein.

The cofactor activity is a result of interaction between the regulator, target protein (C3b or C4b) and factor I. It is therefore conceivable that decrease in the cofactor activity owing to mutation could be a result of decrease in binding to the target protein or factor I. We, therefore, measured binding of the above mutants to C3b and C4b tethered onto the SPR sensor chips in their physiological orientation. Mutants R118A and F144A that showed considerable decrease in C3b cofactor activity did not show any decrease in C3b or C4b binding. We thus suggest that loss in the cofactor activity of these mutants is primarily due to decrease in their interaction with factor I.

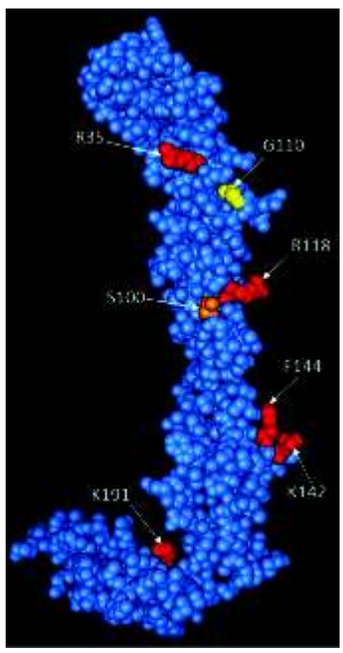


Fig. 1: CPK representation of sCCPH model showing residues important for its function. Residues R118 and F144 play critical role in factor I cofactor activity, while residues R35, K142 and K191 are vital for its decay-accelerating activity. Mutations at positions 100 and 110 (S100K and G110D) lead to gain of function.

Positively charged residues R35, K142 and K191 are vital for decay-accelerating activity of CCPH

Apart from supporting the inactivation of C3b and C4b, CCPH also inactivates complement by accelerating the irreversible decay of C3-convertases (decay accelerating activity; DAA). Previous mapping of domains required for DAAs in CCPH using truncated mutants indicated that domains 1-3 are primarily responsible for driving classical/lectin pathway (CP/LP) DAA, whereas all the four domains are necessary for driving the alternative pathway (AP) DAA. Here, for mapping residues critical for DAAs, we selected residues from domains 1-3 as well as linkers between SCRs 1-2 and 2-3, which were shown to be vital for DAAs in other RCA proteins. The mutants generated were R35A, K40A, K61A, K62A, Y83A, L106A, V113A, K126A, K142A and N156A. These were then examined for their ability to decay the pre-formed classical/lectin (C4b,2a) as well as alternative (C3b,Bb) pathway C3-convertases made on sheep and rabbit erythrocytes, respectively. Among the above ten mutants that were expected to show effect on DAAs, mutants R35A and K142A (Fig. 1) displayed loss in AP DAA as well as CP DAA. In addition, mutant K191A (Fig. 1) which was generated to determine the effect of this residue on CFAs also showed loss in AP, but not CP DAA. Interestingly, similar to that of CFAs, linker mutants that were expected to demonstrate loss in DAAs, did not show any effect on the AP as well as CP DAAs.

Like cofactor activity, DAA is also a result of tri-molecular interaction. Herein, interaction of the regulator with both the subunits of the C3-convertases (C3b and Bb or C4b and C2a) results in dissociation of the protease subunit (Bb or C2a) from the bimolecular enzyme. To understand whether decrease in DAAs of R35A, K142A and K191 mutants is owing to decrease in their binding to C3b or C4b, we looked at their direct binding to these

molecules. Mutant R35A showed moderate decrease in C3b binding whereas the other two mutants displayed substantial decrease in C3b binding. Further, mutants R35A and K142A also displayed substantial decrease in C4b binding. In sum, mutants that showed decreased DAAs also showed decrease in their binding abilities to the target proteins suggesting thereby that loss in DAAs is likely due to decrease in C3b/C4b recognition.

Functional characterization of predicted gain-of-function mutants

We designed eight gain-of-function mutants to determine whether mutations based on gain-of-function in other RCA proteins would result in enhancement of CCPH function. These efforts led to the identification of at least two mutations (S100K and G110D; Fig. 1) which led to robust increase in DAAs. We however would like to point out that these two mutations were based on educated guesses and not having prior knowledge of gain-of-function.

It is therefore obvious from the above results that though certain functional sites are similar in viral and human complement regulators, notable differences exist. Thus, detailed knowledge on functional sites in viral regulators can only be obtained by other conventional methods such as swapping of domains/regions of viral complement regulators with similar domains of proteins which lack cofactor or decay activity.

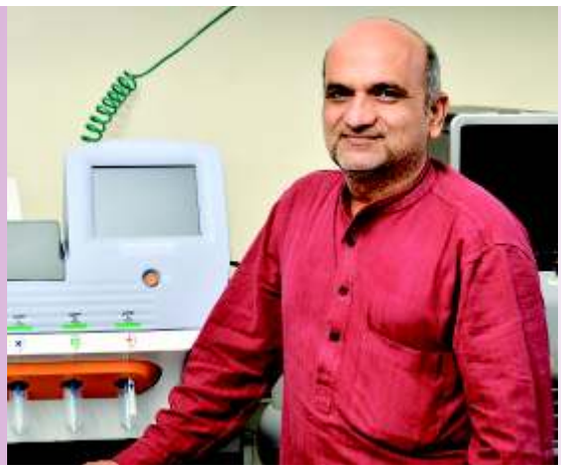
Future Research Plans

1. Role of electrostatics in dictating species specificity in complement regulators.
2. Fine mapping of functional sites in viral complement regulators using domain/region swapping approach.
3. Designing pathway specific complement inhibitors.

Research Report

Microbial Biology

Yogesh S. Shouche



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Human Microbiome Indian perspective

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Background

Microbes colonise various sites on human body such as skin, gastrointestinal tract and vagina. Human gut harbours approximately 10^{14} bacterial cells which out numbers the total number of human cells. Gastrointestinal tract is densely populated by bacteria belonging to *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* divisions. There are increasing numbers of proof that bacteria residing in gut have major impact on human wellbeing. The gut microbiome plays a beneficial role in extracting nutrients from the diet, regulating host fat storage, stimulating intestinal epithelium renewal, and directing the maturation of the immune system. On the contrary, some of these microbes are associated with some systemic diseases, such as obesity & cardiovascular disease, and in intestinal conditions like inflammatory bowel disease. Thus, understanding microbiome structure in the gut is essential to the development of future personalized strategies of healthcare, as well as potentially providing new targets for drug development.

Aims and Objectives

Till now, most of the studies on human gut flora are carried out on European or American populations neglecting Indian subjects. Hence, there is lack of knowledge about composition of gut flora of Indian population. The physiology of Indian population is different from that of western population as suggested by YY- paradox and it is expected that composition of gut microbes would also be different. With this background, studies on the gut flora of Indian population were initiated to understand the genetic and environmental factors that contribute to its development and also the changes that are associated with diseases.

Work Achieved

Changes in Gut flora with age

The gut flora is reported to vary with the genetic makeup of the individual and environmental factors including age. We selected two families each with three individuals belonging to successive generations living together under same roof.

The Denaturing Gradient Gel Electrophoresis (DGGE) analysis revealed the difference in gut flora composition of individuals of different age belonging to same family. The band intensity and number of bands observed in DGGE profile of samples suggest that different bacterial species dominated the gut flora of individuals of varying age.

Clone library analysis

The 16S rRNA clone library analysis showed consistent decrease in the *Firmicutes* and increase in *Bacteroidetes* in both families with an increase in age (Fig. 1). Genus level distribution of gut flora clustered the individuals of same family together.

Fecalibacterium and *Roseburia* dominated in subject T1 (age 14); *Dialister*, *Prevotella* dominated in subject T2 (age 42) and *Prevotella* in subject T3 (age 62) of family T. The genus *Streptococcus* and *Weissella* dominated in the infant and *Fecalibacterium* and *Roseburia* dominated in adult subjects (age 26 and 62 years respectively) of family S.

Firmicutes to *Bacteroidetes* ratio (F/B ratio)

qPCR quantification confirmed that the number of *Firmicutes* decreased while, the number of *Bacteroidetes* increased with the increase in age. Such change in *Firmicutes*/*Bacteroidetes* ratio was different to the previous reports on western populations, as an increase from 0.4 in infants to 10.9 in adults followed by decrease to 0.6 has been reported in the *Firmicutes*/*Bacteroidetes* ratio in a study on European population. Contrasting results have been reported in Italian population on the *Firmicutes*/*Bacteroidetes* ratio, which increased from 3.9 for adults to 5.1 for elderly and decreased to 3.6 for centenarians. In our study, we observed a consistent decrease in *Firmicutes* number and increase in *Bacteroidetes* number with increase in individual's age, which suggested a gradual decrease in *Firmicutes*/*Bacteroidetes* ratio with increase in age of subjects. The observations of clone library analysis were further validated by qPCR, which suggested that the composition of gut flora of Indians responded in different way to that of western populations. We have also isolated 22 anaerobic bacteria from fecal samples of three healthy volunteers. The bacterial isolates were identified using 16S rRNA gene sequencing. The results exhibited that 6 out of 22 isolates (27%) have 97% or less

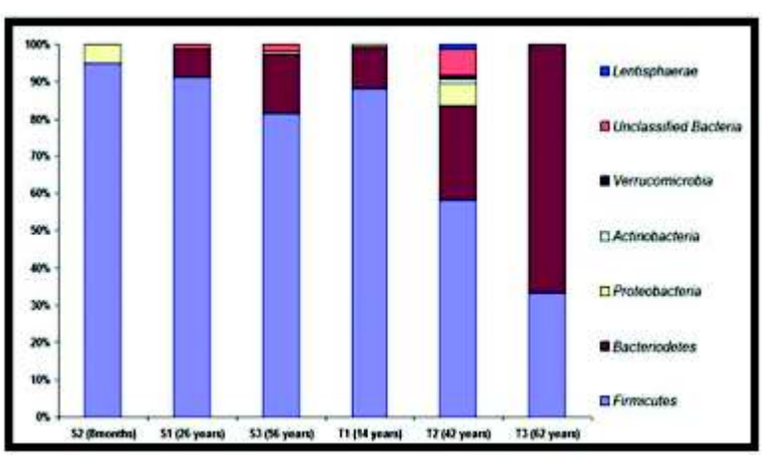

Fig. 1a: Phylum level comparison of gut flora of the subjects. The stacked bars describe the percent distribution of each phylum across the subjects.

sequence similarity to the nearest type strains of 5 different genera suggesting them as potential novel species. We have sequenced and analyzed the genome of two of these, *Megasphaera* sp. NM10 and BL-7. The sequence shows several interesting features that offer an adaptive advantage to these organisms in the gut environment.

Sugar transport and metabolism: The gut acts as a nutrient rich environment which supplies various carbohydrates including simple and complex carbon sources. The gut bacteria have large number of membrane transporters associated with uptake of these metabolilties. *Megasphaera* sp. NM10 and BL7 also have a large number of these membrane transporters. These include a PTS system, maltose and glucose-specific IIC component, mannitol-specific IIC component (EC 2.7.1.69), fructose-specific IIA component, Phosphoenolpyruvate-protein phosphotransferase of PTS system, N-acetylmuramic acid-specific IIB component and others. These transporters are important for the bacteria to survive in the competitive environment of the gut. The pathways for utilization of glucose, maltose, fructose have been identified. In addition, these bacteria have a large repertoire of active carbohydrate utilization enzymes, i.e. Carbohydrate-Binding Modules (CBMs), Carbohydrate Esterases (CEs), Glycoside Hydrolases (GHs), GlycosylTransferases (GTs). Comparative glycobiome analysis indicates that these bacteria have several unique CAzymes that not present either in the host or the related species. (Fig 2)

The other important requirements for survival in gut environment are resistance to antibiotics, phage infection and other extrachromosomal DNA. These bacteria have genes which confer resistance to the most common group of antibiotics i.e Beta Lactamases, in addition these also have genes encoding for resuistance to tetracycline

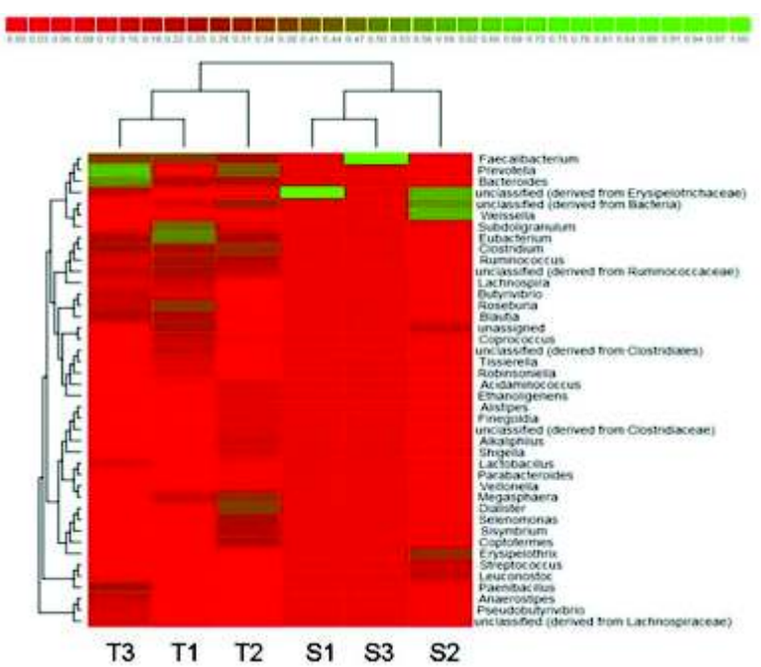

Fig. 1b: Genus level comparision of gut flora

and heavy metals. A large number of multi-drug efflux pumps have been detected in these bacteria. The other major factor is infection by phages, CRISPR elements have been detected major for the cas family which are involved in prokaryotic immunity. All the information that was obtained from the genome sequence of these bacteria suggests a high level of niche adaptation and also importance in the gut environment.

Potential effects on the human host

SCFA's: *Megasphaera* sp. NM10 and BL7 have an ability to produce butyrate, acetate and propionate by fermentation. These (especially butyrate) is involved in functions which decrease colonic pH, inhibit the growth of pathogens; stimulate water and sodium absorption; participate in cholesterol synthesis; provide energy to the colonic epithelial cells, implicated in human obesity, insulin resistance and type 2 diabetes, colorectal cancer.

Choline metabolites: These bacteria are capable of producing various choline metabolites which have been known to modulate lipid metabolism and glucose homeostasis and involved in nonalcoholic fatty liver disease, dietary induced obesity, diabetes, and cardiovascular disease.

Vitamins: Pathways for biosynthesis of various water soluble vitamins such as Ascorbate (vitamin C), Biotin (vitamin H), Folate (vitamin B9), Niacin (vitamin B3), Pantothenic acid (vitamin B5), Pyridoxine and its derivatives (vitamin B6), Riboflavin (vitamin B2), Thiamin (vitamin B1), were identified. These are important for the health of the host and strengthen immune function, exert epigenetic effects to regulate cell proliferation.

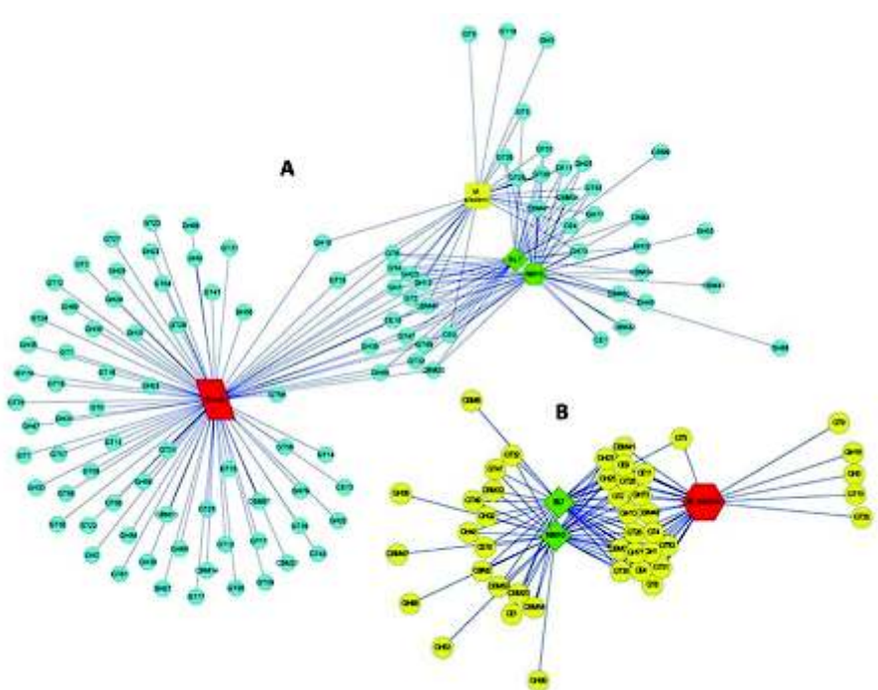

Fig. 2: The Glycobiome network of *Megasphaera* sp. **A)** The glycobiome network of genome of Human (red), *Megasphaera* sp. NM10, BL7 (green) and *M. elsdenii* DSM20460 (yellow). **B)** The glycobiome network of *Megasphaera* sp. NM10, BL7 (green) and *M. elsdenii* DSM20460 (red). CBMs- Carbohydrate-Binding Modules, GHs- Glycoside Hydrolases, GTs- Glycosyl Transferases, CEs- Carbohydrate Esterases. The nodes represent the CAZyme superfamilies and the edges are connecting the nodes based on the presence or absence of respective superfamilies in the organism.

Genome sequence of the two *Megasphaera* sp. NM10 and BL7 isolated from human gut suggests that these are important bacteria in the human gut, which have beneficial effects on health of the host. These bacteria have evolved and highly adapted to the gut environment and further functional characters of these isolates can give detailed insights into the role of these in human gut.

Gut microbiome of diabetic individuals in Indian population

Diabetes is one of the complex multisystem disorders and most common non-communicable disease worldwide that results from pancreatic β-cell dysfunction and/or insulin resistance. Indian population belongs to Asian-Indian phenotype, which refers to the combination of clinical (larger waist-to-hip and waist-to-height ratios signalling excess visceral adiposity), biochemical (insulin resistance, lower adiponectin, and higher C-reactive protein levels) and metabolic abnormalities (raised triglycerides, low high-density lipoprotein cholesterol) that predispose this group to developing diabetes.

In the light of the link between metabolic diseases and bacterial population in the gut studies on the characterization gut microbiome of diabetic individuals of Indian origin were initiated. The study is being carried out in collaboration with Diabetic Unit, King Edward Memorial (KEM) hospital, Pune. The subjects for the study are participants of Pune Children Study (PCS), and are either diabetic or at the high risk of development of diabetes. So far, preliminary analysis of 20 samples has been carried out.

Celiac Disease and gut microbiota

While 20-30% of general population is genetically susceptible for celiac disease (CeD) and majority of population consume wheat, only 1% of them develop celiac disease. At present, gluten free diet (GFD) is the only treatment available. Intestinal microbiota has been implicated in the pathogenesis of celiac disease. We studied the differences in the bacterial community composition in a CeD patient before and after six months on gluten free diet (GFD) using Next Generation Sequencing Fig 3. The OTU based network analysis showed presence of a distinct gut bacterial community in patients with celiac disease at base line and six months after GFD and the control. A relative decrease in *Proteobacteria* was observed after six months of GFD. Pathogenic bacteria such as *Campylobacter sp.* and *Haemophilus sp.* which were present during gluten containing diet (GCD) were not detected after six months of GFD. Additionally, there was an increase in 'beneficial' bacteria such as *Bifidobacterium* after six months of GFD. qPCR analysis confirmed the increase in number of *Bifidobacterium* after GFD. Overall, results suggest that GFD shifted the bacterial community composition towards a healthy gut microbiota by decreasing number of pathogenic bacteria in a patient with CeD. Presently, we are analysing the gut microbiota in active CeD patients before and after GFD treatment, their first degree relatives and functional dyspepsia patients as controls. This study includes duodenal biopsies of subjects from all the four groups. The preliminary results indicate a difference in microbial community composition in the duodenum when compared to stool.

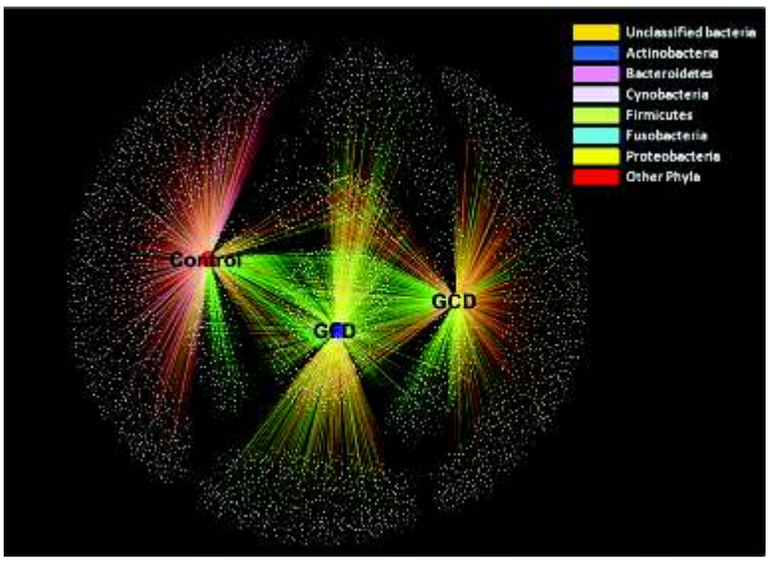

Fig. 3: Network based representation of OTU clustering. The edges corresponding to specific phylum are coloured. Stool samples were collected at two time points for a CD patient i.e. time point 1: patient on gluten containing diet (GCD) and time point 2: after six months of gluten free diet (GFD). Disease control i.e. individuals without CeD.

Furthermore, we expect to observe novel insights as there are no studies that have established the gut microbiota in duodenum of CeD patients.

Future Work

The studies will be further continued to understand association of gut microflora with diseases like diabetes, celiac disease and efforts would be made to understand core microbiome of Indian population.

Research Report

Signal Transduction

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Osteopontin modulates HIF1α-mediated VEGF dependent angiogenesis and breast tumor growth in response to hypoxia

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Background

Hypoxia is a salient feature of most solid tumors and it exerts clonal expansion of highly malignant cells. Cellular adaptation to hypoxia is largely mediated through activation of several genes namely HIF1 and HIF2 that facilitates short-term or long term adaptive mechanisms. HIF1 is a heterodimeric transcription factor comprising of oxygen sensitive alpha sub-unit and the constitutively expressed beta subunit. Proline hydroxylation is inhibited under hypoxia resulting in a stabilized α subunit which then translocates to the nucleus and heterodimerizes with the β subunit and binds to conserved DNA sequence known as hypoxia responsive element (HRE) on the promoters of target genes. HIF1α regulates a wide array of genes involved in tumor angiogenesis, chemoresistance and metastasis. HIF1α is found to be overexpressed in several cancers and is indicative of poor prognosis in breast cancer patients. Recent studies reveal that HIF1α can be selectively regulated at the levels of transcription, translation and post-translation by several growth factors.

Osteopontin (OPN) has been implicated in multiple aspects of tumor progression. Increased serum levels of OPN have been associated with enhanced metastatic burden and poor disease outcome in patients. OPN is a diagnostic marker in hepatocellular, cervical and head and neck carcinoma. It has been reported to instigate indolent tumor growth through bone marrow activation. Elevated plasma OPN levels in head and neck cancer patients were indicative of poor patient outcome and also correlated with tumor hypoxia. OPN is known to confer cancer cell resistance to hypoxia and reoxygenation induced cell death. Hypoxia enhances $\alpha v\beta_3$ integrin expression in B16F10 cells and also silencing $\alpha v\beta_3$ or $\alpha v\beta_5$ integrin results in decreased HIF1α expression in glioma. Integrin-linked kinase (ILK) is closely associated with integrin signaling and has been implicated in hypoxia-induced tumor angiogenesis.

We have shown that hypoxia-induced OPN is required for maximal expression of HIF1α in breast cancer cells. OPN modulates HIF1α expression at transcriptional level via ILK and

Akt dependent NF-κB p65 signaling pathway. Our results delineated the role of OPN under hypoxia and its implication in breast tumor growth and angiogenesis through NF-κB-mediated HIF 1α dependent VEGF expression (Raja et al, Oncogene, 2013).

Aims and Objectives

1. To study how hypoxia regulates OPN expression in breast cancer cells.
2. To decipher the molecular mechanism by which OPN regulates hypoxia responsive genes leading to breast tumor growth and angiogenesis under hypoxic condition.
3. To examine the differential expression profiles of OPN and HIFs in different grades of human breast cancer specimens and its correlation with breast tumor progression.

Work Achieved

We have identified the existence of a positive inter-regulatory loop between hypoxia and OPN. Our results suggested that hypoxia induces OPN expression in breast cancer cells (Fig. 1), however the expression was found to be HIF1α independent. OPN enabled transcriptional upregulation of HIF1α expression both under normoxia and hypoxia whereas stability of HIF1α protein in breast cancer cells remained unaffected (Fig. 1). Moreover, we have shown that OPN induces ILK/Akt-mediated NF-κB, p65 activation leading to HIF-1α dependent VEGF expression and angiogenesis in response to hypoxia. These *in vitro* data are biologically significant as OPN expressing cells induce greater tumor growth and angiogenesis through enhanced expressions of proangiogenic molecules (Fig. 2). Immunohistochemical analysis of human breast cancer specimens revealed significant correlation between OPN and HIF1α but not HIF2α. Elevated expression of HIF1α and OPN was observed in pre-neoplastic and early stage infiltrating ductal carcinoma implicating the role of these proteins in neoplastic progression of breast cancer. In summary, our results corroborate the prime role of OPN in cellular adaptation

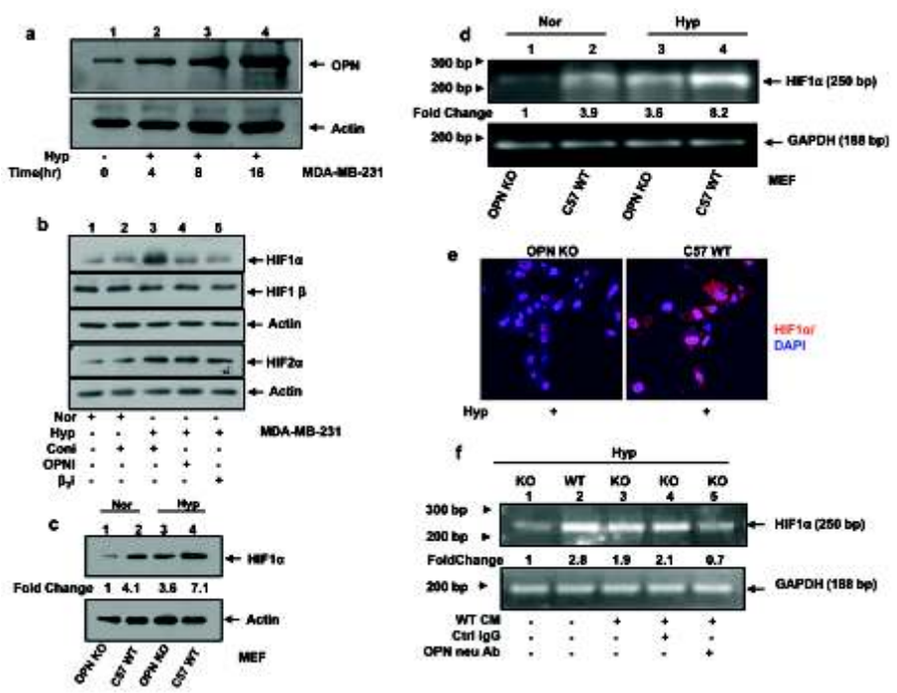


Fig. 1: Hypoxia induces OPN expression and OPN dependent HIF1a expression in breast cancer cells. **(a)** Western blot analysis of OPN expression in MDA-MB-231 cells treated with hypoxia for 0-16 h. **(b)** MDA-MB-231 cells were transfected with siRNA specific to OPN or β_3 integrin and immunoblotted for HIF1α, HIF1β and HIF2α levels. Actin was used as control. **(c)** MEF cells isolated from OPN KO and C57 WT mice were subjected to hypoxia treatment for 16 h and HIF1α expression was determined by western blot. **(d)** HIF1α mRNA levels were analyzed by RT-PCR in OPN KO and WT derived MEF cells exposed with hypoxia. GAPDH was used as control. **(e)** Nuclear translocation of HIF1α in OPN KO and WT MEF cells in response to hypoxia was analyzed by confocal microscopy. Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). **(f)** WT conditioned media were added to OPN KO derived MEF cells and then supplemented with either IgG or OPN neutralizing antibody (20 μg/mL) and exposed with hypoxia. HIF1α expression in response to hypoxia was analyzed by semi-quantitative RT-PCR.

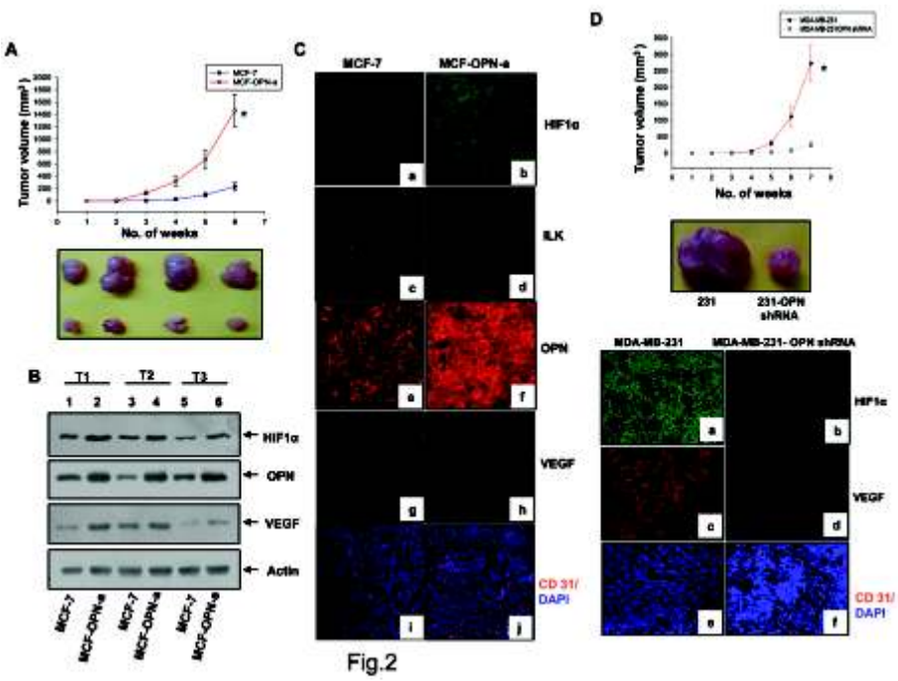


Fig.2

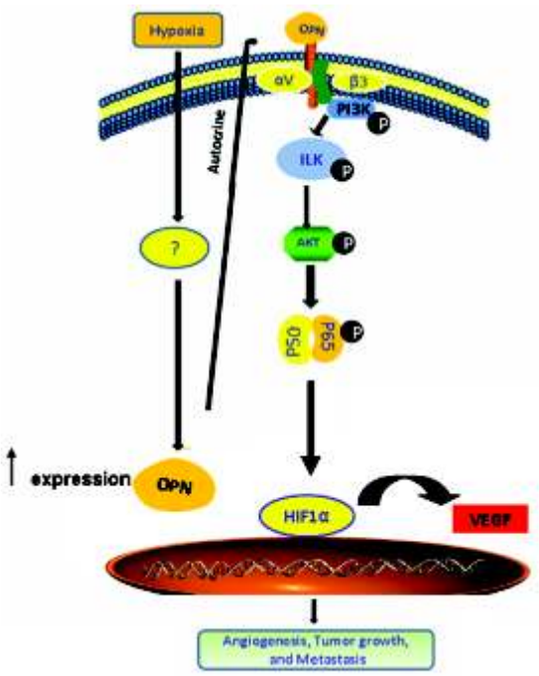
Fig. 2: OPN, HIF1 and VEGF play crucial role in breast tumor progression. (A) MCF-7 or MCF-OPN-a cells were injected into lower mammary fat pads of female NOD-SCID mice (n=7) and tumor growth kinetics is graphically represented for a period of 6 weeks. * *P< 0.0006*. Lower panel: the excised tumors were photographed. **(B)** Immunoblots showing relative expression of HIF1α, OPN and VEGF in three separate tumors (T1-3) derived from MCF-7 or MCF-OPN-a cells. **(C)** Immunofluorescent photographs of MCF-7 or MCF-OPN-a derived tumors sections for expressions of HIF1α, ILK, OPN, VEGF and CD31. Cell nuclei stained blue with 4',6-diamidino-2-phenylindole (DAPI) (a-j). **(D)** Top panel: MDA-MB-231 or MDA-MB-231 cells stably transfected with OPN shRNA were injected into lower mammary fat pads of female NOD-SCID mice (n=7) and tumor growth kinetics is graphically represented. * *P< 0.001*; Middle panel: the excised tumors were photographed; Bottom panel: Tumors derived from MDA-MB-231 or MDA-MB-231 cells stably transfected with OPN shRNA were analyzed for expressions of HIF1α, VEGF and CD31 by confocal microscopy (a-f).

through ILK and NF-κB-mediated HIF1α dependent VEGF expression in response to hypoxia that ultimately controls breast cancer progression and angiogenesis (Fig. 3) and targeting this signaling network may have potential therapeutic implication in management of breast cancer (Raja et al, Oncogene, 2013).

Future Work

1. To examine the role of semaphorin 3A in regulation of breast tumor growth and angiogenesis through FOXO 3A dependent MelCAM expression.
2. To study the role of CD20 and CD133 positive cancer stem cell in control of melanoma growth, angiogenesis and metastasis.

Fig. 3: Schematic representation of OPN regulated HIF1α expression leading to enhanced angiogenesis and breast tumor growth through ILK/NF-κB, p65 dependent VEGF signaling pathway in response to hypoxia.



Musti Krishnasastry

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Exploration of virulence traits of *tlyA* gene product of *Mycobacterium tuberculosis*

Background

The hall mark of *Mycobacterium tuberculosis* (Mtb) is that it blocks the phagosome acidification by blocking the fusion of phagosome with the lysosome referred as maturation in the literature. This helps the mycobacterium to avoid the proteases of lysosomes to prevent degradation and hence processing and successful presentation of mycobacterial antigens to host's immune system. We have recently hypothesized that the tlyA gene product of H37Rv, Rv1694, is a 'hemolysin like' molecule in addition to being a ribosomal RNA methylase. It has the ability to assemble into ordered oligomers, capable of lysing red blood cells and also on phagosomes of macrophages. Interestingly, the Rv1694 is expressed and exported to the cell wall of *tlyA* transformed *E. coli*. These observations led us to speculate that the Rv1694 can play an important role in compromising the integrity of phagosomal compartments during the initial phases of Mtb infection. This is because it has the capability to induce target compartment lysis by acting as a bridge between the microbe and the target membrane such as the phagosomal membrane.

The aim of the present work is to understand the mutated forms of Rv1694, which are catalytically deficient in rRNA methylation, vis-à-vis cell surface appearance. Since E. coli has no natural homolog of Rv1694, any new property acquired by *E. coli* can be attributed to the property implanted by Rv1694 due to its expression. In addition, this approach, to the best of our thinking, can also eliminate the problem of redundancy of molecules, if any expressed by Mtb, involved in compromising the integrity of phagosomal membranes.

Aims and Objectives

1. Detailed characterization of Rv1694 homologues from *M. tuberculosis*.
2. Role of cell surface Rv1694 homologues.
3. Mechanism of action of Rv1694 and its homologous proteins.

Participants

Aejazur Rahman, *SRF*
Santosh Kumar, *SRF*
Ekansh Mittal, *SRF*
Anil Kumar, *SRF*
Shika Nag, *SRF*
Sapna Deore, *SRF*
Anil Lotke, *Technician*
Tanuja Bankar, *Technician*

Work Achieved

Surface staining of H37Rv for Rv1694

Consistent with our hypothesis the Rv1694 is present on the cell wall of H37Rv. We have stained the H37Rv with Rv1694 specific immune rabbit serum (IRS) or pre-immune rabbit serum (NRS) followed by immuno fluorescence microscopy, as shown in Fig. 1A. It is relevant to mention here that we have specifically looked for well isolated bacteria for the signal than clumps or clusters for higher degree of clarity. It is also important to mention here that staining of H37Rv with NRS or fluorescent secondary antibody showed any staining of H37Rv. This result suggests the presence of a common mechanism which targets the Rv1694 to the cell wall of H37Rv and *E. coli*.

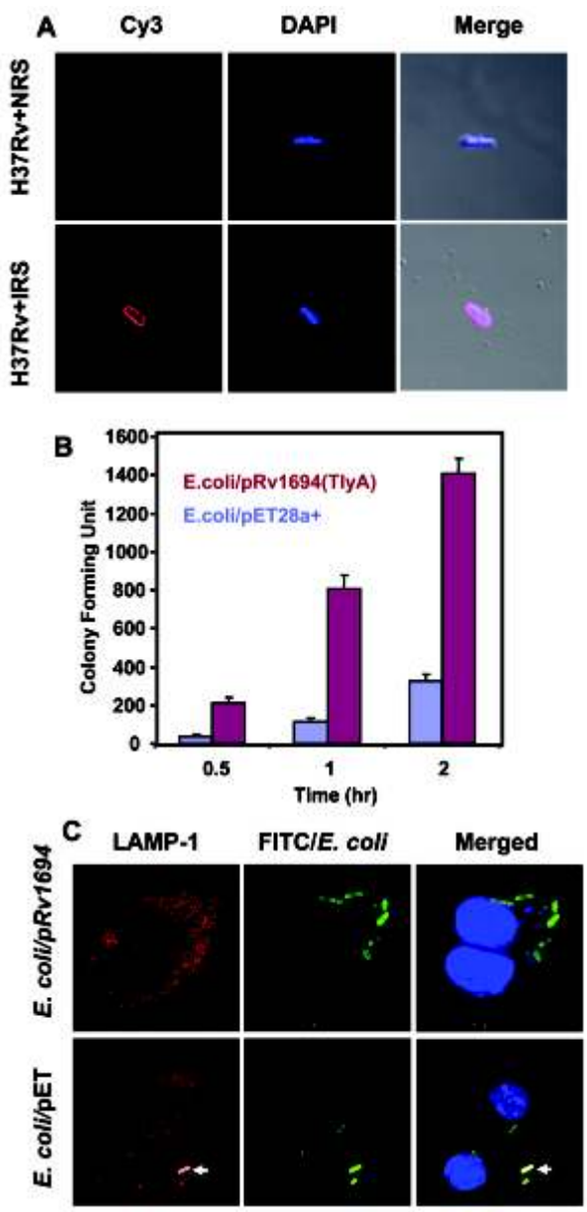


Fig. 1: (A) Immunofluorescence staining of H37Rv: H37Rv was immuno-stained with pre-immune rabbit serum (NRS) orimmune rabbit serum(IRS) and visualized with Cy3-anti-rabbit IgG (red). Panels from left (upper and lower) indicate staining for Rv1694, DAPI for nucleic acid, phase contrast and merged images. H37Rv was not stained with NRS or Cy3-anti-rabbit antibody which indicates that there was no non-specific background of the antibodies used in our study.

(B) Intracellular survival of Rv1694 in *E. coli*: *E.coli* / pRv1694 (TlyA) and *E.coli*/pET were incubated with RAW264.7 macrophages at an MoI of 1:20 at 37^{0}C for 30, 60 and 120 min. After incubation, free bacteria were washed and macrophages were lysed to release the engulfed bacteria, serially diluted and plated on LB agar plate to observe bacterial colonies. Each colony represents live bacteria after phagocytosis by macrophage.

(C) *E.coli*/pRv1694 inhibits phagolysosome formation. RAW264.7 macrophages were incubated with FITC labelled *E.coli*/pRv1694 or *E.coli*/pET at an MoI of 1:20 at 37oC for 60 minutes. Phagolysosome formation was observed for LAMP-1 (red) colocalization with FITC labeled *E. coli*. Macrophages were observed on Zeiss LSM 510 Meta confocal microscope equipped with 100x objective. Arrow indicates phagosome maturation.

Intracellular survival of *E.coli*/pRv1694

To assess the role played by Rv1694 after infection of macrophages, we compared the survival of *E.coli*/pRv1694 and *E. coli* expressing the mock vector pET28a+ (*E.coli*/pET) after phagocytosis by RAW264.7 macrophages. After phagosytosis, macrophages were lysed and engulfed bacteria were recovered by counting the number of colonies forming units on agar plates. Fig. 1B, shows that the level of phagocytosis and survival was 5 to 7 fold more for *E.coli*/pRv1694 than that of *E.coli*/pET. This observation clearly suggests that the expression of Rv1694 in *E.coli* has resulted in higher adhesiveness/invasion into macrophages.

E.coli/pRv1694 inhibits phagolysome formation in macrophages

In order to examine the phagolysosome formation with immunofluorescence, *E.coli*/pRv1694 and *E.coli*/pET were labeled with FITC and infected the RAW264.7 cell line. The respective *E. coli* were incubated with macrophages for 1hr and washed thoroughly to remove the extra-cellular bacteria. Phagolysosome formation was ascertained by visualization of co-localization of FITC labelled E. coli with LAMP-1 protein, a lysosome marker. The data in Fig. 1C clearly shows a diffused staining or no co-localization of LAMP-1 in case of *E.coli*/pRv1694 in comparison to *E.coli*/pET, which showed prominent co-localization with LAMP-1 (marked with white arrow).

Mutation of D^{154}, K^{182} and E^{238} of Rv1694 abolish rRNA methylation activity but not hemolytic activity

Like all 2'-O-ribose methyl transferases, the Rv1694 also has the conserved K-D-K-E tetrad. The Rv1694 can methylate 2'-O-ribose of both 16S and 23S ribosomal RNA at nucleotide positions C1409 and C1920 respectively. The methylation confers susceptibility to capreomycin. In order to understand any link between methyl transferase activity and hemolytic activity vis-à-vis cell surface appearance, we mutated three amino acids viz., D^{154}, K^{182} and E^{238} which form the catalytic domain, important for its rRNA methylation activity and expressed in E.coli as shown in Fig. 2A.

All these mutants of Rv1694, upon close contact with rabbit red blood cells, showed significant hemolytic activity without and with pre-immune rabbit serum after ~30 hours of incubation at 37^0C (Fig. 2B) while immune serum has been able to neutralize the hemolytic activity. There was no significant difference in hemolysis between wild-type and mutants studied here. Interestingly, all the three mutants have reached the cell wall of *E. coli* (Fig. 2C). This also implies that the mechanism responsible for cell-wall localization is independent of rRNA methylation activity (Fig. 2C).

We next examined the methylation activityof Rv1694 mutants. TlyA expressing *E. coli* has exhibited retarded or no growth in the presence of 100 µg/ml capreomycin in comparison to *E. coli*/pET28a. The growth rates of TlyA, D154A and *E. coli*/pET28a were same in absence of capreomycin as shown in Fig. 3A (growth-rate of *E. coli* expressing K182 and E238 were also same as that of the wild-type and vector control). Further, in presence of capreomycin, the growth-rates of *E. coli* expressing D154, K182 and E238 mutants was same as that of the vector control (the growth-rate of *E.coli* was always

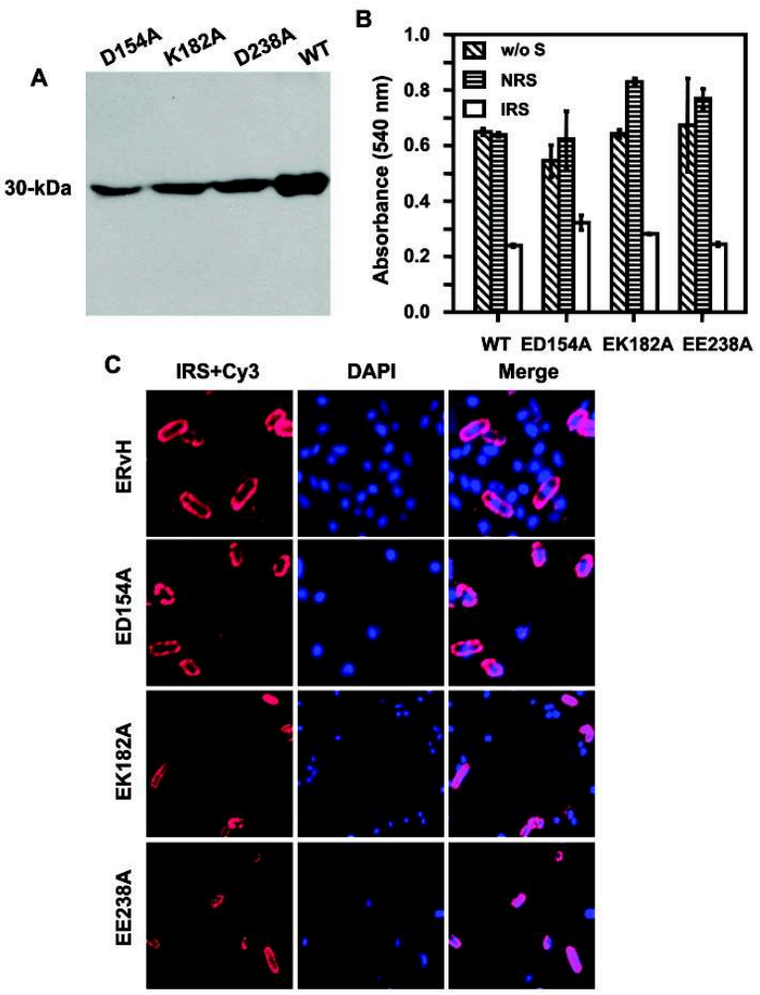


Fig. 2: (A) Expression of mutant Rv1694 in *E. coli*: Expression of Rv1694mutants *viz*. D154A, K182A and E238A in *E. coli*. Cultures of recombinant *E. coli* were processed for SDS-PAGE and visualized by immuno-blotting with anti-6xHis antibody. WT represents wild-type TlyA.

(B) Contact dependent hemolytic activity of TlyA mutants: Contact dependent hemolytic activity of Rv1694, D154A, K182A and E238A was performed by mixing the bacteria expressing the indicated mutant or wild-type protein in the presence or absence of NRS or IRS with rabbit red blood cells at 37°C for 24 to 30 hours. RBC lysis was monitored by measuring the absorbance at 540 nm of a cell-free supernatant. Error bars represents the standard deviation of three independent experiments.

(C) Surface localization of Rv1694 mutants: Transformed *E. coli* was incubated with anti-Rv1694 antibody followed by Cy3-anti-rabbit IgG (red) and DAPI staining (Blue) for bacterial DNA. These labeled bacteria were observed under confocal microscope with 100x objective.

marginally slower in presence of capreomycin than in the absence), where as *E. coli*/Rv1694 has retarded growth in comparison to all the mutants and vector control. All these observations indicate that point mutations at D154 or K182 or E238 of Rv1694 abolish its ribosomal RNA methylation activity conferring resistance to capreomycin.

Ribosomes of D154A exhibit normal translation

Capreomycin inhibits translation activity by binding to 16S and 23S ribosomal RNA only when methylated at nucleotide positions C1409 and C1920 respectively by Rv1694's wild-type methylase activity. We have made S30 extract of wt and D154A of Rv1694 expressed *E. coli* and carried out the *in vitro* transcription and translation assay with supercoiled plasmid of α-hemolysin from *Staphylococcus aureus* as a reporter gene as shown in Fig. 3B (since the kinetics of RBC hemolysis of α-hemolysin is well studied and very fast as compared to Rv1694). Moreover, the assay is fast and reliable as the efficiency of translation can be compared with literature. *In vitro* translation reaction was done without and with 80 ng/ml capreomycin and after the completion of this reaction, kinetics of RBC hemolysis was examined with the *in vitro* translated α-hemolysin which is directly proportional to the amount of α-hemolysin present in the tube and reflects the

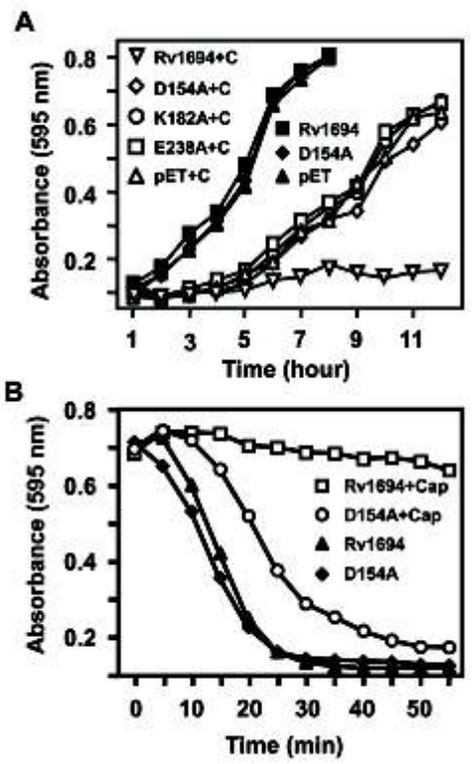


Fig. 3: (A) D-K-E mutant of Rv1694 is capreomycin resistant. Mutation at D154, K182 and E238 residues of Rv1694 showed no methylation activity and hence becomes capreomycin resistant. Filled symbols represent growth curves of *E.coli*/pRv1694 (■), *E.coli*/pET(▲), *E.coli*/pD154A (♦) in the absence of capreomycin and open symbols represent the same mutants in the presence of 100 g/ml capreomycin are *E.coli*/pRv1694 (▽), *E.coli*/pD154A(◊), *E.coli*/pK182A (○), *E.coli*/pE238A (□) and *E.coli*/pET (Δ).

(B) Ribosomes of D154A mutant *E.coli* has near normal translation in presence of Capreomycin. S30 extracts of E.*coli*/pRv1694 and *E.coli*/pD154A were supplied with a super-coiled plasmid of staphylococcal α-hemolysin and *in vitro* transcription and translation was carried out in the absence or presence of 80 ng/ml capreomycin. Various lines represent the translational activities in the absence of capreomycin for *E.coli*/pRv1694 (♦) and *E.coli*/pD154A (▲) and in presence of 80ng/ml capreomycin for *E.coli*/pRv1694 (□) and *E.coli*/pD154A (○).

translation efficiency in the absence and presence of capreomycin. The lytic activity of the S30 extracts of ribosomes of normal *E.coli* and D154Awas same in absence of capreomycin, where as in presence of capreomycin (80 ng/ml) abolished the translational activity of the wt S30 extract while the D154A S30 extract is normal (positive for hemolysis) and hence, resistant to capreomycin.

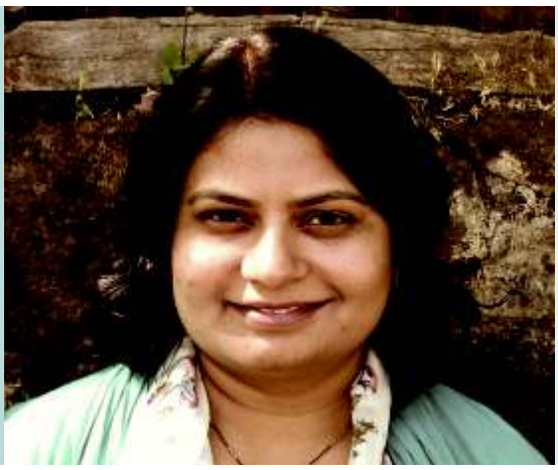
In summary, how the TlyA protein reaches the cell-wall of the bacterium is interesting because TlyA does not contain any signal sequence. Moreover, the TlyA can in principle compromise the integrity of the phagosomal compartments, thereby paving way for the intracellular-survival.

Future Work

We are going to investigate the mechanism of intra-cellular survival of TlyA expressing bacteria by studying phagosomal maturation pathway.

Research Report

New Faculty



Radha Chauhan

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Structural and Functional Studies of the nuclear pore complex proteins

Research Interests

The nuclear pore complexes (NPCs) embedded in nuclear membrane bilayer solely mediate transport of all kind of macromolecules between nucleus and cytoplasm, and regulate nearly most cellular processes such as gene expression, mitosis, cell differentiation etc. Additionally, alternations in NPC and its associated proteins have been linked to several human diseases, such as cancer, genetic disorders and viral diseases. The architecture of the NPC is evolutionarily conserved from yeast to human and is a highly modular structure. Each NPC is comprised of ~30 different proteins called nucleoporins (Nups) that are arranged in multiple copies to yield a size of 65 MDa (yeast) or 125 MDa (vertebrate). In order to understand the molecular mechanisms of NPC assembly formed by these ~30 nups and its versatile functions, the high-resolution structures are highly desired but complexity and the size of the NPCs pose tremendous challenges. A rational strategy therefore would be to disintegrate the components of NPC based on their structural and functional specificity and employ integrative approaches to learn about the roles of Nups in NPC assembly and cellular physiology.

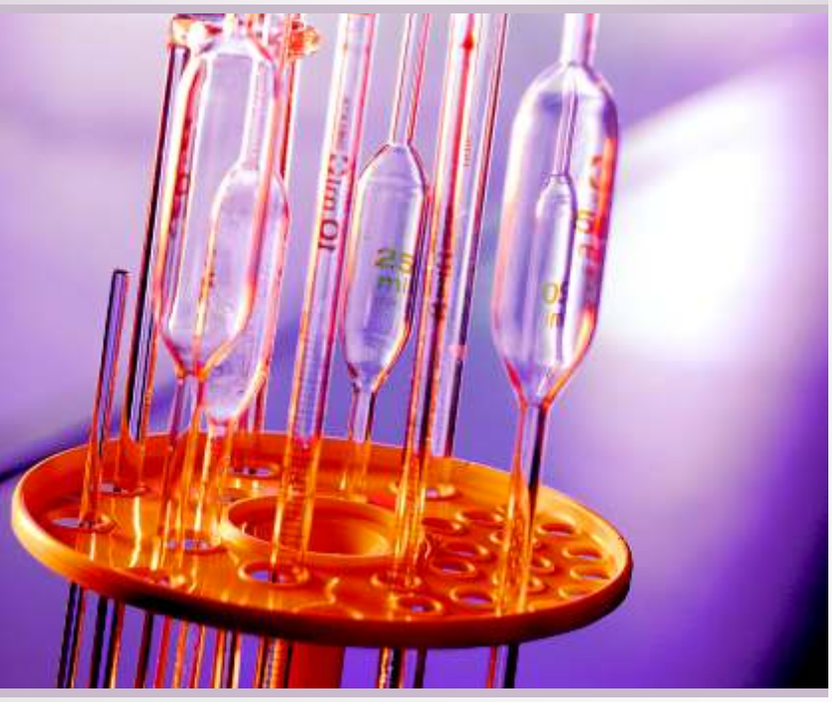
Our laboratory routinely utilizes various structural biology tools such as X-ray crystallography, spectroscopic methods etc and we work in collaboration with cell biologists to understand the versatile functions of NPCs, such as how Nups participate in nucleocytoplasmic transport, gene regulation and cell differentiation

Participants

Hima Girija, *CSIR JRF*
Ankita Chouksey, *Project JRF*
Pravin Devangan, *UGC JRF*
Gayatri sagare, *Technician*

Collaborators

Deepa Subramanyam, *NCCS*
Jomon Joseph, *NCCS*



Support Units & Other Facilities

Experimental Animal Facility

Ramanamurthy Bopanna

Rahul Bankar

The Experimental Animal Facility is a core scientific department of the Institute with an objective to breed, maintain and supply quality laboratory animals for research and development. The section also extends complete research support to facilitate animal experimentation in the Institute. It is a barrier-maintained facility for the breeding, maintenance and supply of laboratory animals viz. inbred and mutant mice, rats, rabbits etc. for the ongoing research projects of the Institute. The following is the list of various laboratory animals maintained at the facility:

MICE:

- BALB/cJ
- C57BL/6J
- DBA/2J
- DBA/1(2 lines)
- 129/SvJ
- FVB/NJ
- SWISS#
- BALB/c*
- NZB
- AKR#
- CF1

Genetically engineered mutant mice (knock-out, transgenic and mutant mice -33 lines)

RATS:

- WISTAR

RABBITS:

NEWZEALAND WHITE

* BALB/c with cataract mutation.

Outbred

Defined barrier practices are followed in the maintenance of the laboratory animals.

The Team

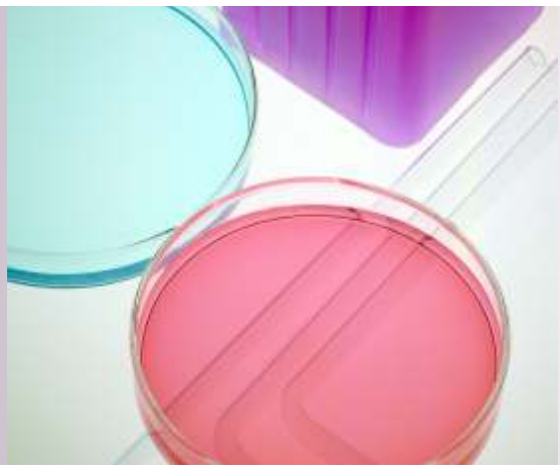
Mr. Md. Shaikh
Mr. A. Inamdar
Mr. P.T. Shelke
Ms. Vaishali Bajare
Mr. Mahavir Rangole
Mr. Rahul B. Kavitake
Mr. Ganesh B. Yadav
Mr. Sanjay Gade
Mr. Harshal G. Gaonkar
Mr. Dilip B. Thorat

The breeding program for the propagation of the inbred mice is based on the established principles of genetics and breeding. The breeding program involving mutant mice is structured as per the genetic requirement of the specific strain concerned.

The total number of mice strains, inbred, outbred, mutant and hybrids, being maintained at the Experimental Animal Facility stands at *45*. The foundation/nuclear colonies of mice are housed in Individually Ventilated Caging systems.

Complete scientific support and advice has been extended regularly to Scientists and their group members for the conduct of experiments under IAEC approved projects. The following services have been provided as per user requirements: supply of timed pregnant mice, blood and tissue sampling, immunizations, surgical procedures, injection of tumor cells in SCID/nude mice etc., assistance in the writing of Animal Study Protocols and assistance regarding interpretations of animal use regulations and the procurement of animals.
In the areas of education and training the facility has imparted training for 65 research scholars in laboratory animal handling and experimentation, ethics and rules governing use of animals for experiments.

The breeding of laboratory animals has been planned to meet the needs of Scientists / Research Scholars for various animal experiments. A total of 12,739 laboratory animals were supplied on demand for the ongoing research projects during the period.



Proteomics Facility

Srikanth Rapole

The proteomics facility is a core service facility of the institute with an objective to provide mass spectrometric analysis of biological samples. The following is the list of various instruments available at the facility:

4800 LC-MALDI TOF/TOF system (AB Sciex) is a tandem time-of-flight MS/MS system that is used for high-throughput proteomics research. The system identifies proteins by determining accurate masses of peptides formed by enzymatic digestion. Additionally, the system can more definitely identify and characterize proteins by isolating and fragmenting a molecular ion of interest and measuring the fragment ion masses. The number of samples analyzed is approximately 893 samples including 206 external samples from April-2012 to March-2013.

4000 Q-Trap LC-MS/MS system (AB Sciex) is a hybrid triple quadrupole/linear ion trap mass spectrometer. The system is ideal for proteomic applications including post-translationally modified proteins discovery, protein identification, and biomarker validation. The number of samples analyzed is approximately 149 samples including 23 external samples from April-2012 to March-2013.

Eksigent Tempo Nano MDLC system is a high performance, reliable, nano-scale liquid chromatography for proteomic applications. This Nano-LC system delivers precise and reproducible micro-scale gradients at constant flow-rates, creating ideal conditions for optimized mass analysis and a stable ionization spray. It is connected to 4800 MALDI-TOF/TOF system for proteome analysis.

Eksigent Express Micro LC-Ultra System is an advanced micro-LC technology with its pneumatic pumps, integrated autosampler, ultra-sensitive, full-spectral UV detector, and temperature-controlled column oven. And with Eksigent's intuitive software, users get full system control as well as complete analysis and reporting capabilities. The advantage of Eksigent's state-of-the-art micro-LC is running fast analysis with excellent reproducibility and only a small fraction of the solvent used.

Eksigent EKSpot MALDI Spotter couples Nano MDLC to MALDI mass spectrometer which results in an extremely powerful tool for the analysis of complex peptide/protein samples. This spotter holds 16 AB SCIEX 4700 targets or eight microtiter plate size targets.

Participants

Dr. Vijay Sathe, *Analyst*

Snigdha Dhali, *Technician*

4800 MALDI-TOF/TOF

4000 Q-Trap LC-MS/MS

Each of the targets can hold up to 1,000 spots and it generate up to 8,000 spots on an overnight run.

Shimadzu Prominence UFLC is higher speed and uncompromised separation liquid chromatography instrument. It provides ten times higher speed and three times better separation when compare with normal conventional HPLC. In addition to ultra fast analysis, UFLC is also used for many applications such as conventional HPLC analysis and semi-preparative analysis.

2-D DIGE proteomics set-up including Ettan IPGphor isoelectric focusing unit, Ettan DALT unit, DIGE Typhoon FLA 9000 scanner, DeCyder 2-D DIGE analysis software, and Ettan spot picker. This set-up is used for differential protein expression studies, biomarker discovery, quantitative proteomics etc.

Bioinformatics and High Performance Computing Facility

Shailza Singh

Technical Staff

Mrs. Virashree Jamdar, *Technician*

The bioinformatics facility at NCCS provides access to high-performance computing resources and programming expertise. The compute infrastructure serves scientists at NCCS to master the informatics needs of their research in a proficient and cost-effective manner.

Hardware Infrastructure

SGI Altix XE 1300Cluster
Head Node:
SGI Altix XE 270 Serve.
Dual Quad Core XEON 5620 @ 2.4GHz / 12MB cache,12GB Memory,5 x 2TB SATA Disk @ 7.2K RPM RAID 5

Compute Nodes:
SGI Altix 340 Servers
2 x HEXA Core XEON 5670 @ 2.93GHz / 12MB cache, 24GB Memory, 250GB SATA Disk @ 7.2K RPM, Dual Gigabit Ethernet Card

SGI Cluster Software Stack:
SLES Ver 11
SGI ProPack 7
SGI Foundation Software Ver 2.0
Interconnect:
24-Ports Gigabit Ethernet Switch

GPU Computing HP Proliant SL6500
2x Intel Xeon X5675 @3.06GHz/6 core/12MB L3 Cache
96 GB (8 GB x 12) PC3 – 10600 (DDR3 – 1333) Registered DIMM memory
2 x 1 TB hot Plug SATA Hard Disk @7200 rpm
Integrated Graphics ATI RN50/ES1000 with 64 MB memory
2x NIVIDIA Tesla 2090 6 GB GPU computing module

Specialized Workstations:

HP Elite 8200 CMT PC
Second generation Intel core i7-2600 processor 3.40 GHz, 8M cache, 4 cores/8 threads
Intergrated 4 port SATA 6GBs controller
Intergrated Intel HD graphics

HP Z800 High End Work Station
2x Intel Xeon E5649 6 core @2.53 GHz, 80 watt 12MB cache
5.86GTs QPI, DDR3 1333 MHz, HT Turbo
NVIDIA Quadro FX380 Graphics with 256MB memory
SATA 6 GBs controllers with RAID 0/105 & 10 support
19" LCD wide Display with Windows OS

HP Z820 High End Work Station
2x Intel Xeon E5-2690@2.9GHz, 8 core/20MB L3 cache
8 GTs QPI, DDR3 1600 HT Turbo 2 with vPro support
NVIDIA Quadro 4000 Graphics with 2GB DDR memory
SATA 6 GBs controllers with RAID 0/105 & 10 support
22" LCD wide Display with Windows OS

High End Desktop
Intel Core 2 Duo @3.00GHz with 8 GB of DDR2 memory, 320 GB of SATA storage and 19" LCD wide Display with Linux/Windows OS

Desktop Computers
Desktop computers with Intel core 2 duo processor @1.8Ghz to 2.8GHz with 2 GB to 4 GB of DRR2 memory, 160GB to 320GB of SATA storage with 17" wide LCD display and with Windows XP OS

iMAC: For running specialized software like Biojade

Printer: HP Laser jet M1136MFP, Canon Network Printer, HP laserjet pro 8000 color printer

APC UPS 10 KVA for supporting the HPCF

Software infrastructure
The Bioinformatics Facility at NCCS has procured several software for scientific research having commercial and/or academic license. These are:

Sequence analysis: BLAST, CLUSTAL-W, MEGA, Eisen

Molecular Modeling: Modeler (DISCOVERY STUDIO 3.0), Protein Families (DISCOVERY STUDIO 3.0), Protein Health (DISCOVERY STUDIO 3.0), Protein Refine (DISCOVERY STUDIO 3.0), Profiles-3D (DISCOVERY STUDIO 3.0)

Molecular Docking: Flexible Docking (DISCOVERY STUDIO 3.0), LibDock (DISCOVERY STUDIO 3.0), Ludi (DISCOVERY STUDIO 3.0), LigPrep (DISCOVERY STUDIO 3.0), LigandFIT (DISCOVERY STUDIO 3.0), LigandScore (DISCOVERY STUDIO 3.0), AUTODOCK, Database of 1.5million Compound Library (DISCOVERY STUDIO 3.0)

Pharmacophore Modeling: Auto Pharmacophore generation, Receptor-ligand pharmacophore egeneration,3D QSAR pharmacophore generation, Steric Refinements with excluded volumes. (DISCOVERYSTUDIO3.0)

Toxicity Prediction: ADMET (DISCOVERY STUDIO 3.0), TOPKAT (DISCOVERY STUDIO 3.0)

QSAR: Create Bayesian Model, Recusrive Partioning Model, Multiple Linear Regression Model, partial least squares model, genetic function approximation model, 3D QSAR model (Discovery Studio 3.0). Intelligent QSAR using molecular fragments of interest and their features, evaluation of descriptors from template scaffold to form relationship with the activity.

Molecular Dynamics: CHARMM, GROMACS, NAMD, MOIL

Molecular Visualization: Rasmol, MolMol, WinCoot, Swiss PDB viewer, MolScript, VMD

***ab initio* modeling:** GAUSSIAN

Systems Biology Tools: Virtual Cell, M-cell, Cell Designer, GEPASI, Cytoscape, Osprey, E-Cell, SimBiology

Artificial Intelligence: SVM^{light} and SNNS

Material Modeling and Simulation: Material Studio 5.5

Graphs and Graphics: Sigma Plot, GNU Plot, Corel Draw and Adobe PhotoShop

Statistical packages: MATLAB and R

Library

The NCCS library has a collection in frontier areas of biotechnology having relevance to NCCS research activities. The library holds approximately twelve thousand four hundred bound journals, two thousands eight hundred books, one hundred ninety NCCS Ph.D. theses and subscribes to twenty three scientific journals and twenty eight other periodicals in print form.In addition, it also subscribes to nine scientific online journals independently, and 722 online journals including one book series, 'Methods in Enzymolgoy', from various publishers such as Springer, John Wiley, Nature Publishing group, Mary & Libert, Oxford, Elsevier Science Direct etc., through the 'DeLCON' DBT Online Journal Consortium of the Department of Biotechnology (DBT), Govt. of India.

In the development of its collection, the library's priority is to support NCCS research activities. The library collection is expanded in consultation with NCCS scientists. The library's print collection is growing by approximately 1200 volumes per year. The library is equipped with Linux based SLIM21 with RFID-interfaced library software for library house keeping operations and Web-OPAC for online searching of the library documents. The library has also installed the barcode technology for circulation (issue & return) of library documents. The library maintains library information (in Hindi & English) on its webpage, which includes free Online Medical database link, NCCS research publications' list, library forms, 'NCCS in the News', Ph.D. theses, list of NCCS alumni and other Scientific Grants/ Funds and fellowships-related links. During the period under review, the library has created a Digital Archive of NCCS Ph.D. theses. Moreover, it now also provides the service of scanning the Ph.D. thesis and other documents for plagiarism using the plagiarism detection software, iThenticate.

Additional documentation facilities include local area network for library activities and PubMed database access and a number of CD-ROM for books, journals & Ph.D. theses. The library is listed in the Union Catalogue of Biomedical Serials in India, created by the National Informatics Centre, New Delhi, and continues to be a member of the Medical Library Association of India.

Team

Mr. Krupasindhu Behera, *Technical Officer*
Mr. Rameshwar Nema, *Technical Officer*
Mrs. Aparna V. Panse, *Office Assistant*
Mr. M.V. Randive, *Helper*

Computer Section

New Work Done

NCCS Hindi website developed:
The Hindi website of NCCS has been developed and hosted on Hindi Diwas 20th Sept. 2012.

New High speed STM-1 (155Mbps) Internet connectivity:
New Fiber optic based STM-1 Internet (155Mbps Bandwidth) link from M/s Power grid Corp., Banglore was installed, configured and made functional for NCCS use. This link has been terminated on NCCS firewall and operated through a proxy server according to NCCS security policy for web access.

NCCS LAN Upgradation:
The old 3COM switches have been replaced with installation and configuration of Cisco catalyst 2960 Gigabit switches (total 12 no.) for upgrading the LAN speed to 1Gbps in all laboratories, guest house and experimental animal facility. The Cat-6 Cables redressing with labeling each LAN point on all the network switches racks was completed. This Cisco switches were integrated with Cisco Access Control server (ACS) for hardware MAC id. based desktop authentication.

Establishment of LAN / Internet connectivity in Guest House
NCCS Guest House has been connected to NCCS LAN with the installation of Cisco Switch for internet / E-mail usage. In each room, 2 LAN points have been provided by laying Cat-6 cables from the switch. Additionally Wireless LAN access has been provided in two VIP suites by configuring Linksys router.

Installation of Iomega Network Attached Storage (NAS):
A new 16TB Network Attached Storage (NAS) server was installed and configured for E-mail / website backup and also a new 9TB data storage enclosure was added to HP MSA 2000 server for augmenting VMWARE data storage capacity.

Expansion of LAN:
The various new laboratories and departments. viz. Store and Purchase, Academic Section, New Repository, Administration were included in NCCS LAN for Internet / E-mail usage with configuration of VLAN on core switch.

Paperless Governance Initiative
As an attempt to move towards paperless office, NCCS has installed an online booking software for all its facilities. In addition, development has been completed for launch of 'e-jobcard' for needs arising for instrumentation, maintenance and civil works at NCCS. In addition, All NCCS staff members have been provided E-mail ID and soft copy salary slips and paper copies have been discontinued.

Providing technical support to Internet/WWW, E-mail, hardware, operating systems, anti virus, network, application software to NCCS users for their desktops, laptops and printers.

Future Plans
In the next financial year (2013-14), NCCS is expected to achieve complete paperless office for its human resource management system that covers salary, promotion, leave and allowances.

Team

Mr. Rajesh Solanki
Mr. Shivaji Jadhav
Mrs. Rajashri Patwardhan
Mrs. Kirti Jadhav

NCCS Facilities

a) DNA Sequencer

Around 6, 000 samples were run on the machine during this period.

b) FACS Core Facility

There are seven equipments in the FACS core facility of the institute under the supervision of Dr. Lalita Limaye. These are operated on a rotation basis by six dedicated operators. The Calibur equipment is also operated by trained students.

Technicians in the facility:

1. Hemangini Shikhare.
2. Pratibha Khot.
3. Amit Salunkhe.
4. Rupali Jadhav.
5. Ashwini Kore.
6. Vikas Mallav (operator provided by BD and posted in NCCS under BD-NCCS STEM CELL COE from Dec. 2011)

The usage of the seven equipments for the period under consideration is summarized below:

IMMUNOPHENOTYPING & CELL CYCLE analysis

Equipment	Surface / Intracellular staining	DNA Cell cycle	CBA flex	CBA	Total Samples Acquired
FACS Calibur	2374	3591	-	-	5965
FACS Canto II (Old)	6773	78	-	-	6851
FACS Canto II (New)	4637	-	-	-	4637

STERILE SORTING

EQUIPMENT	SORTING	ACQUISITION **	TOTAL
FACS Aria II SORP	377	2000	2377
FACS Aria III SORP	331	1268	1599
FACS Aria III Standard	212	1160	1372

** Includes analysis of samples that require UV laser, as we do not have UV analysers.

BD Pathway 855:

Around twelve users from NCCS have used this instrument during the period under consideration. Imaging of samples was done for live cell staining (96 well plates) as well as for fixed cells in the form of sections on slides. Images were captured using mercury arc

lamp and transmitted light in montage form (e.g. 8X8, 10X10). Macros are done as per the user's request.

Samples from outsiders:

Since the workload of outsider's samples has increased, NCCS had made a policy to charge for these from June 2012 . For academic and research institute, the charges are less and for private institutes/ companies the charges are higher. Institutes like NCL, IBB, IISER, IRSHA, Rasayani Biologist Pvt. Ltd., geneOmbio Technologies Pvt. Ltd. are utilizing our facility. We have received 251 samples, including Surface/ Intracellular staining and DNA cell cycle analysis.

Activities under BD-NCCS COE programme:

1. Calibur training

Training on Calibur for NCCS students was condected every month throughout the year, by BD application specialist. 40 students from NCCS have received training on Calibur and have started using it independently.

2. Canto-II training and examination

Training was organized on Canto-II during this period. 18 students from NCCS received this training. An examination was also conducted for the trained students on Canto II, on 30th & 31st August 2012. 11 students successfully qualified this exam and started using the instrument independently.

3. National workshop

A national workshop was organized on Hematopoietic and Mesenchymal Stem Cells Analysis & Sorting at the "BD-NCCS Centre of Excellence in Stem Cell Research". This hands-on training was conducted at NCCS, Pune, from April 9-13, 2012. The workshop was supervised by Dr. Limaye, Head, FACS Central Facility. Dr. Kale and Dr. Wani also participated as faculty in the workshop. About 30 applications were received, from which 15 participants were short listed for the program: 7 from NCCS and 8 from other institutes. The following topics were covered, with hands-on training for sample preparation and acquiring & analysis of samples:

- Side population analysis and sorting by Fluorescence Activated Cell Sorting (FACS) - Dr. Limaye
- Mesenchymal stem cell characterization and differentiation into bone cells - Dr.Wani
- Isolation and characterization of mouse hematopoietic stem cells using cell surface markers (KTLS) - Dr. Kale
- Demo-based training on Hi-content Bioimaging on the BD Pathway 855 Bioimager – Dr. Paresh Jain from BD

4. Pathway Training

- The basic training on Pathway was organized for FACS operators (Ashwini, Rupali) on 30th January 2013, which was carried out by Mr. Swapnil Walke (BD Application Specialist).

- The advanced training on pathway was organized for FACS operators (Ashwini, Rupali) on 19th February 2013, which was carried out by Dr. Paresh Jain (BD Application scientist).

5. Seminar

A seminar was arranged at NCCS on 'FlowJo - Overview & Fundamentals' (Flow cytometer data analysis software) on 5th October, 2012, by FlowJo application scientist Mr.Hemant Agrawal.

c) Confocal Microscopy

Technical Officer: Ashwini N. Atre

The imaging facility has three scanning confocal laser microscopes.

All the systems, which include Zeiss 510 Meta and Leica TCS SP5, areinverted microscopes and have a wide range of lasers such as Blue Diodelaser 405nm), Argonlaser(458/477/488/514 nm), He-Ne [543nm, 594nmand633nm] and DPSS 561nm.The systems are capable of doing FRET, FRAP, 3D imaging and reconstruction,and live imaging, which is required for most cell biology research. TheLeica SP5 II microscope has been upgraded with two hybrid detectors. The FLUOVIEW FV10i microscope from M/s Olympushas a compact design anddoes not require a dedicated darkroom.

All three instruments are used by in-house users as well as by users fromneighboring organizations.

The facility is now housed in a new Repository building.

d) IVIS Imaging System Facility

Scientist In-charge: Dr. Gopal C. Kundu
Technical Staff: Dr. Mahadeo Gorain, Technician-B.

The IVIS imaging system facilities is a central facility of NCCS which is used for bioluminescent and fluorescent imaging of cells or whole small animal under in vitro or in vivo conditions.

The Xenogen IVIS-200 System is capable of imaging bioluminescence and fluorescence in living animals. The system uses a novel in-vivo biophotonic imaging to use real-time imaging to monitor and record cellular and genetic activity within a living organism. A light-tight imaging chamber is coupled to a highly-sensitive CCD camera system.

In this IVIS Imaging System include a custom lens with 5-position carousel and adjustable field of view (FOV) of 4-26cm, more uniform light collection, and improved resolution with single cell sensitivity for *in-vitro* use. An integrated fluorescence system and 24-position

emission filter wheel allow easy switching between fluorescent and bioluminescent spectral imaging, while a laser scanner provides 3D surface topography for single-view diffuse tomographic reconstructions of internal sources. A 25 mm (1.0 inch) square back-thinned CCD, cryogenically cooled to -90^{0}C (without liquid nitrogen), minimizes electronic background, and maximizes sensitivity. This camera system is capable of quantitating single photon signals originating within the tissue of living mice. Up to five or six mice can be imaged simultaneously and an integrated isoflurane gas manifold allows rapid and temporary anesthesia of mice for imaging.

IVIS Imaging System

Standard filter sets for IVIS Imaging System

Fluorescence Filters

Set	Name	Excitation (nm)	Emission (nm)
1	GFP	445-490	515-575
2	DsRed	500-555	575-650
3	Cy5.5	615-665	695-770
4	ICG	710-760	810-875

Spectral Imaging Filters

Set	Name	Emission (nm)
5	560 nm	550-570
6	580 nm	570-590
7	600 nm	590-610
8	620 nm	610-630
9	640 nm	630-650
10	660 nm	650-670

Bioluminescent and Fluorescent Imaging: This system can be used for in-vitro and in-vivo studies based on bioluminescence and fluorescence techniques. The lens system includes user-accessible filter wheels that accept up to 22 filters including a large set of fluorescence filters and set of bioluminescent filters to use in spectral imaging studies.

Establishment of Microbial Culture Collection

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Participants

Tapan Chakrabarti, *Consultant*
Kamlesh Jangid, *Scientist*
Omprakash Sharma, *Scientist*
Ashish Polkade, *Scientist*
Dhiraj Dhotre, *Scientist*
Amaraja Joshi, *Scientist*
Neeta Joseph, *Scientist*
Rohit Sharma, *Scientist*
Amit Yadav, *Scientist*
Mahesh Chavdar, *Scientist*
Avinash Sharma, *Scientist*
Praveen Rahi, *Scientist*
Venkata Raman, *Scientist*
Shrikant Pawar, *Technical Officer*
Hitendra Munot, *Technical Officer*
Dimple Notani, *Technician*
Vishal Thite, *Technician*
Sonia Dhage, *Technician*
Mahesh Sonawane, *Technician*
Madhuri Vankudre, *Technician*
Shalilesh Mantri, *Technician*
Vikram Kamble, *Technician*
Umera Patawekar, *Technician*
Vikas Patil, *Technician*
Sunil Dhar, *Technician*
Ashok Shinde, *Technician*
Vipool Thorat, *Technician*

Background

Department of Biotechnology established a Culture Collection in June 2008 with broader charter to preserve, characterize and authenticate microbial resources. These are valuable raw materials for the development of biotechnology in India, because of its vast area with varied topology and climate has a rich reservoir of biological diversity which needs to be conserved judiciously and carefully, to prevent enormous economical loss. It is most important to build and enhance human and technological capabilities to isolate, preserve and characterize microorganisms in order to accrue a greater share of the benefits from such microbial resources.

The Microbial Culture Collection (MCC) was started at National Centre for Cell Sciences (NCCS), Pune and currently occupies an interim laboratory facility of approximately 5000 sq.ft. at National Centre for cell sciences (New building) for its culture preservation activities. It has established complete infra structure for microbial growth, long term preservation and identification.

Aims and Objectives

The main objectives of this MCC are to act as a national depository, to supply authentic microbial cultures and to provide related services to the scientific community working in research institutions, universities and industries.

Work Achieved

At present, MCC preserves and supplies microbial cultures to the Department of Biotechnology's (DBT) microbial prospecting project. These cultures were originally collected by the nine institutes involved in the project from various ecological niches using different media. At present MCC receives cultures from these nine institutes in four forms,

1. Ordinary/Normal Cultures: All the cultures which are isolated by each institute.
2. 'Three star' Cultures: All normal cultures are then screened by Piramal Life Science Limited (PLSL), Mumbai for four different activities. Three star cultures are the ones which show one or more of these activities
3. 'Re-fermented' Cultures: All the three star cultures undergo second level of screening (fermentation) to check whether they retain the activity. Re-fermented cultures are those three star cultures which retain their activity after second fermentation.

4. 'Scale-up' Cultures: Re-fermented cultures that have shown potentially novel molecules/compounds during screening at PLSL and have been selected for large scale fermentation by PLSL are designated as 'Scale-up' cultures. These cultures are being sent by PLSL to MCC.

MCC is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM, registration number 773).

The MCC was recognized by the World Intellectual Property Organization (WIPO), Geneva, Switzerland as an International Depository Authority (IDA) on April, 2011. The deposit of microorganisms under the Budapest Treaty is recognized to fulfill the requirement of patent procedure in 55 member countries.

At present MCC is providing following services to all:

1. General deposit
2. Safe deposit
3. IDA/Patent deposit
4. DNA sequencing based Identification services

Number of cultures in each category are shown in Table 1

Category	Number Preserved
DBT's Microbial Mission Project	1, 46, 668
General Deposit	
Bacteria	179
Fungi	65
Patent Depisits under Budapest Treaty	14

16S rRNA gene sequencing results for 'Three Star' cultures

Total 7730 pure 'Three Star' cultures has been received at MCC, so far. Out of them, 6407 'Three Star' cultures have been sequenced. The DNA sequencing is done using three/four different universal 16S rRNA gene specific primers. Out of 6407 sequenced three star cultures, 3955 have sequence length above 1200bp. Preliminary classification of these three star sequences was done using Ribosomal Database Project (RDP) using Bayesian naïve classifier.

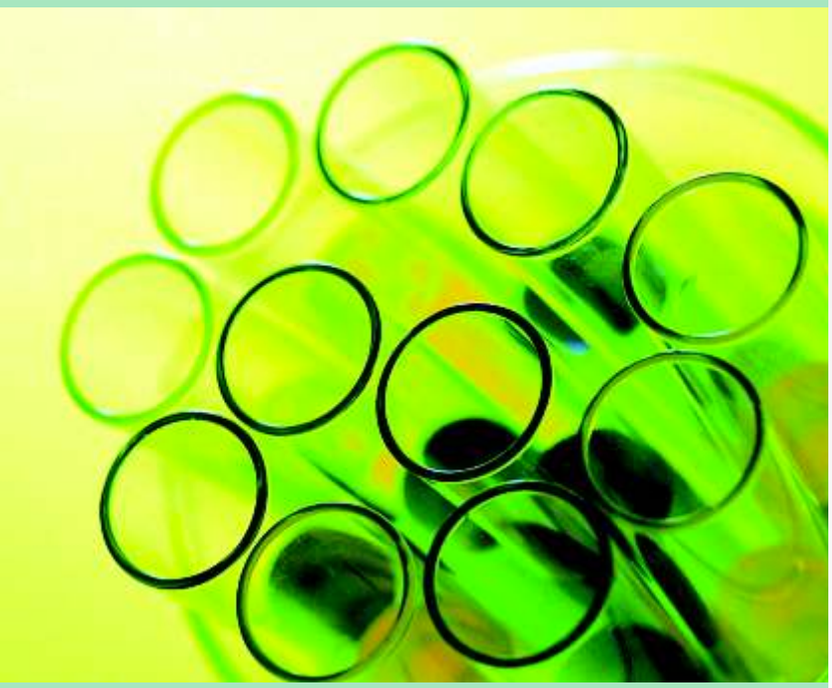
Future Research Plans

- Further characterization of DBT's Microbial Misson Project cultures.
- Enriching the repository by receiving more cultures.
- Offer more services like DNA-DNA hybridization, G+C analysis, Fatty Acid Methyl Ester profiling.

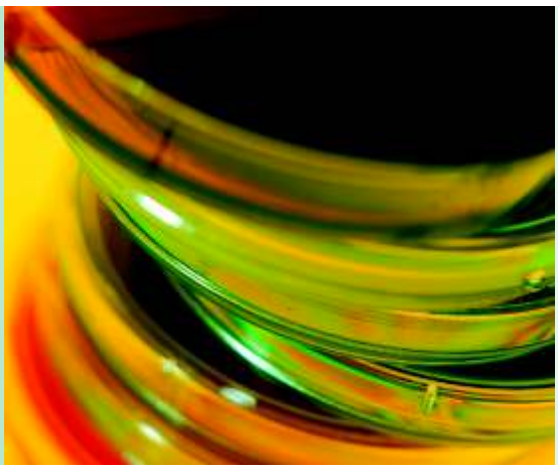
Fig. 1: Inaugural session of the Symposium on "Microbes: Molecular Ecology & Systematics" L to R Prof. W. N. Gade, VC Pune University, Dr. Manoj Modi DBT, Dr. G. B. Nair Executive Director THSTI Gurgaon, Dr. Shekhar Mande Director NCCS, Dr. G.C. Mishra Ex Director NCCS, Dr. Yogesh Shouche

Fig. 2: Visit by Prof. M.K.Bhan to MCC. L to R Shri. Raghavan, JS, (DBT), Prof. Bhan, Dr. Avinash Sharma

Fig. 3: Students interacting with scientists during workshop at Nashik



Other Information



Publications / Book Chapters / Patents

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Book Chapters / Invited Reviews

1. Mande, S. C., Kumar, A., and Ghosh, P. (2013). Analysis of dihedral angle variability in related protein structures. In Biomolecular Forms and Functions, a celebration of 50 years of the Ramachandran map, M. Bansal and N. Srinivasan, ed. (World Scientific Publishing) pp. 107-115.

2. Rao, K. V. S., Kumar, D., and Mande, S. C. (2012). Probing gene regulatory networks to decipher host-pathogen interactions. In Systems Biology of Tuberculosis Ed.s McFadden, Beste and Kierzek. (Springer) Pp. 37-54.

3. Dinasarapu AR, Chandrasekhar A, Sahu A, Subramaniam S. (2012) Complement C3. *UCSD Molecule Pages*, doi:10.6072/H0.MP.A004235.01

4. Rattan A, Kasbe R, Mullick J and Sahu A (2013)The complement system as a viral target for immune evasion. Chapter 1. In Microbial Pathogenesis: Infection and

Immunity. U. Kishore and A. Nayak, editors. (Landes Bioscience, Austin, Texas, U.S.A.), pp. 1-27.

5. Sonali, Shinde., Vineetha, Mandlik and Shailza, Singh(2013) Integrating Bioinformatics and Systems Biology for exploring novel lipid pathways in infectious diseases. Advances in Mathematical Chemistry, Bentham Science Publishers.
6. Vineetha, Mandlik., Milsee, Mol., Sonali, Shinde and Shailza, Singh. (2013)Gene Regulatory Network: A New Paradigm in Bioinformatics. Biotechnology, 6, Studium Press LLC, USA.
7. Shailza, Singh. (2012). Quantum pharmacology for infectious diseases: a molecular connectivity approach. Curr Comput Aided Drug Des, 8(3), pp. 249-54 [Invited Review]

Patents Filed / Sealed

Dr. Samit Chattopadhyay

Chattopadhyay S and Jalota-Badhwar A., Tumor suppressor Activation polypeptide and uses thereof Indian patent file number: IPR/4.19.20/06083/2006 (Indian patent); United States patent, Patent Number # US 8420100 B2, PCT filed September 2010, Patent accepted April 16, 2013.

Vaijayanti Kale

Electrospun Nano-fiber scaffold for repair of skin legions (No: 3343/MUM/2012), 22/11/2012

Prof. JayeshBellare, IIT Bombay, Dr. Meghana Kanitkar, NCCS, Pune, Mr. AmitJaiswal, IIT Bombay.

Dr. Sandhya Sitasawad

Manish S. Lavhale, Santosh Kumar, Shrihari Mishra, Sandhya Sitasawad

* IN2008DE02261A - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*. (Indian)

* PCT/IN2008/000795- Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*.

* WO2010035277A1 - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*.

* US2010311987A1 - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*. (USA)

* EP2337782A1 - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*. (European)

* EP2337782A4 - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*. (European)

* JP2012503645A - Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus Excelsa *in vitro* and *in vivo*. (Japanese)

Awards/ Honours/ Memberships/ Extramural Funding

Awards / Honours / Memberships

Rahul Bankar

- Member, Laboratory Animal Scientists Association (LASA)

Manoj Kumar Bhat

- Khorana Program Technology Transfer Course Awards: 2012

Samit Chattopadhyay

- Fellow of National Academy of Science (FNA), Indian National Science Academy (INSA), New Delhi, 2012
- Awarded the prestigious J. C. Bose Fellowship by the Department of Science and Technology, Government of India, in recognition of PI's active outstanding performance and contribution to science, 2013
- Ph.D. Student: Ms. Nidhi Chaudhary
  1st prize "Prof. V C Shah Award" for best oral presentation, "Regulation of DNA Damage Repair and Apotosis by Nuclear Matrix Binding Proteins", at All India Cell Biology Conference held at BARC, Mumbai on 19th October, 2012.
- Former PhD student: Dr Pavithra Lakshminarasimhan Chavali
  INSA Young Scientist Award: Department of Oncology, University of Cambridge, Cancer Research UK-CRI, Li Ka Shing Center, Robinson Way, Cambridge, CB2 0RE, UK. She identified that the tumor suppressor protein, SMARI is dysregulated in breast cancer and that its over expression arrests the cells at G1/S phase.

Jomon Joseph

- Member - Indian Society of Cell Biology

Sanjeev Galande

- ? Appointed as Honorary Associate of the Sydney Medical School, University of Sydney, Sydney, Australia since March 2013.

Gopal Kundu

- International Young Investigator Award, 5th Mayo Clinic Angiogenesis Symposium, USA 2012
- 7th National Grassroots Innovation Award-2013: NIF-NCCS Partnership Award for the contribution in the area of Cancer Biology, Rashtrapati Bhavan, New Delhi, 7th March, 2013.

The 'Partnership Award' from the National Innovation Foundation-India was awarded to Dr. Gopal Kundu at the 7th National Grassroots Innovation Awards ceremony, 2013

Girdhari Lal

Memberships

- Life member, Biological Chemists (SBC), India (since 2011).
- Life member, Indian Society of Cell Biology (ISCB) (since 2010).
- Life member, Association of Microbiologist in India (AMI) (since 1999).
- Life member, Indian Immunology Society (IIS) (since 2003).
- Trainee member of The Transplantation Society (TTS), USA (2006-2007).
- Member of American Association for the Advancement of Science (AAAS), USA (2007-2008).
- Member of American Society of Transplantation (AST), USA (since 2012).
- Member of American Association of Immunologists (AAI), USA (since 2012).
- Life Member, The Biotech Research Society (BRSI), India. (since 2011).

Nibedita Lenka

- Life Member, Indian Academy of Neuroscience.
- Active Member, International Society for Stem Cell Research (2005 – continuing).
- Life Member, Stem Cell Research Forum of India (SCRFI).

Lalita Limaye

Annual member of

- International Society of experimental Haematology

Life member of

- Indian society of cell biology
- Biotechnology society of India
- Indian women scientists association
- Indian association of Microbiologists of India

Debashis Mitra

- DBT Tata Innovation Fellowship, April 2012
- OPPI Scientist Award, September 2012
- Fellow, Indian Academy of Sciences, January 2013

Dr. Debasish Mitra was awarded the OPPI Scientist Award (2012)

B. Ramanamurthy

- Life Member of Laboratory Animal Science Association of India
- Executive Member, Laboratory Animal Scientists Association (LASA)

Arvind Sahu

- Review Editor of Frontiers in Molecular Innate Immunity, a specialty section of Frontiers in Immunology (since 2011).
- Fellow, National Academy of Sciences, India (since 2009)
- Member of the International Complement Society (since 1993)
- Member of the Molecular Immunology Forum (since 2005)
- Member of the American Society for Microbiology (since 2005)

Anjali Shiras

Awards

- Travel Award for participation at International Society Stem Cell Research Meeting (ISSCR); Yokohoma, Japan- 2012

Memberships

- International Society for Stem Cell Research (ISSCR), USA
- Indian Society of Neuro-oncology (ISNO), India
- Indian Association of Cancer Research (IACR), India

Shailza Singh

- DST Young Scientist Award
- International Travel Award by CSIR and DBT
- RGYI Award 2012
- Life Member -Biotechnology Society of India (BSI)
- Life Member-Society of Biological Chemists, India (SBC)
- Life Member-Association of Microbiologists of India (AMI)
- Life Member-Association for DNA Fingerprinting and Diagnostics
- Ph.D. Student: Milsee Mol J.P.
- Third prize for Oral presentation in 'Accelerating Biology 2013:The Next Wave' organized at CDAC from 20 - 22nd February 2013

Mohan Wani

Awards and Honours

- Academic Editor, PLOS ONE journal, 2013.
- Recognized PhD research guide in Dental Medicine for 2013-2017, Dr. D. Y. Patil Vidyapeeth, Pune.

Memberships

- Fellow, National Academy of Sciences (FNASc)
- Member, Guha Research Conference (GRC).
- Member, Molecular Immunology Forum (MIF).
- Member of the American Society for Bone and Mineral Research (ASBMR), USA.
- Member of International Chinese Hard Tissue Society.
- Life Member of Indian Society of Cell Biology (ISCB).

Vasudevan Seshadri

- DBT-CREST award 2012-13

Extramural Funding

Sharmila Bapat

Molecular pathways regulating ovarian cancer cell plasticity and stem cell properties (2012-2015); Indo Australia Biotechnology Fund (Round 6), funded by Department of Biotechnology, Government of India and DIISRT, Commonwealth of Australia.

Jomon Joseph

1. Regulation of RNA metabolism by Dishevelled, a critical player of Wnt signalling. 2011-2014. (DBT)
2. Exploring the functional connection between Par polarity proteins and Nup358 in cell polarity. 2012-2015. (DBT)

Manoj Kumar Bhat

Relationship between obesity and cancer, its ramifications in cancer progression and chemotherapy. 2013-2016,(Project under DST-SERB)

Radha Chauhan

Understanding the role of Nup93 subcomplex in Nuclear Pore Complex assembly and Functions. 2012-2017. (DST Ramanujan Fellowship).

Vaijayanti Kale

The role, and mechanism, of free radical scavengers and/or cell death cascade regulators in mitigating the Diabetes Mellitus-induced endothelial progenitor cell (EPC) dysfunction. (DBT -2012-2015)

Gopal Kundu

1. Role of Splice Variants of Osteopontin in Regulation of PI 3 Kinase Dependent/Independent ILK-mediated VEGF Expression Leading to Breast Tumor Growth and Angiogenesis. 2010-2013 (CSIR)
2. Gold nanostructures and polymeric nanoparticles for breast cancer therapy. 2009-2012 (DBT)
3. Peptide nanoparticle mediated drug/siRNA delivery to tumor vasculature that suppresses tumor growth and angiogenesis in breast and prostate cancers. 2011-2014 (DBT)
4. STAT3 a key regulator and novel therapeutic target in osteopontin-induced tumor growth and angiogenesis in breast cancer. 2010-2013 (DST)
5. Role of small molecule inhibitor(s) as targeted therapy in pancreatic and prostate cancers using invitro and in vivo models. 2010-2013 (DBT)
6. Therapeutic application of targeted shRNA libraries in treatment of breast and prostate cancers. 2012-2015 (DBT)

Girdhari Lal

1. Cellular and molecular mechanism of CD4 T cell and endothelial cell interaction to control inflammation and autoimmunity (2012-2015) (Department of Biotechnology, Government of India; BT/PR4610/MED/30/720/2012)
2. Role of chemokine and its receptors in the pathogenesis and regulation of autoimmunity (2011-2016) (Department of Biotechnology, Government of India; BT/RLF/Re-entry/41/2010)
3. CCR6 as therapeutic target to control inflammation and autoimmunity. (2011-2014) (Department of Biotechnology, Government of India; BT/03/IYBA/2010)

Lalita Limaye

1. Functional characterization of the in vitro generated dendritic cells from cord blood derived hematopoietic stem cells.2012 (DBT)
2. Evaluation of the effect of apoptotic inhibitors on ex vivo expansion and cryopreservation of Hematopoietic stem/progenitors cells in a co-culture system with cord derived Mesenchymal stem cells. 2013- 2016 (DRDO LSRB, New Delhi)
3. Generation of Mesenchymal stem cells from human umbilical cord tissues, their characterization and differentiation to neural cells. 2012-2015 (BRNS, Mumbai)

Nibedita Lenka

Recipient of Indo-Australia Biotechnology Fund (Round 6): (2012-2015).

Shekhar Mande

1. Construction of regulatory networks in *Mycobacterium tuberculosis* analysis of gene expression data and transcription regulation predictions. 2010 - 2012 (DST)
2. *Mycobacterium tuberculosis* bioinformatics and structural strategies towards treatment. 2010 - 2013 (DBT)
3. DBT centre of excellence for microbial biology. Mar 2008 - Mar 2013 (DBT)
4. Addressing functional properties of *E. coli* through genome-wide protein-protein linkage analysis. Mar 2008 - Mar 2013 (DBT)

5. Virtual centre of excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis* - Molecular pathogenesis of tuberculosis. Dec 2008 - Dec 2013 (DBT)
6. Discovery of bioactive natural products from microbes especially actinomycetes in niche biotopes in Manipur. Mar 2011 - Mar 2014 (DBT)
7. Sys TB: A Network program for resolving the intracellular Dynamics of host pathogen interaction in TB infection. Jan 2012 - Jan 2017 (DBT)

Debashis Mitra

1. Identification of novel cellular targets and new lead molecules to inhibit HIV-1 infection. DBT-Tata Innovation Fellowship grant, (2012-2015)
2. Studies on modulation of Heat Shock Proteins during HIV-1 infection and their functional role in virus replication and pathogenesis. 2010-2013 (DBT).
   Principal Investigator: Debashis Mitra
3. Structure based discovery of novel antiviral molecules with potential to inhibit drug resistant viruses. 2010-2013 (DBT)

P.I: Dr. Ashoke Sharon, BITS, Mesra. Co-PI: Debashis Mitra, NCCS and Dr. D. Chattopadhyay, ICMR Virus Unit, Kolkata.

Srikanth Rapole

1. An attractive and promising strategy for early cancer diagnosis through the assembly of the human cancer volatome. 2013-2016 (New INDIGO)
2. Metabolomic profiling for identification of novel potential biomarkers in breast cancer using mass spectrometry and bioinformatics. 2013-2016 (RGYI Grant)

Bhaskar Saha

Inter-TLR intereactions in the regulation of Leishmania infection: Department of Biotechnonlogy; 2012- 2015

Arvind Sahu

Studies on species specificity in poxviral complement regulators. 2011-2014 (DBT)

Manas Kumar Santra

Molecular characterization in the expression of mRNAs for ornithine-urea cycle enzymes and enzyme proteins, multiple glutamine synthetase genes and signaling cascades under hyper-ammonia stress in the air-breathing walking catfish, *Clarias batrachus*, 2011 – 2014, (DBT)

Vasudevan Seshadri

1. Functional assessment of adult human pancreatic islets following autologous transplantation. June 2010-June 2012 (DBT)
2. Role of protein disulfide isomerase in glucose stimulated insulin biosynthesis. June 2011-May2014 (DBT)

Padma Shastry

Role of Prostrate Apoptosis Response-4 (Par-4)and its interactive proteins in chemo resistance/ sensitivity in gliomas using multi-cellular spheroids (MCS) as model. 2011-2014 (ICMR)

Anjali Shiras

1. Derivation of functional hepatocytes from human induced pluri-potent cells. 2013-16: ICMR, India.
2. Identification of biomarkers for diagnosis and prognostication by Next Gen sequencing of oligo-dendroglial tumour exome. 2011-14: DBT; India
3. Deciphering Cancer Stem cells - Endothelial Niche Interactions in Glioblastoma; 2012-2014 : DBT, India
4. Unraveling the role of miRNAs in self-renewal and tumorigenicity of brain tumor stem cells derived from Neuroepithelial tumors of the Central Nervous System (CNS). 2008-2012: DBT; India.

Yogesh Shouche

1. Establishment of Microbial Culture Collection and Biological Research Centre. 2009-2013 (DBT)
2. Screening of bio-molecules from bacterial diversity isolated from various niches of Western Ghats. 2012-2014 (DBT)
3. Small intestinal and whole gut met genome in patients with celiac disease, their first degree relatives and controls.2011-2013 (DBT)
4. The origins and process of microbiota development in different geographic areas: creating new nutritional tools for microbiota modulation. 2011-2014 (DBT)

Samit Chattopadhyay

1. ATM dependent DNA damage repair by tumor suppressor SMAR1. Council of Scientific and Industrial Research. (CSIR)
2. Regulation of T cell Development and Differentiation by Nuclear Matrix Protein SMAR1: Its Implications in Immune Responses. 2012- 2014, (Department of Biotechnology, New Delhi)
3. Regulation of CD44 splicing by tumor suppressor SMAR1: Implications in cancer metastasis. 2012- 2014. (Department of Biotechnology, New Delhi)

Dr. Sandhya Sitasawad

Investigation of anti-angiogenic efficacy/ potencyand molecular mechanism of the novel anti-cancer compound AECHL-1. 2013- 2016. DST, Government of India

Shailza Singh

1. Synthetic Biology for Leishmania. 2012 (DST-SERB)
2. Systems Biology of *L. major*: Therapeutic Implications. 2012-15 (DBT)

Mohan Wani

Studies on understanding the role of IL-3 in regulation of human osteoclasts and osteoblasts differentiation. 2010-2013 (DBT)

Commemorating the 25th Year of NCCS

Scientists/Academicians who gave talks at NCCS

Prof. Jim Smith
Director, MRC National Institute for Medical Research, London, UK
27th March, 2013

Dr. Jyothsna Rao
Research Officer
Ovarian Cancer Biomarker Group, Prince Henry's Institute for Medical Research, Australia.
18th March, 2013

Raghavendra Gadagkar gave a talk on 'Understanding an Insect Society'

Prof. Raghavendra Gadagkar
IISc & JNCASR, Bangalore
8th March, 2013

Dr. Amit Ghosh
Emeritus Scientist, National Institute of Cholera and Enteric Diseases, Kolkata, India
25th February, 2013

Prof. Saumyadipta Pyne
C.R. Rao Advanced Institute of Mathematics, Statistics and Computer Science, Hyderabad
21st February, 2013

Prof. Jake Tu
Dept. of Biochemistry, Virginia Polytechnic Institute and State University, USA
19th February, 2013

Mr. Arvind Gupta
Children's Science Center, IUCAA, Pune
9th February, 2013(Lecture-demonstration for making science interesting through toys and fun activities)

Arvind Gupta gave a lecture-demonstration on making science fun through toys

Dr. Sathees C. Raghavan
Department of Biochemistry, Indian Institute of Science, Bangalore-560 012.
30th January, 2013

Prof. Siddhartha Gadgil
Dept. of Mathematics, IISc, Bangalore
26th December, 2012

Prof. Rajeeva Karandikar
Director, Chennai Mathematical Institute, Chennai
24th December, 2012

Prof. Vaskar Saha
Paterson Institute for Cancer Research, University of Manchester, UK
21st December, 2012

Prof. Lucio Miele
Director, The University of Mississippi Medical Centre Cancer Institute, Jackson, USA
17th December, 2012

Nobel laureate, Jules Hoffmann visited NCCS in October 2012

Prof. Jules Hoffmann
Nobel Laureate - Physiology or Medicine (2011)
9th October 2012

Prof. William Kerr
Dept. of Biology and Molecular Medicine, University of South Florida, Tampa, USA
3rd October, 2012

Prof. D. Balasubramanian
Director of Research, L. V. Prasad Eye Institute, Hyderabad
26th September, 2012

Prof. Madhav Gadgil
28th May, 2012

Prof. Anindya Dutta
University of Virginia School of Medicine, Charlottesville, USA
27th April, 2012

Events organized on the National Science Day (28th February, 2013)

Talks by NCCS faculty:

- The Life and Times of Sir. C. V. Raman: Dr. Shekhar Mande
- Paving the Way to Pluripoteny: Contributions of Sir John B. Gurdon and Shinya Yamanaka' Dr. Anjali Shiras
- 'The Odyssey of GPCR to the Nobel Prize 2012': Dr. Radha Chauhan

Debate for Research Scholars:

'Space exploration/colonization OR Education for all - What should India fund?'

Students' debate organized on the National Science Day, 2013

Other Events

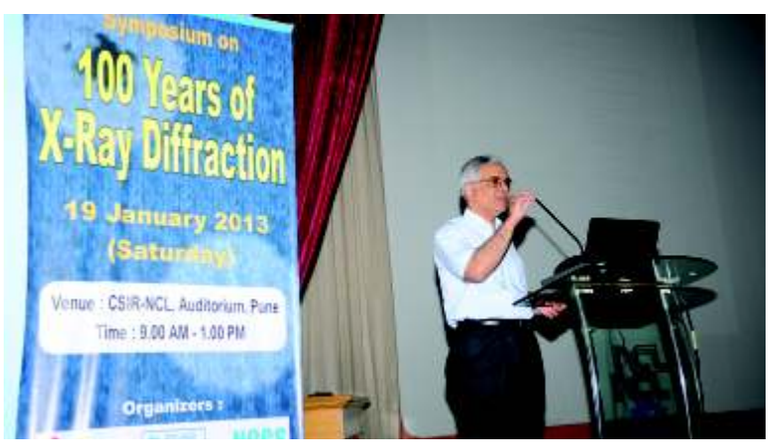
- Retreat for NCCS faculty, research scholars and postdocs: Organized at Fort Jadhavgarh, Pune (12-13 August, 2012)
- Essay writing & T-shirt design competitions were organized to encourage everyone at NCCS to capture the essence of the 25th year of NCCS and voice their vision for the next 5 years of NCCS, respectively.

Display of students' posters at the NCCS retreat organized in August 2012

Symposia / Workshops / Training Programmes Organized

Symposium: '100 years of X-Ray Diffraction'
(organized in collaboration with IISER-Pune and NCL)
19 January, 2013

The symposium organized in January, 2013, to commemorate 100 years of X-Ray Diffraction



Hands-on workshop on 'Molecular Identification of Microbes, Bioinformatics & Phylogeny'
21, 22 December, 2012

MIDI Training programme for Microbial Identification System (MIS)
20 - 21 November, 2012

Symposium on 'Microbes: Molecular Ecology and Systematics'
Organized at YASHADA, Pune. 6 - 7 September, 2012

Workshop on 2D DIGE Technologies
29 - 30 May, 2012

Dr. S. A. Bapat coordinated a series of 6 workshops on "Cancer Informatics".
Funding was through a special grant received from the Department of Biotechnology, Government of India. These were conducted as follows:

Convener(s)	Topic	Dates
Dr. Sharmila Bapat, NCCS, Dr. Deepti Deobagkar, BIC	Analysis and Informatics of Cancer Microarray Data*	13th - 14th September, 2012
Dr. Biplab Bose, Indian Institute of Technology, Guwahati	Analyses of Biological Networks	6th - 7th November 2012
Prof. K. Satyamoorthy, Manipal University	Epigenomics and Cancer Informatics	10th - 12th January, 2013
Prof. Radhakrishna Pillai, Rajiv Gandhi Centre for Biotechnology, Trivandrum & Oklahoma Medical Research Foundation, U.S	Cancer informatics: analysis of microarray data	16th - 18th January, 2013
Dr. Amit Dutt, Tata Memorial Centre, ACTREC, Navi Mumbai	Cancer informatics: SNP / NGS / Transcriptome / miRNAs	28th - 30th January, 2013
Dr. Ravi Sirdeshmukh, Institute of Bioinformatics, Bangalore	Proteomics and Cancer informatics : Data Generation, Analysis and Interpretation	27th - 29th January 2013

* Workshop and Mini-Symposium organized jointly by NCCS and Bioinformatics Centre (BIC), University of Pune.Organizing Committee: Dr. Payel Ghosh, Dr. Jyoti Rao& Dr. Manoj K. Bhat (NCCS); Dr. Abhijeet Kulkarni, Dr. Vijay Baladhye, Ms. Sunita Jagtap &Mr. Sanjay Londhe (BIC).

Organizers and participants of the mini-symposium & workshop on 'Cancer Informatics' held in September, 2012

Workshop on sample preparation for mass spec analysis

May, 2013 (3 batches)

National Workshop on Hematopoietic and Mesenchymal Stem Cells Analysis & Sorting

A 5-days hands-on training program conducted at the BD-NCCS Centre of Excellence in 'Stem Cell Research and Cell Sorting', NCCS

9 - 13 April, 2012

Miscellaneous

- 119 volunteers from NCCS donated blood during the blood donation camp held on 30th August, 2012. NCCS was awarded a Certificate of Appreciation from the Poona Serological Institute Blood Bank for the same.

Award given by the Poona Serological Institute Blood Bank, in appreciation of volunteers who donated blood at the camp held in NCCS on 30th August, 2012

- A lecture and demonstration on fire-fighting was was arranged at NCCS for the students, staff and other personnel, which was conducted by Mr. Chaitanya More on the 22nd of Feb, 2013. This was organized by Mr. Basutkar through the 'Great Wall' Services.

Demonstration of using a fire-extinguisher, organized at NCCS in February 2012

Conferences / Workshops / Seminars

Participation in Conferences / Seminars / Workshops by NCCS Scientists

Sharmila Bapat

- "Where are the missing women?" Plenary Talk as Chief Guest at the inaugural program of the DST-Women Scientists Program ("Statistical and Mathematical Modeling including Data Collection and Analysis" conducted at C.R.Rao Advanced Institute of Mathematics, Hyderabad, India, 14-15th October, 2012
- "Identification of molecular classes in cancer: a case study of high-grade serous ovarian adenocarcinoma", Invited talk at C-DAC symposium on "Accelerating Biology 2013: The Next Wave" at Yashada, Pune, 20th-22nd February, 2013.
- "Intra-tumor heterogeneity in ovarian cancer", Invited talk at the Indo-US Symposium on Flow Cytometry, conducted by the Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, 4th-5th, October, 2012
- "Heterogeneity of serous ovarian adenocarcinomas", Invited talk at the Indo-French Seminar on "Recent Trend in Proteomics" conducted by IISC, Bangalore, 6th - 8th April 2012.
- "EMT-MET systems networks in ovarian cancer" invited talk at workshop and symposium on "Analysis of Biological Networks" at IIT Guwahati, 6th - 7th November, 2012.
- "Ovarian cancer stem cells and regulation of transformation-associated pathways" invited talk at workshop and symposium on "Epigenomics and Cancer Informatics" at Manipal Life Sciences, Manipal, 10th - 11th January, 2013.
- "Molecular Classification of high-grade serous ovarian adenocarcinoma" invited talk at workshop and symposium on "Cancer informatics: analysis of microarray data" at Gandhi Centre for Biotechnology, Trivandrum, 16th - 18th January, 2013.
- Attended the Cold Spring Harbor Laboratory meetingon The Cell Cycle (May 15th-19th, 2012); poster titled "Heterogeneity in Tumor sub-populations identifies distinct functional networks and new drug targets".

Manoj Kumar Bhat

- DST supportedKhorana Program Technology Transfer Course 2012 under IUSSTF (University of Wisconsin 15th July-29th July 2012)

Samit Chattopadhyay

- Chattopadhyay S and Nakka K K, Expression and modulation of CD44 variant isoforms in human cancers. Indian Academy of Sciences, Bangalore, 13th and 14th July, 2012. Invited speaker.

- Chattopadhyay S. and Kiran K Nakka, CD44 alternate splicing: Implication in cancer cell metastasis, Carcinogenesis, International Conference, Dr Ram Manohar Lohia Hospital, Post Graduate Institute Medical Education and Research, New Delhi, November 19-21, 2012.
- Chattopadhyay S., Epigenetic regulation of pro-inflammatory and anti-inflammatory genes, Chromatinasia, at CCMB, Hyderabad, November 22-24, 2012.
- Attended Guha Research Conference, 30th November to 2nd December, 2012 NEHU, Shilong, Meghalaya.

Sanjeev Galande

- "Signaling to chromatin: Tale of a genome organizer". Invited talk at IISER Trivandrum. October 8th, 2012.
- "Role of chromatin organizer SATB1 in T lymphocyte development and differentiation". At the L'Oreal-inStem Symposium on Epithelia and the Immune System: The Biology of Pigmentation, Aging and Stem Cells. December 13th and 14th 2012, InStem, Bangalore.
- "Tale of a genome organizer". In "Control in Biological Systems". NCBS Annual Scientific Meeting, 3-5 January 2013.
- "Epigenetic regulation of non-communicable diseases". March 9th 2013, AUT university, Auckland, NewZealand
- International meeting on 'Gene Expression and Signaling in the Nucleus". September 26-30, 2012, Cold Spring Harbor Laboratory, NY, USA.
- Young Investigator Meeting (YIM) Boston, 6-8 October 2012, MIT, USA.
- 4th Meeting of the Asian Forum of Chromosome and Chromatin Biology on 'Epigenetic Mechanisms in Development and Disease' November 22-24, 2012, at the Centre for Cellular and Molecular Biology, Hyderabad, India
- Science & Communication Workshop by the Wellcome Trust-DBT India Alliance. March 20 - 22, 2013, Hyderabad.

Vaijayanti Kale

- Invited to participate as a speaker in 5th Annual Stem Cell Asia and Regenerative medicine Congress. Topic: Applications of artificial bone marrow microenvironments (ABME) in regenerative medicine. 19th -20th March 2013
- Application of in vitro niches (IVNs) in regenerative medicine. Vaijayanti Kale. Indo-Brazil Workshop on Biomedical Sciences. 11th -13th September 2012, Goa, India

Gopal Kundu

- Application of nanotechnology and nanomedicine in cancer: osteopontin as important therapeutic target. School of Pharmacy, Manipal University, Manipal, 21st April, 2012.
- Osteopontin as important therapeutic target in cancer: Application of nanomedicine in cancer. DBT Brain Stroming Meeting, Kolkata, 25th June, 2012
- Role of Tumor and Stroma-derived Osteopontin in Regulation of Tumor Progression and Angiogenesis, FASEB-SRC on Osteopontin Biology, Vermont, USA, 6th Aug, 2012

- Osteopontin and related proteins: inflammation, cancer and pathological angiogenesis. Cleveland Clinic Foundation, Cleveland, USA, 14th August, 2012
- Osteopontin: an important therapeutic target in cancer and pathological angiogenesis. UMMC Cancer Institute, Jackson, USA, 16th August, 2012
- Osteopontin contributes to breast cancer progression through modulation of HIF 1 alpha -mediated VEGF dependent angiogenesis. 5th Mayo Clinic Symposium on Angiogenesis and Tumor Microenvironment, Minnesota, 18th August, 2012
- Cancer Research: current strategies and future prospects. PSG UG-PG Biotech, PSG Institutions, Coimbatore, 30th August, 2012
- Cancer Awarness in India: INSPIRE Talk, KIIT University, Bhubaneswar, 15th Sept, 2012
- Osteopontin: an Important Therapeutic Target in Cancer. Carcinogenesis Conference, Ram Manohar Lohia Hospital, New Delhi, 19th November, 2012
- Hypoxia Driven and Tumor Associated Macrophage-derived Osteopontin Plays Crucial Role In Angiogenesis and Tumor Progression: Guha Research Conference, Shillong, 28th Nov-2nd Dec, 2012
- Therapeutic Implication of Osteopontin in Cancer and Pathological Angiogenesis. 3rd Intl Cancer Research Symposium, Swissotel, Kolkata, 19-21st Dec, 2012
- Osteopontin Activated Macrophages Plays Crucial Role in Melanoma Progression and Angiogenesis. MIF Meeting, JNCASR, Bangalore, 17th Jan, 2013
- Osteopontin Signaling Network in Cancer: Redefining the Molecular Targets. DBT Workshop, Institute of Bioinformatics (IOB), Bangalore, 27th Jan, 2013
- Osteopontin: an Important Therapeutic Target in Cancer and Pathological Angiogenesis. Natl Conference, Banaras Hindu University, Varanasi, 4th Feb, 2013
- Osteopontin Signaling Network in Cancer: Redefining the Molecular Targets. Intl. Symp. On Molecular Signaling, Visva Bharati, 19th Feb, 2013
- Novel Strategies for Nano-drug delivery systems in Oncology, DBT Brain Storming Meeting on Nanomedicine and Cancer, AIIMS, New Delhi, 5th March, 2013
- Redefining the Molecular Target (s) and Target Based Therapy in Cancers Based on Chemical Biology Approach. Royal Soc. Chem Med Chem, IICT, Hyderabad, 23rd March, 2013

Giridhari Lal

- Lal G. (2013) Non-humoral function of B cells in transplantation tolerance. Alumni Symposia organized by Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore on 16th Feb. (*Invited Talk*).
- Lal G. (2012) A story of T cells from discovery to therapy (Cytokines and autoimmunity), held at Department of Biotechnology, Modern college, Ganeshkhind, Pune. India on 6th Oct.(*Invited Talk*).
- Lal G. (2012) B cell and follicular helper T cell (Tfh) cross-talk is required for generation and maintenance of tolerance. 3rd International conference on stem cells and cancer (ICSCC 2012) held at New Delhi, India on 28th Oct. (*Invited talk*).
- Lal G, Nakayama Y, Burrell BE, Singh AK, Ding Y, and Bromberg JS (2012) "Follicular helper T cell (Tfh) and B cell cross-talk in germinal centers is required for co-stimulatory blockade induced tolerance" in American Transplant Congress 2012, held at Boston, USA, on 2nd June. (*Oral Presentation*).

- Lal G, Nakayama Y, Burrell BE, Sethi A, Ding Y, and Bromberg JS (2012) "B cell derived IL-10 is required for co-stimulatory blockade induced tolerance" in American Transplant Congress 2012, held at Boston, USA, on 2*nd* June. (*Oral Presentation; Plenary Session*).

Nibedita Lenka

- N. Lenka. 2012. Stem cells therapy in cardio-vascular diseases: the possibilities and challenges. 1st East Zonal Conference of Indian Pharmacological Society, College of Veterinary Science, Guwahati, Assam (Invited Lead Speaker).
- Invited participant at the DBT-sponsored Brain Storming Meeting on 'Induced Pluripotent Stem Cells' and 'Haematopoietic Stem Cell Fate in the Niche', CMC, Vellore, 2012

Lalita Limaye

- Participated in DST sponsored workshop for women scientists on FUTURE CHALLEGES TO SOCIETY-Resources and Development Scope and challenges - held in Feb.2013 in NIAS (National Institute of Advanced Studies) in Bangalore.
- Kedar Limbkar attended ICMR's one day Workshop on Biomedical Communication held at National Institute for Research in Reproductive Health (NIRRH), Mumbai on 25/08/12.
- Invited speaker in Modern College Shivaji Nagar- delivered lecture on "hematopoietic stem cells"

Shekhar Mande

- Biotechnology and its applications, Technology Day address, URDIP, Pune, 11-May-2012
- Protein structures, Special invited talk, Manipur University, Imphal, 18-May-2012
- Genome-wide interactions and communication signals during Mycobacterial latency, India-Spain-Portugal workshop, talk delivered over the web, 24-May-2012
- Molecules of Life, INSPIRE camp, Shivaji University, Kolhapur, 31-May-2012
- The structural basis of universality of cAMP mediated signaling, invited talk, ACTREC, Mumbai, 2-July-2012
- Interdisciplinary sciences in modern biology, Sunil Newaskar and Jaydeep Naik Oration, Garware College, Pune, 24-Aug-2012
- Networks of protein interaction and potential drug targets, Meeting in honour of Prof. Wim GJ Hol, Amsterdam, talk delivered on the web, 4-Sept-2012
- Biotech and Health, Special talk in celebration of Biotechnolgy Day, University of Pune, Pune, 21-Nov-2012
- Systems analysis to address Mycobacterial latency, invited talk in the Indo-German symposium on systems biology, University of Hyderabad, Hyderabad, 27-Nov-2012
- Recombinant DNA technology and modern healthcare, Inaugural address, Interacademy Science Workshop, Kakatiya University, Warangal, 30-Nov-2012
- Network analysis and mycobacterial latency, Silver Jubilee Bioinformatics Conference, University of Pune, Pune, 6-Dec-2012
- Analysis of dihedral angle variability in evolutionarily related protein structures, International conference on Biomolecular Forms and Functions, Indian Institute of Science, Bangalore, 11-Jan-2013

- Historical perspectives, Invited talk, National colloquium on 100 years of Crystallography, Thiruvananthpuram, 8-Feb-2013
- Genomics approaches towards understanding Tuberculosis, National Science Day address, National Institute of Virology, Pune, 28-Feb-2013
- Historical developments in the understanding or Structure-Function relationship in proteins, Invited talk, Sri Venkateswara College, University of Delhi South Campus, New Delhi, 14-Mar-2013

Debashis Mitra

- Identification of novel anti-HIV molecules and their potential as microbicide candidates Invited Talk delivered at Birla Institute of Technology, Mesra, 27th June 2012
- HIV-1 Tat protein regulates cellular gene expression by interacting with NFκB enhancer sequence. 81st Annual Meeting of Society of Biological Chemists (India), 8-11 Nov 2012, Kolkata.
- Cellular Stress proteins in HIV-1 replication. 3rd Molecular Virology Meeting, 10-11 January 2013, National Institute of Virology, Pune,
- Reporter cell based assays for screening of potential anti-HIV molecules Workshop on Analytical Techniques for Characterization of biological Macromolecules from January 30 to February 1, 2013, Department of Biotechnology, Heritage Institute of Technology, Kolkata.
- Cellular heat shock proteins regulate HIV-1 gene expression and replication National perspective of Microbiology Research in India, March 14 -15, 2013, Department of Microbiology, University of Calcutta, Kolkata.

Arvind Sahu

- Species selectivity in poxviral complement regulators is dictated primarily by the charge reversal in the central complement control protein modules, XXIV International Complement Workshop, Oct 10 - Oct 15, 2012, Crete, Greece.
- Molecular determinants of species selectivity in poxviral complement regulators, Invited talk, 3rd Molecular Virology Meeting at National Institute of Virology, Pune, Jan 10, 2013.
- Species selectivity in poxviral complement regulators, Invited talk, 21st Molecular Immunology Forum at Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, Jan 19, 2013.

Manas Kumar Santra

- Manas K. Santra. The F-Box protein FBXO31 directs degradation of MDM2 to facilitate p53-directed senescence induction, Invited talk, Indian Association for the Cultivation of Science, Jadav Pur, India, December 14, 2012.
- Manas K. Santra, K. A journey from chemistry to cancer biology: FBXO31 functions as a novel checkpoint protein in genotoxic stress induced genome instability, Invited talk, Vidyasagar University, Midnapore, India, December 17, 2012.

Padma Shastry

- mTORC2 signaling cascade in survival, proliferation and invasion of human gliomas in -Carcinogenesis and Preventive Oncology: Molecular Mechanisms to Therapeutics- held in New Delhi from 19th to 21st November 2012.
- Driving the human bone marrow derived MSC towards neuronal lineages" inXXX Annual Conference of Indian academy of Neurosciences and International Symposium held in Amristar from 27th Oct 2012-30th, 2012
- Complex role of mTORC2 in invasion of human gliomas in the Symposium on Neuro-oncology in 32th Convention of Indian Association for Cancer Research held in ACBR in New Delhi from 13th Feb 2013 to 17th Feb. 2013

Anjali Shiras

- ? Activation of Wnt/β-catenin Signaling Induces Malignant Transformation in Gliomas. Indian Association of Cancer Research (IACR) Meeting; New Delhi, India, 2013.
- Wnt3a as an oncogene in Glioma Progression Asian Society of Neuro-oncology (ASNO) Meeting: Mumbai, India, 2013
- Identification of group of miRNAs functioning through Wnt Signaling pathway and downregulated in Glioma Stem cells. 4th Annual Conference of The Indian Society of Neuro-Oncology (ISNO) on Targeted Therapies in Neuro-oncology, Bangalore, 6-8 April, 2012

Srikanth Rapole

- ? Copper role in β-2-Microglobulin Amyloid Formation Studied by Mass Spectrometry: Invited talk at International symposium on proteomics beyond IDs and 4th annual meeting of *Proteomics Society of India*, *National Chemical Laboratory, Pune, India, November 22-24, 2012*.
- Copper role in β-2-Microglobulin Amyloid Formation Studied by Mass Spectrometry: Invited talk at Workshop on metals in health and disease , Agharkar research Institute, Pune. India, 8 January *2013*.
- DBT workshop on cancer proteomics organized by Institute of Bioinformatics, Bangalore.
- The University of Warwick workshop on practical proteomics organized by Waters India, Bangalore.

Yogesh Shouche

- Tools and techniques in Microbial Ecology - Invited Talk at the Symposium on "Microbes: Molecular Ecology and Systematics." at YASHADA, Pune. September 5-6, 2012
- Probiotics in Prevention of Lifestyle Disorders, 15th-16th December 2012, Bengaluru, India. Start date-End Date, Month, Year, City, Country.

Shailza Singh

- Invited Talk on "Biochemical and Structural Target of *Leishmania*: A Systems and Synthetic Biology Perspective" at International Interdisciplinary Science Conference, JMI, New Delhi, India, 8th-10th December 2012.
- Invited Talk on " Systems Biology for Modular Modeling of Signaling Pathways in *Leishmania* macrophages and their cross talk: A Mathematical Modeling Approach" at Seventh Indo-US Workshop on Mathematical Chemistry, Thanjavur, India, 4th-6th December 2012.
- Invited Talk on "High Performance Computing Resources in Drug Delivery Systems" at CDAC, Pune, 28th May 2012.

Sandhya Sitaswad

- Attended a workshop on 'Discovery Development and Commercialization of Biologicals' from March 11- March 15, 2013 at IIT, Delhi.

Mohan Wani

- "Biosafety guidelines for teaching and research laboratories" in IBB-Safety Symposium 2012, Institute of Bioinformatics and Biotechnology, Pune, November 3, 2012.
- "IL-3 inhibits human osteoclast differentiation and promotes osteoblast differentiation", 81st Annual Meeting of Society of Biological Chemists, Science City, Kolkata, November 9, 2012.
- "Regulation of Th17 cell development by IL-3" at Molecular Immunology Forum, Jawaharlal Nehru Centre for Advances Scientific Research, Bangalore, January 19, 2013.
- "Regulation of bone remodeling by immune cells", 3rd Interdisciplinary Research Conference, Krishna Institute of Medical Sciences Deemed University, Karad, March 2, 2013.

Conferences / Workshops Participated in by Students / Postdoctoral Fellows / RAs

- Himanshu Kumar attended the 1st annual conference of PAi and the InternationalSymposium on Probiotics for Human Health 27-28th August 2012. He also won the best presentation award in the Conference

- Akshada Gajbhiye presented a poster entitled 'Investigation of Serum and Tissue Proteome Alterations in Breast Cancer using iTRAQ-LC-MS/MS' at International symposium on proteomics beyond IDs and 4th annual meeting of *Proteomics Society of India, 22 Nov -24 Nov, 2012 organized by National Chemical Laboratory, Pune.*

- Debasish Paul presented a poster entitled 'Identification of Candidate Proteins Involved in Chemotherapeutic Resistance Through Global Proteomic Profiling' at International symposium on proteomics beyond IDs and 4th annual meeting of

Proteomics Society of India, 22 Nov -24 Nov, 2012 organized by National Chemical Laboratory, Pune.

- Milsee Mol J.P. presented a poster entitled 'Understanding Signaling dynamics in Leishmania through systems biology'at 'Accelerating Biology 2013: The Next Wave', CDAC, Pune, 20 -22 Nov, 2013. She was awarded third prize for best oral presentation.

- Sonali Shinde presented a poster entitled 'Evolutionary dynamics of IPCS in Leishmania: A bioinformatics perspective' at 81st Annual meeting of The Society of Biological Chemists and Symposium on Chemistry and Biology: Two Weapons Against Diseases, Kolkata, 8 - 11 Nov, 2012

- Vineetha Mandlik presented a poster entitled 'Structural Conservedness and stastical coupling analysis of IPCS in *Leishmania major*' at 81st Annual meeting of The Society of Biological Chemists and Symposium on Chemistry and Biology: Two Weapons Against Diseases, Kolkata, 8 - 11 Nov, 2012

- Ajitanuj Rattan presented a poster entitled 'Role of complement in neutralization of pandemic influenza virus' at NCCS Retreat, 12 Aug 2012 at Fort Jadhavgadh , Pune.

- Ashish Kamble presented a poster entitled 'Role of complement pathways in vaccinia virus neutralization and mechanism involved in neutralization' at 3rd Molecular Virology Meeting, 10-11 Jan 2013 at National Institute of Virology, Pune.

- Ajitanuj Rattan presented a poster entitled 'Role of complement in neutralization of pandemic influenza virus' at 3rd Molecular Virology Meeting, 10-11 Jan 2013 at National Institute of Virology, Pune.

- Jayashree C Jagtap, presented a poster "Expression of prostate apoptosis response-4 (par-4) in human glioma stem cells and regulation during drug- induced apoptosis" atannual meeting of Australian Neuroscience held in Melbourne from 3rd to 6th Feb 2013 She was awarded Travel grant by FAONS (Federation of Asian Ocean Neuroscience Symposium) for participation in the meeting.

- Natesh Kumar presented a poster "The role of STAT-3 in Oncostatin-M (OSM) and TNF-α mediated regulation of MMP-9 activity" at 32nd Annual Convention of Indian Assoiciation for Cancer Research (IACR) held in New Delhi on Feb 13-16, 2013.

- Parveen. D and Reecha D Shah presented a poster "Combinatorial effect of tamoxifen and sodium valporate on the expression pattern of chemoresistant genes in human gliomas using multi-cellular spheroids as model" at 32nd Annual Convention of Indian Assoiciation for Cancer Research (IACR) held in New Delhi on Feb 13-16, 2013.

- Priyanka Chaudhary presented a poster in 3rd Molecular Virology Meeting in NIV, Pune.
  10-11 January 2013 and was awarded best poster award.
  Regulation of HIV-1 replication and gene expression by HspBP1 protein

- Surya Srivastava presented a poster in 3rd Molecular Virology Meeting in NIV, Pune, 10-11 January 2013. Deciphering Nef induced changes in landscape of cellular transcriptome during HIV-1 infection
- Avinash Kumar attended the workshop on "Mouse Embryology Workshop" organized by InStem, 10 – 23rd March 2013, Bangalore.

- Meghana Kanitkar presented a poster entitled "A polycaprolactone-gelatine nano-fiber matrix as a combined growth and delivery system for endothelial progenitor cells (EPCs) in treatment of diabetic wounds" at the Tissue Science-2012 conference, 1st to 3rd October, 2012, Chicago, USA. She was awarded the ICMR travel Award for participation.

- Jinu Mary Mathai presented a poster entitled 'Regulation of microRNAs and histone marks in cancer by the nuclear matrix protein' at the 4th Asian Chromatin Meeting, CCMB, Hyderabad. November 22-24, 2012.

- Sagar Varankar attended 2nd Cancer Genomics conference:GCGC organised by GCGC consortium, 19th-20th Nov, 2012, ACTREC, Mumbai.

- Mr. Ashwani Kumar, Dr. Shubhada Hegde and Dr. Payel Ghosh attended the workshop on 'Introduction to Systems and Synthetic Biology for Scientists and Engineers' organized by Indian Institute of Technology Bombay, from April 30, 2012 to May 3, 2012, Mumbai.

- Dr. Shubhada Hegde and Dr. Payel Ghosh conducted two sessions in a workshop on 'Cancer Informatics: Analysis and Informatics of Microarray Data' jointly organized by NCCS, Pune and University of Pune during 14th-15th September, 2012

- Mamata Khirade attended workshop on microarray data analysis using R and Bioconductor packages organised by Ragiv Gandhi Centre for Biotechnology on 15th-18th Jan, 2013, Thiruvananthapuram.

- Brijesh Kumar attended workshop on Cancer Proteomics organised by DBT workshop series on Cancer Informatics, 27-29th Jan, Bangalore.

- Aman Sharma participated in a Workshop on Microarray data analysis using R/bio-conductor from 16-18 January, 2013, Trivandrum, Kerala.

- Sachin Rathod participated in DBT funded "Cancer Informatics Workshop" from 28th to 30th January 2013 held at Advanced Centre for Treatment Research and Education in cancer (ACTREC), Navi Mumbai India.

- Sachin Rathod participated in DBT funded "Workshop on Analysis of Biological Networks" during 6th -7th November 2012, held at IIT Guwahati, organized by Department of Biotechnology and Indian Institute of Technology Guwahati India.

- Aman Sharma: Oral presentation "Perivascular niche interactions of glioma stem cell-endothelial cell promote angiogenesis response in glioblastoma" at 32nd Annual Convention of Indian Association of Cancer Research, 13-16 February, New Delhi (Short-listed for ACBR Young Scientist Award)

- Sachin Rathod and Phalguni Rath presented a poster entitled: "Wnt3a functions as an oncogene and induces tumor progression in Glioma" at 32nd Annual Conversion of Indian Association for Cancer Research (IACR) & International Symposium: Infection & cancer held on 13-16 February 2013.

- Suchismita Panda presented a poster entitled: "Role of Genomic INstability Inducing RNA (Ginir) in cellular transformation" at Second Global Cancer Genomics Consortium Symposium 2012 held at ACTREC, Kharghar, Navi Mumbai from 19th - 20th November-2012.

Students Awarded Ph.D.

Loka Raghukumar Penke

Title of the Thesis: Modulation of chemokine and chemokine receptor expression in Leishmaniasis

Guide: Bhaskar Saha

Neetu Srivastava

Title of the Thesis: Role of dual-specific MAPK phosphatases in CD40 signaling in macrophages

Guide: Bhaskar Saha

Raki Sudan

Title of the Thesis: Role of Protein Kinase C (PKC) in regulation of CD40-mediated functions in leishmaniasis

Guide: Bhaskar Saha

Sushmita Pahari

Title of the Thesis: Invovlement of small GTPases in CD40 signaling in Leishmaniasis

Guide: Bhaskar Saha

Kiran K. Nakka

Title of the Thesis: Role of SMAR1 protein in Pre-mRNA processing

Guide: Dr. Samit Chattopadhyay

Mukesh Mani Tripathi

Title of the Thesis: Novel roles for -catenin and Nup358 in Wnt signaling

Guide: Dr. Jomon Joseph

Roli Mishra

Title of the Thesis: Molecular mechanisms involved in the regulation of tumour growth by exogenously expressed truncated eNOS proteins.

Name of guide: Dr. Vaijayanti Kale

Santosh Kumar

Name of the guide: Studies on the Role of Stroma and Tumor derived Osteopontin in tumor progression : an Important Terapeutic Target in Cancer.

Name of guide: Dr. Gopal Kundu

Smita Kale

Title of the Thesis: Studies on the role of Osteopontin – activated Macrophages in Regulation of Melanoma Growth and Angiogenesis.

Name of guide: Dr. Gopal Kundu

Shubhada Hegde

Title of the Thesis: Studies on the structure and dynamics of protein interaction networks

Guide: Shekhar C. Mande

Neeru Dhamija

Title of the Thesis: Studies On The Role Of HIV-1 Tat In Viral And Cellular Gene Expression: Regulation At The Chromatin Level

Guide: Dr. Debashis Mitra

Swarup Saumya Srivastava

Title of the Thesis: Studies on the modulation of cell signaling by a hemolysin : Mechanism of action

Guide: Dr. M. V. Krishnasastry

Kaustubh Gokhale

Title of the Thesis: Characterization of Hox cluster from mosquito

Guide: Dr. Yogesh S. Shouche

Monika Sharma

Title of the Thesis: Effect of microenvironment-mediated signaling on growth and development of hematopoietic stem cells

Guide: Dr.Vaijayanti P. Kale

Neesar Ahmed

Title of the Thesis: Understanding the dynamics of caveolae by external stimulants and stress

Guide: Dr. M. V. Krishnasastry

Ganeshkumar Rajendran

Title of the Thesis: Studies on understanding the epigenetic mechanisms contributing to gliomagenesis using known and novel glioma cell-lines

Guide: Dr. Anjali Shiras

Aejazur Rahman

Title of the Thesis: Cloning, Expression and Characterization of Hemolysin(s) of Mycobacterium tuberculosis H37Rv

Guide: Dr. M. V. Krishnasastry

Himansu Kumar

Title of the Thesis:	Characterization of probiotics from indigenous biomedicines
Guide:	Dr. Milind S. Patole

Mruthunjaya S

Title of the Thesis:	Differentiation of Human Bone Marrow Mesenchymal Stem Cells (MSC) towards Neural Lineages".
Guide:	Dr. Padma Shastry

Arvind Gupta

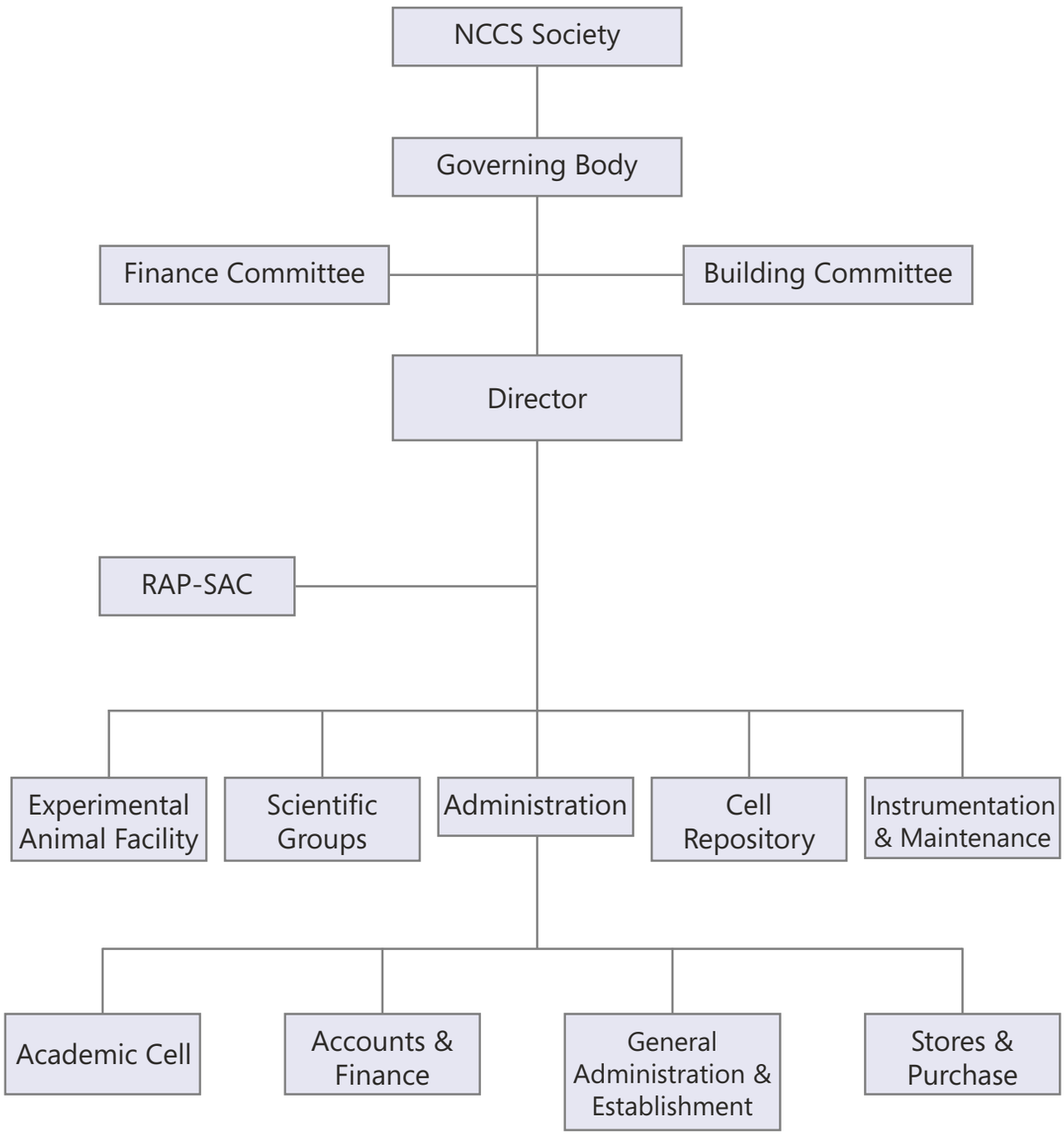
Title of the Thesis:	Molecular studies on gut associated microbiota inhousefly and fleshfly
Guide:	Dr. Yogesh Shouche

C P Antony

Title of the Thesis:	Methylotrophy and associated bacteria in Lonar lake, a meteorimpact crater
Guide:	Dr. Milind S. Patole

NCCS Organization

NCCS Organization



NCCS Committees

NCCS Society Members

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17. **Prof. Ashok Venkitaraman** — Member
Ursula Zoellner Professor,
Director- MRC Cancer Cell Unit,
Hutchinson, MRC Research Centre,
Hills Raod, Cambridge,
UK- CB2 0XZ.

Administration

The NCCS Administration consists of the following sections: General Administration & Establishment, Civil Maintenance, Accounts & Finance, Stores & Purchase, and an Instrumentation & Maintenance unit. All these sections provide support and services to the main scientific activities of the centre.

As on date, the centre has the following staff strength:

Scientists	:	29
Administrative	:	43
Technical	:	69
Total	:	141

Reservation Policy

NCCS follows the Government of India orders on reservation matters. For direct recruitments, we follow respective rosters, with reservation as follows: 15% for SC, 7.5% for ST and 27% for OBC, on an All India Basis by Open Competition. Liaison officers have been nominated to ensure compliance with the reservation orders issued in favour of SC/ST/OBC. The Centre also follows the Government of India reservation policy for physically handicapped candidates.

Right to Information Act 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. V.S. Shinde, Officer 'B' (Administration) as the CPIO and Dr. (Mrs.) V.P. Kale, Scientist 'F', has been nominated as the First Appellate Authority.

Vigilance Matters

Dr. Lalita Limaye, Scientist 'F', has been nominated Chief Vigilance Officer with effect from 24/01/2013 of the centre. Vigilance reports are sent regularly to the nodal ministry, i.e. the Department of Biotechnology, Government of India, New Delhi.

Security

NCCS has engaged a private Security Agency for providing security services on a contractual basis. All important places in the complex have been manned by security personnel throughout 24 hours in a day. As on date, there is no security-related problem at the centre.

Disciplinary Matters

The centre follows CCS (Conduct) rules 1964, CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the centre.

Implementation of Official Language

NCCS has constituted the Official Language Implementation Committee to implement the Government of India order to use the Official Language in day to day official work.

Most of the staff members have undergone the Hindi Typing training conducted by Hindi Teaching Scheme & the remaining staff members are being nominated for the same.

The Centre also observes Hindi Week (Hindi Saptah) every year. In the year 2012-13 Hindi Essay and Hindi Dictation competitions were held during the Hindi Week and the winners were awarded cash prizes and certificates. Mrs. Seema Deshpande, Assistant Director, Hindi Teaching Scheme, Pune, was invited as Chief Guest for the Hindi Day Function held on 20th September, 2012. On this occasion the Hindi Website of the Institute was launched by Dr. G. C. Mishra, Eminent Scientist, NCCS, who was the special invitee.

The Director, NCCS, strongly supports the use of the Official Language in official work, and the other related activities carried out at the Institute.

Committees

The centre has formed the following committees as required under various statutes and guidelines for smooth functioning of the institute:

1. Grievance Committee
2. Committee for prevention of sexual harassment of working women employees
3. Animal Care and Use Committee
4. Biosafety Committee
5. Institutional Ethics Committee

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