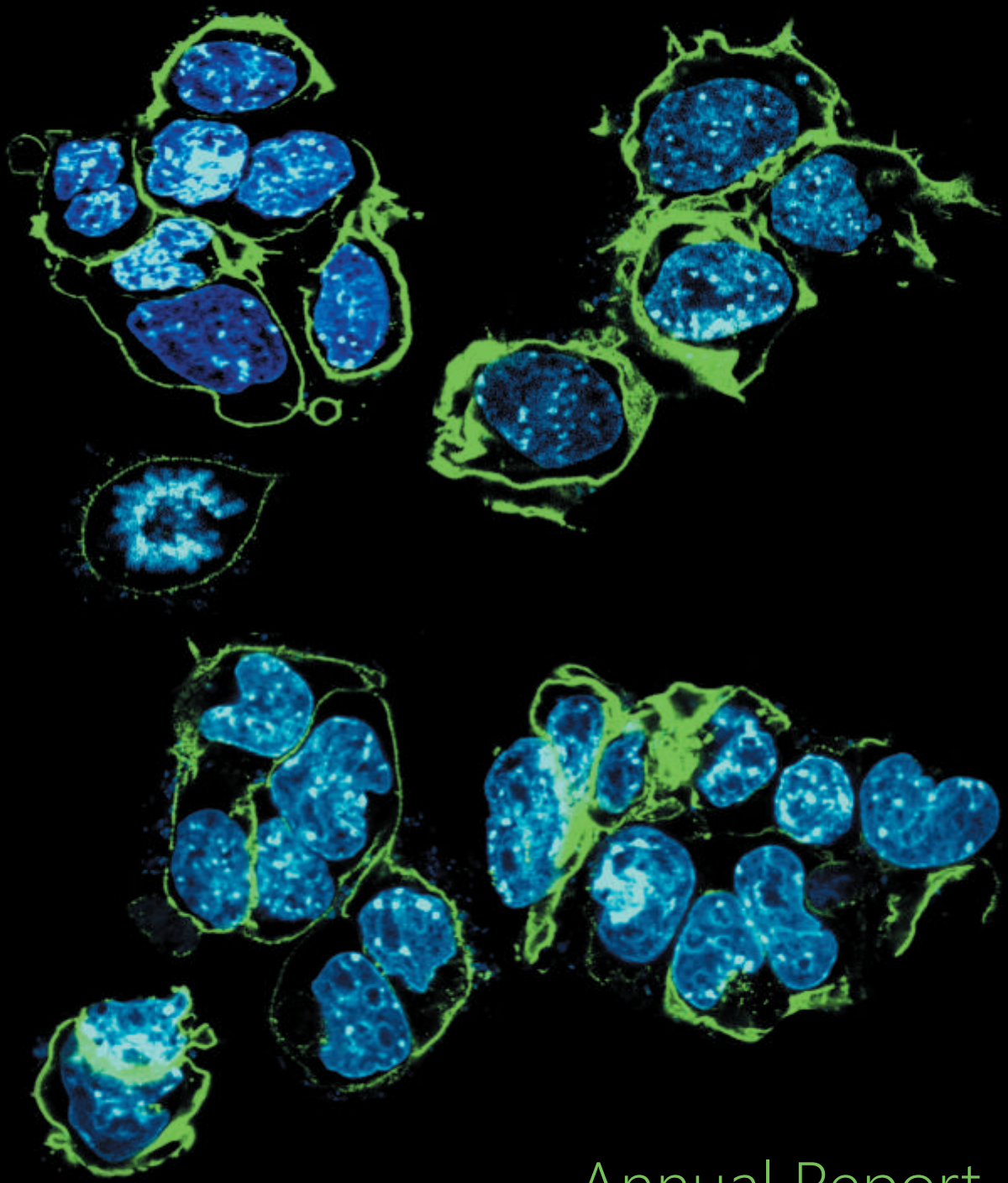
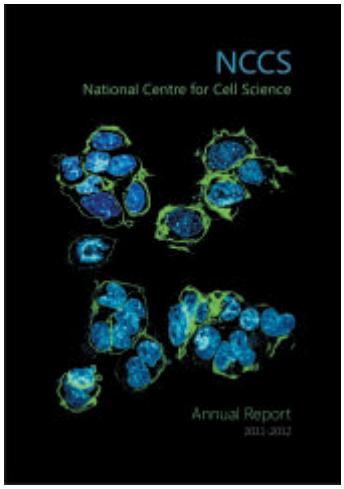
NCCS

National Centre for Cell Science



Annual Report

2011-2012



Cover page image

Derivation of human induced pluripotent stem cells from reprogrammed fetal dermal fibroblasts.
Characterization of iPS cells by immuno-cytochemistry for expression of stage specific embryonic antigen-4 (SSEA-4), an ES cell specific marker. Nuclei were stained with DAPI (blue) and Alexa Fluor 488 secondary antibody was used to stain SSEA-4 (green).
(Courtesy: **Dr. Anjali Shiras** and **Mr. Phalguni Rath**)

National Centre for Cell Science

Annual Report 2011-2012

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.

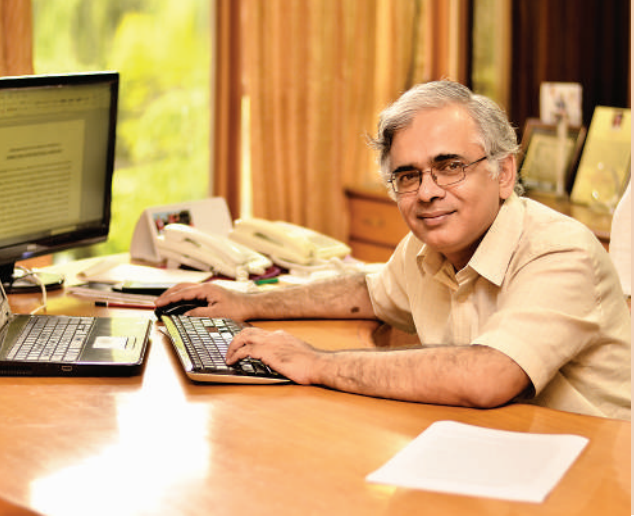
- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

- 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 is my proud privilege to present the Annual Report of the National Centre for Cell Science (NCCS), Pune, for 2011-12. NCCS has been, and continues to be a fine institution which supplies cell lines to researchers across the country, provides training to develop high quality manpower in animal tissue culture and carries out frontline research in major areas of modern Cell Biology. In the last year, NCCS provided 2780 cell lines to 127 organizations as a part of its commitment. Towards developing high quality manpower, NCCS admitted 33 research scholars into its PhD programme, bringing the total number of research scholars who have registered for a Ph.D. to 105. NCCS also trained 4 summer trainees and 15 students as project trainees.

NCCS scientists have continued their pursuit to understand cancer at the molecular level. In one of the research projects, a gene expression-based signature of serous ovarian adenocarcinoma tumour classification was resolved through metastases-associated gene expression clustering and metastases-independent identification of correlating modules of genes that exhibit similar expression patterns. This analysis suggested the epithelial to mesenchymal transition (EMT) to be a differential function between the two tumour groups thus resolved. Identification of appropriate cell models led to validation of the predictive differential invasive capability between the two classes. Further, network-based approaches hold promise for the resolution of additional class-specific biological functions and new therapeutic approaches in this disease.

Among the class of transcription factors which exhibit loss of control in different cancers are the nuclear matrix attachment region binding proteins. Scaffold matrix attachment region binding protein 1 (SMAR1), belongs to this class of repressors and is correlated to higher grades of breast cancer. Research carried out at NCCS has demonstrated that SMAR1 modulates p53 activity in order to dictate cell fate towards arrest or apoptosis. New insights have been obtained into the mechanisms by which SMAR1 modulates DNA repair activity and thereby maintains genomic integrity. As enzymes involved in DNA repair are attractive targets for developing anti-cancer therapy, different cellular factors that can regulate DNA damage checkpoints in cancer stem cells, such as SMAR1, are also potential novel targets.

Among the major contributions made by NCCS scientists in the past few years are those towards understanding the intricate balance between host-pathogen interactions and their repercussions in different disease models. In one such model, research carried out at NCCS has revealed that CD40 signaling plasticity - a property inherent to a reciprocal system - can be exploited for designing novel therapies. The *Leishmania major* MAP kinase 4 perturbs the CD40 signaling reciprocity and thereby assists the parasite in survival within the hostile environment of host macrophages. Virtual screening on the modelled enzyme identified a compound that targeted MAP kinase 4 of *L. major* and exerted anti-leishmanial activity *in vitro* and *in vivo*. Thus, the *L. major* MAP kinase 4 appears to be an excellent target for novel therapeutics.

Similar host-pathogen interplay is exhibited by viruses and the host complement system, where viruses successfully subvert the host complement system before gaining entry into the host cells. Work carried out at NCCS has shown that DNA viruses encode homologs of human regulators of complement activation. Molecular analyses of these regulators reveal interesting differences that can contribute to host tropism of animal and human viruses. Surprisingly, only 3 residues in the poxviral complement regulators are sufficient in defining host-complement specificity. This observation is startling not only for understanding differences among viruses and their host specificity, but also for the fact that only a few mutations are capable of modulating the host specificity of viruses, which could lead to major health care repercussions.

As this is the first time that I am presenting the Annual Report of NCCS, it my pleasure to place on record my deep appreciation for the strong foundation that has been laid for NCCS. The leadership provided by the immediate past Director, Dr. G. C. Mishra and the founder Director, Dr. U. V. Wagh continues to inspire us for our growth. It will be our endeavour to embrace the future, remain competitive and make NCCS one of the foremost Centres of Excellence known worldwide.

S. C. Mande
Director

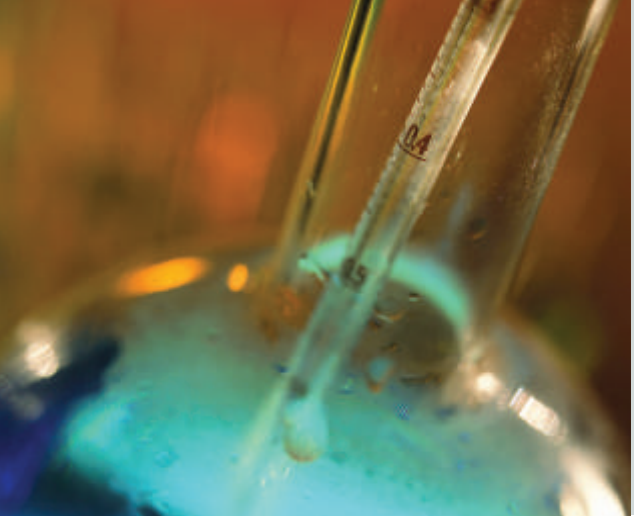
Human Resource Development

During the year 2011-12, thirty-three students joined NCCS for pursuing a Ph.D. under the guidance of various faculty members. During this year, twenty nine students were registered as Ph.D. students under the University. The total number of Ph. D students as on 31st March 2012 was 105. During this year, a total of nineteen students either submitted their thesis to the University or were awarded a Ph.D.

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 2011-12 was:

Project Training: 15
Summer Training: 04

Seventeen Research Fellows attended seminars / conferences / symposia / meetings conducted by various reputed organizations in India and three Research Fellows attended International seminars / conferences / symposia / meetings. Three Research Fellows participated in workshops / lecture series held in India.



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 2011-12, we have supplied 2870 cell lines to 126 research institutions in the country.

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Research Report

Bioinformatics & Proteomics

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Structure-function properties of *Mycobacterium tuberculosis* proteins

Background

Our laboratory has been involved in two major projects: (1) structure-function characterization of proteins of *M. tuberculosis*, and (2) development of novel computational methodologies to understand large scale high throughput data from various biological experiments. Towards the first objective, we have undertaken work on heat shock proteins and proteins involved in redox reactions of *M. tuberculosis*. Towards the second objective, we have begun analyses of diverse microarray experiments of *E. coli*. These analyses are also being integrated with the protein:protein interaction data from different sources.

Aims and Objectives

1. To identify important proteins of *M. tuberculosis* involved in redox reactions and in heat shock response. To purify these recombinant proteins and undertake structure-function analyses on them.
2. Analysis of large scale data available in the literature to find common patterns, if any.
3. To evolve methodologies for the analysis of protein: protein interactions, especially the dynamics of changes in these interactions.

Work Achieved

Crystal structure of NrdH

The *M. tuberculosis* NrdH protein (encoded by the Rv3053c open reading frame) is a 79 residue long thioredoxin-like protein with a glutaredoxin-like sequence. NrdH in *M. tuberculosis* is speculated to supply electrons required for the essential biochemical reaction of ribonucleotide reduction (RR). RR is one of the most fundamental biochemical processes required for the existence of a DNA-based life form. In any given cell, Ribonucleotide Reductases (RNR) use ribonucleotides to make deoxyribonucleosides, which then act as precursors for DNA synthesis. RNRs are thus essential for both DNA replication and repair.

Participants

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There are three main classes of RNR (class I, class II and class III), which differ in their subunit composition, co-factor use and oxygen requirements. The catalytic mechanism of RNR consists of a complex, conserved (amongst all classes) reaction mechanism. Amongst these three classes, class I enzymes constitute the most widely studied RNRs, and are present in a large number of organisms ranging from viruses to prokaryotes to higher eukaryotes. Class I enzymes differ from class II and class III in several aspects. Most importantly, all class one enzymes require oxygen to function. While the class I enzymes majorly reduce ribonucleoside 5'-diphosphates, most of the class II and all class III enzymes reduce ribonucleoside 5'-triphosphates (NTPs).

Based on differences in allosteric regulation and utilization of different electron donors, class I RNR are subdivided into Ia and Ib. Class Ia is present in Eukaryotes, Eubacteria, Bacteriophages and Viruses, while class Ib is present in Eubacteria alone.

At the end of each cycle of RR, RNR needs to be reduced in order to be primed for the next cycle of reduction. An external co-factor, a glutaredoxin/thioredoxin, usually performs this step for the Class1a RNR. The oxidized glutaredoxin/thioredoxin is then reduced by the glutaredoxin/thioredoxin reductase which can then 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, even though NrdH appears to be glutaredoxin-like, it behaves like a thioredoxin and can accept electrons from thioredoxin reductase.

The crystal structure of NrdH from *M. tuberculosis* was solved and refined to crystallographic R-factor of 17.1% (Rfree 21.2%) at 1.67Å resolution (Figure 1). The 3D structure is similar to the structures of other NrdH proteins from *E.coli* and *Corynebacterium*, and has a typical thioredoxin fold. Structural superimposition of *M.tb* NrdH over Thioredoxin A (TrxA) and *M.tb* Thioredoxin reductase (TrxR) over the already solved structure of *E.coli* TrxR - TrxA complex explains the ability of *M.tb* NrdH to accept electrons from *M.tb* 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.

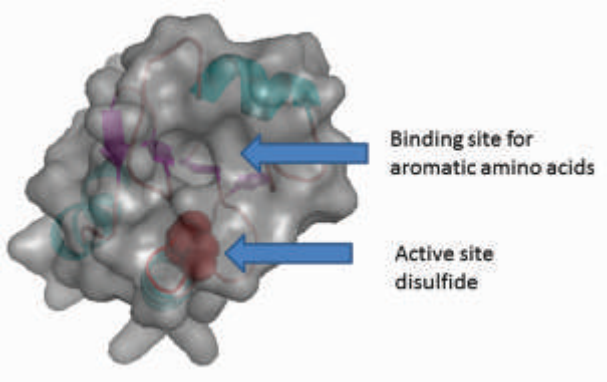

Fig. 1: Surface representation of the crystal structure of *M. tuberculosis* NrdH. The conserved active site disulfide, which is conserved across all the thioredoxin-like proteins, is shown. The cavity for binding of aromatic amino acids, which are present in the electron transfer partner, thioredoxin reductase, is also shown.

Microarray of GroEL-1 knock out strain

One of the long standing aims of my laboratory has been to decipher the functional role of GroEL proteins of *M. tuberculosis*. We had shown a few years ago that apart from performing their canonical role as chaperonins, they also possess DNA-binding properties. To comprehend the role of GroEL-1 in binding to DNA, we carried out comparative studies on *M. tuberculosis* H37Rv (WT) and *M. tuberculosis groEL-1* knockout (KO). Growth curve analysis with the WT, KO and *groEL-1* complemented strain (Comp) in 7H9 media showed that there was no growth defect shown by the KO. Gene expression profiling of the KO and WT were done under five stress condition (Table1) using

Table 1: Genes differentially expressed under stress conditions

Condition		WT vs KO
Cold shock	On ice	398
Mild cold shock	At room temperature	316
Low pH	pH= 5.5	35
Low aeration	Standing culture at 37C	53
Stationary phase	OD 2.2	643

Nimblegen arrays. Five biological replicates were taken for each of the stress conditions. All genes which showed a 2 fold change and had a p value <0.01 were taken as significant differentially expressed genes in KO in comparison to the WT. A large number of genes were found to be differentially expressed in the stationary phase, cold shock and mild cold shock compared to the conditions, low pH and low aeration. While PE PGRS genes were highly upregulated in pH stress, ribosomal genes showed an increased expression at stationary phase in the KO. A heat map was generated for all the five stress conditions (Figure 2). A total of 1175 genes were differentially expressed in KO under various stress conditions as compared to the WT.

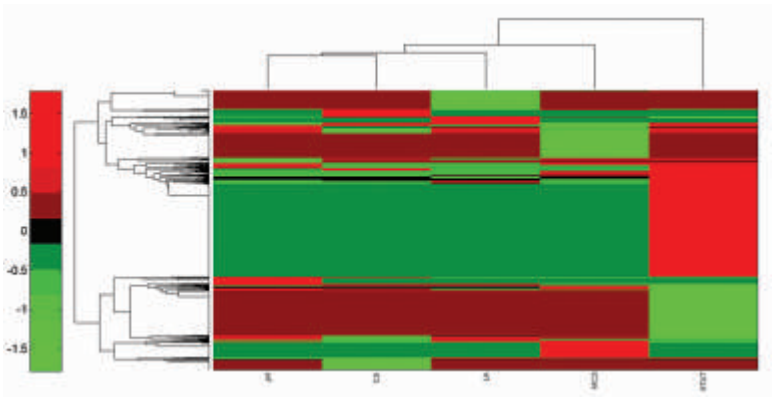

Fig. 2: Difference in expression ratios of 1175 genes. Columns represent stress conditions; Rows represent genes.

Future Work

1. 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 improving the outcome of this disease. 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. In general, MS based proteomic approaches begin with the collection of biological specimens representing two different physiological conditions. One of the samples is obtained from cancer patients and the other reference subjects, such as normal individuals, or patients with benign conditions. Proteins or peptides from plasma,

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Collaborator

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tissue or cell line samples are extracted and separated to reduce sample complexity. Protein profiles are obtained after separation and then compared against each other using statistical algorithms in order to detect differentially expressed proteins.

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, phosphorylation plays an important role in normal physiological states as well as aberrant signaling pathways in cancer and other diseases. It is important to identify and characterize the post-translational modifications (PTMs) of biomarkers that are involving in breast cancer. In this work, using recent development of mass spectrometry based quantitative proteomics technology, precise and absolute quantification of phosphorylation sites of biomarkers will also be investigated. 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. Identification and characterization of phosphoproteins involving in breast cancer using mass spectrometry based phosphoproteomic approaches.
3. 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-Dimensional Electrophoresis

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 evaluated different protein extraction protocols from published papers to obtain maximum coverage of the serum proteome by removing the highly abundant proteins. A combination of sonication, depletion, acetone precipitation and desalting offered reproducible separation of over 1000 spots, which was almost four times more as compared to the crude serum. This optimized protocol was used to process the patient and healthy control serum samples for further proteomic analysis. 2D profiling of serum samples was performed using 24 cm IPG strips

with pI values in the range of 4–7 to achieve a better resolution in protein separation and to eliminate the interference of basic immunoglobulins. After staining with GelCode Blue Safe Protein Stain, over 1000 protein spots were detected reproducibly in each gel by IMP7 software. Representative 2DE images of the serum profiles of healthy control and breast cancer are shown in Fig. 1.

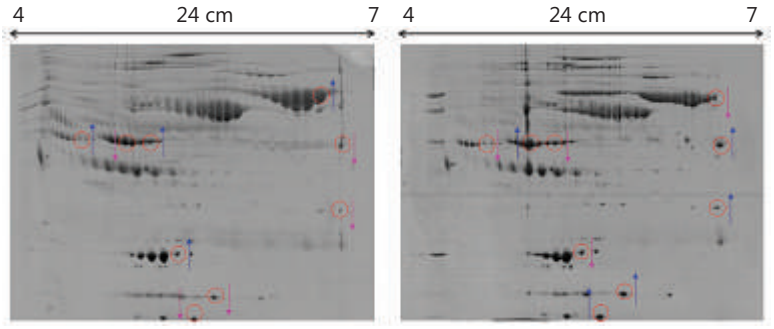

Fig. 1 : Representative 2D gels of serum from healthy control and breast cancer patient.

To identify differential protein expression between healthy subjects and breast cancer patients, individual gel images were grouped in two classes (control and test) and analyzed using IMP7 software. Descriptive statistics per class, histograms, overlapping measures, and statistical tests were performed to identify significant protein expression changes. Using the IMP7 software, 9 differentially expressed and statistically significant ($p \leq 0.05$) protein spots (5 up-regulated and 4 down-regulated) were identified (Fig. 2). 3D view representation of fold change for selected gel regions or areas around the selected spots are depicted in Fig. 2. Identification of 9 differentially expressed and statistically significant (Student's t-test; $p<0.05$) protein spots was established by MALDI-TOF/TOF mass spectrometry. The identified up-regulated proteins are Transthyretin, Haptoglobulin, Ig gamma-1 chain C region, Complement C4-B, Alpha-1-antitrypsin, and down-regulated proteins are apolipoprotein A-1, Vitamin D binding protein, Serotransferrin, Alpha-2-HS-glycoprotein. These identified proteins are interesting from a diagnostic point of view and could further be studied as potential serum biomarkers.

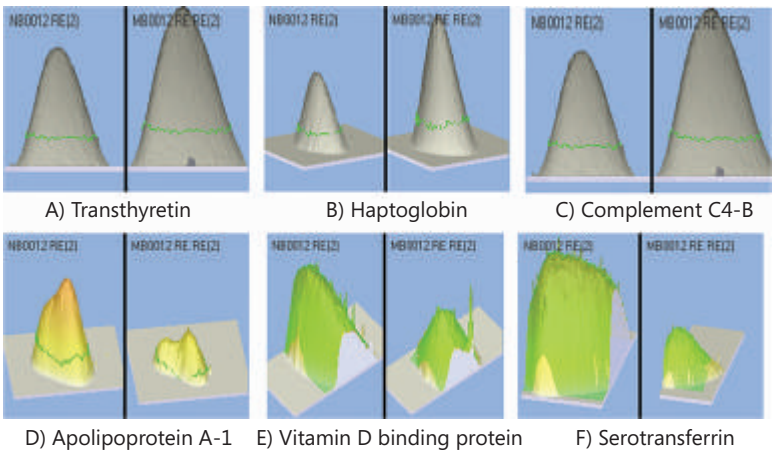

Fig. 2: The 3D images of statistically significant differentially expressed selected spots analyzed using IMP7 software in breast cancer (up-regulated spots shown above and down-regulated spots shown below).

Biomarker discovery in biological fluids greatly depends on the reproducibility, selectivity and sensitivity of the detection technique. Lack of reproducibility and insufficient

coverage of whole serum proteome are the major challenges for 2DE approach. In order to overcome the poor reproducibility of 2DE due to the substantial gel-to-gel variations, 2D-DIGE technique will be further used where two (or more) differentially labeled samples will be combined and co-separated on a same gel under identical conditions.

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

In order to circumvent the challenges associated with the breast tissue sample such as it consists of a number of various cells and extracellular matrix, non-protein elements, extremely high content of lipids and to obtain a comprehensive coverage of the tissue proteome on gels, multiple processing steps are required prior to the proteomic analysis. In this study, we evaluated different protein extraction protocols from published papers to obtain maximum coverage of the breast tissue proteome. For the first, we optimized the tissue extraction protocol using 4 types of buffers viz. RIPA buffer, T-PER buffer, Lysis A buffer and Lysis A+B buffer. In this optimization, T-PER extraction buffer showed best resolution of proteins as well as maximum number of spots. Secondly, we optimized the protocol with T-PER extraction followed by Trizol method with desalting and without desalting. 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 and normal tissue samples for further proteomic 2-dimensional electrophoresis analysis. 2D profiling of tissue samples was performed using 24 cm IPG strips with pI values in the range of 3–10 to achieve a better resolution in protein separation and maximum number of spots. After staining with GelCode Blue Safe Protein Stain, over 1000 protein spots were detected reproducibly in each gel by IMP7 software. So far, we have identified 3 differentially expressed and statistically significant (Student's t-test; $p<0.05$) protein spots by MALDI-TOF/TOF mass spectrometry. The identified up-regulated proteins are Actin, Annexin A2, Superoxide dismutase (Fig. 3).

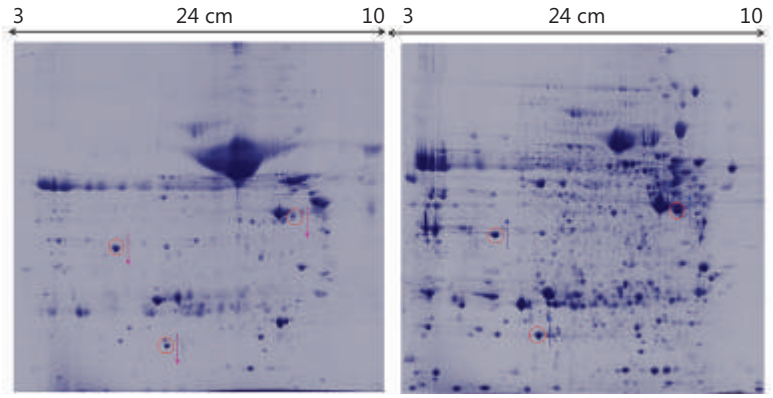

Fig. 3 : Representative 2D gels of breast cancer malignant tissue and normal tissue.

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

LC-MALDI-MS/MS has been used for the analysis of 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 (Control: NT0010 and Malignant: MT0010) 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 was 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 Pilot software. We identified 452 non-redundant 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. Representative spectra of down-regulated and up-regulated peptides are shown in Fig 4. The work is under progress.

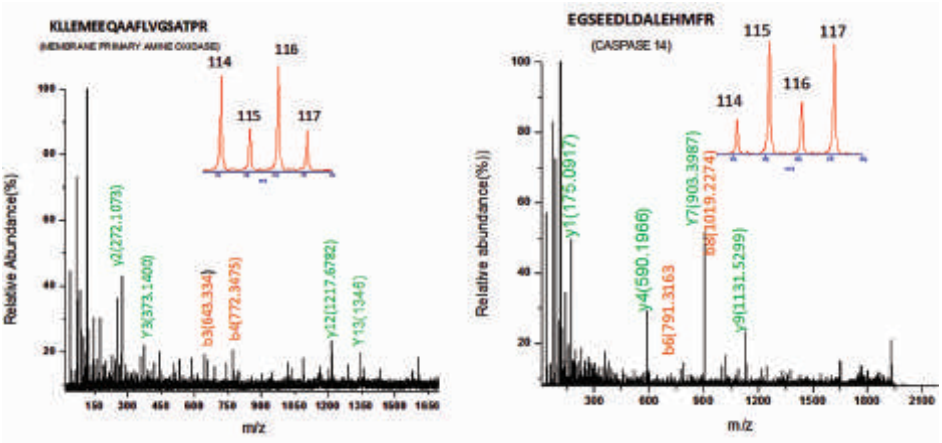

Fig. 4 : Representative spectra of down-regulated and up-regulated peptide/protein analyzed by iTRAQ LC-MALDI-MS/MS. The inset shows a zoom into the *m/z* region of the MS/MS spectrum where reporter ions appear.

Future work

1. Discovering novel potential biomarkers for breast cancer using 2-D DIGE approach.
2. Validating novel potential biomarkers using western blotting and MRM based LC-MS/MS.
3. Identification and characterization of phosphor proteins involving in breast cancer.
4. Subjecting novel potential biomarkers to functional pathway analysis using bioinformatics tools.

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

Background

In recent years, the disciplines of systems biology and synthetic biology together with bioinformatics have gained prominence as the embodiments of the future of biological science. Building circuits and studying their behavior in cells is a major goal of systems and synthetic biology in order to evolve a deeper understanding of biological design principles from the bottom up. Collectively, these developments enable the precise control of cellular state for systems studies and the discovery of novel parts, control strategies, and interactions for the design of robust synthetic function. As a consequence, formal methods and computer tools for the modeling and simulation of genetic regulatory networks (genetic circuits) will be indispensable. There are reports and evidences for the synthetic circuit in prokaryotes and eukaryotes like *E.coli* and yeast systems but to the best of our knowledge there are no reports in the literature suggesting the synthetic circuit construction for protozoan parasites. This serves as the first attempt made to use synthetic biology approach for the construction of genetic circuit of targeted enzyme IPCS for the protozoan parasite *Leishmania*. In spite of many therapeutic approaches exist for leishmaniasis, there is no cure for the disease. Severity of these lesions is widely distributed all over the world especially sub-tropical regions of the world. This report reviews formalisms that have been employed in mathematical biology and bioinformatics, systems and synthetic biology to describe genetic regulatory systems, in particular a directed graphs, Bayesian networks and Boolean network construction for the genetic circuit. In addition, it discusses how these formalisms have been used in the simulation of the behavior of actual regulatory systems. This aims for the construction of the genetic circuit of IPCS (Inositol Phosphoryl Ceramide Synthase) in *L. major*, its simulation to explore the dynamic behavior of the circuit and validation of genetic circuit constructed for IPCS. Genetic circuit was simulated by stochastic and deterministic approaches followed by circuit validation using qualitative and quantitative methods. Qualitative was done by using Boolean method and quantitative was done by using Bayesian method with the aid of ODE (Ordinary Differential Equations) of the constructed genetic circuit for IPCS. Quantitative analysis gave hints as to which parameters offer the

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Mrs. Virashree Jamdar, *Technician*

Collaborator

Dr. Bhaskar Saha, *NCCS, Pune*

best success in achieving a desired behavior that shows robustness to variations. Qualitative behavior of the circuit can be very informative in models having multiple steady states and showing switch-like or oscillatory behavior which was deciphered by using Boolean Network construction for the genetic circuit constructed. Based on the current approaches dealt, herein, we describe how different approaches of bioinformatics and systems biology could enable novel synthetic biology applications in *Leishmania*.

Aims and Objectives

1. To characterize dynamical properties of small gene networks (genetic circuit) displaying oscillations or bistability.
2. To construct artificial genetic systems displaying oscillations and bistability through repressilator and the Toggle switch models.
3. To investigate the various properties of built in genetic switch/circuit including the robustness to noise, signal amplifications and tuneable frequency which will ultimately deal with the synchronization ability of the constructed genetic circuit.

Work Achieved

Model Summary

Genetic toggle switch consists of IPCS_1 and IPCS_2. Repressilator consist of Lactose repressor (LacI, Tetracycline repressor (Tetr), Lamda repressor (Phage lamda). In the circuit (fig.1), IPCS_1 encodes for the repressor for the opposite gene IPCS_2 and IPCS_2 represses IPCS_1. In the circuit, it is considered that IPCS_1 and IPCS_2 mutually repress each other that reflect the bistable behavior of the genetic switch for IPCS (fig.1). Repressilator genes acts in cyclic manner where LacI represses Tetr, Tetr represses LamdaR and LamdaR in turn represses LacI, thus repressilator tends to produce oscillatory behavior. Coupling between genetic toggle switch and repressilator is developed by LacI

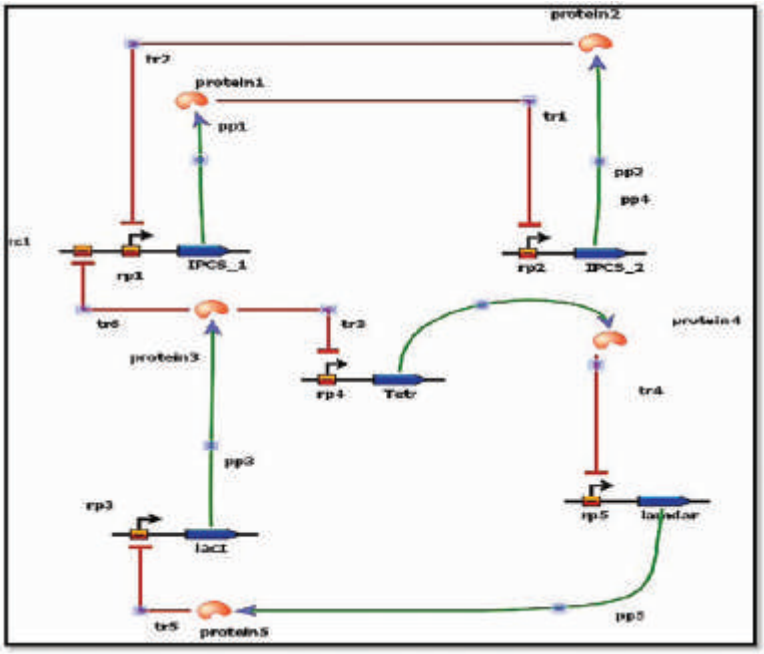


Fig. 1 : Genetic circuit for IPCS [Biomodels Database (MODEL 1208030000)]

and IPCS_1 where LacI regulates the IPCS_1 and represses it. Parameters considered for the construction of circuit are summarized in Table 1 below. Default parameters were

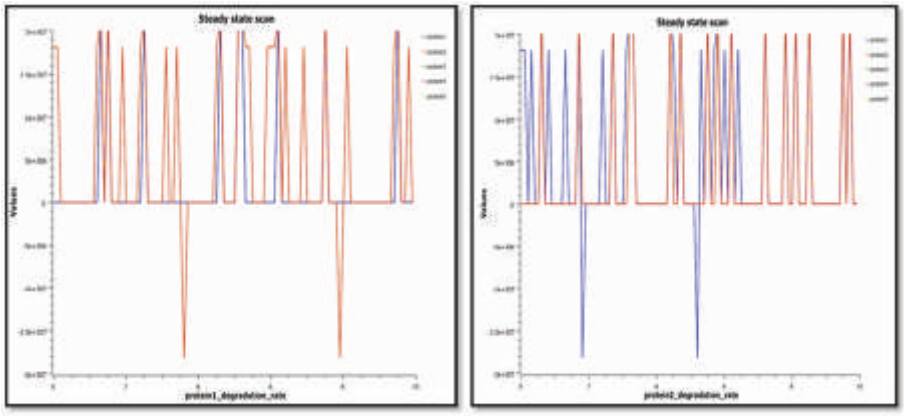
Table 1. Model summary of the genetic circuit

Module no	Promoter strength	Kd (Dissociation constant)	H(Hill/coefficient)	Degradation rate
IPCS_1	5.054	2.99	4.89	0.1835
IPCS_2	5	1.9	3.116	0.087
LacI	11.73	1.9	1.126	0.1867
Tetr	5	1.38	1.387	0.124
LamdaR	5	2.029	1.54	0.125

initially assigned to the genetic modules on the basis of connectivity of the genetic circuit. Initially the Kd values for the genes are set to 1 and hill's coefficient value was set to 2. Parameter scan was done for the steady state analysis and the parameters values considered for the model are summarized in table 1 below. The translation rates and transcription rates are set to 1 for all the genetic modules as the steady state analysis is reflected by degradation rates of the proteins.

Steady state Analysis: Steady state scan analysis for the degradation rates of all the proteins was carried out. Fig.2 represents degradation graphs for IPCS_1 and IPCS_2 proteins. Figure 2 (a) shows the bistable behavior of the genetic circuit. When the degradation of IPCS_1 protein decreases there is rise in the expression of the opposite

Fig. 2: Degradation Rates graphs



a) IPCS_1 degradation graph **b)** IPCS_2 degradation graph

Legends of the graphs

IPCS_1 :

IPCS_2 :

LacI :

Tetr :

LamdaR :

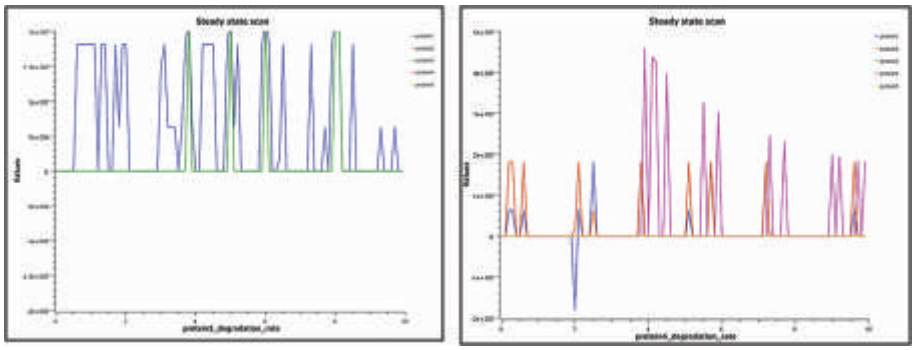
x= time points (in seconds)

y= values of degradation rate

toggle gene IPCS_2. There are two points of time when there is peak fall in IPCS_2 and there is switching of the states.This desired graph was obtained at 0.18 degradation rate of IPCS_1. Fig 2(b) shows degradation rate of IPCS_2.When there is increase in the level of IPCS_2 at particular interval of time, there is decrease in the level of IPCS_1.Two points of time in graph shows there is peak fall in the degradation of the IPCS_1 which shows that IPCS_2 represses IPCS_1 and flipping of the switch occurs. The desired behavior was obtained at 0.08 degradation rate of IPCS_2.

In figure 3 degradation graphs for LacI shows the coupling behavior between IPCS_1 and LacI. Intially there is no increase in the level of LacI as a result of which there is no coupling between LacI and IPCS_1. This results in the increase in the level of IPCS_1.At time interval of 4, there is an increase in the level of LacI and drop in the level of the IPCS_1.This change in the regulation of IPCS_1 with respect to LacI shows the coupling between genetic toggle switch and the repressilator. Fig 3 (b) is degradation rate graph for Tetr in which there is less oscillation between LamdaR and Tetr but it shows the repression of LamdaR.
Simulation : Simulation of the genetic circuit was performed for 100 time points using tau-leap stochastic simulation at differernt concentration. Results of fluctuations in the protein level with respect to change in dissociation constant(Kd) is dealt in Case 1 and Case 2.

Fig. 3: Degradation Rate graphs



a) LacI degradation graph

b) Tetr degradation graph

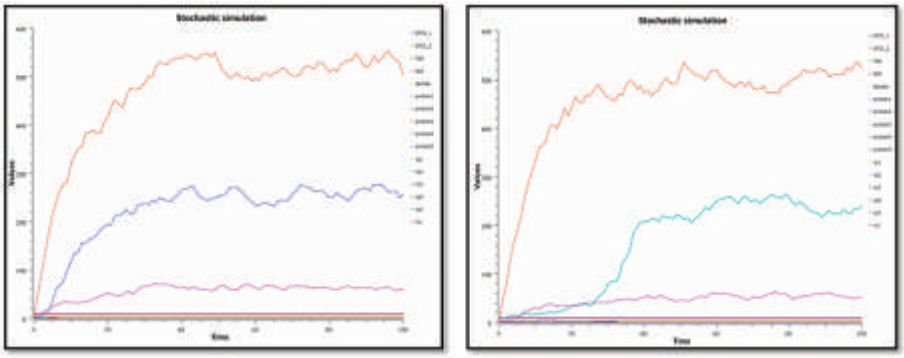
LacI : IPCS_1 : Tetr : LamdaR :

Case 1 : *Change in the Kd values of IPCS_1*

Case 2: Simulation for the change in kd values of IPCS_2 were also performed that caused fluctuation in the levels of IPCS_1

In Fig 4, there is a change in the proteins level with respect to change in the Kd values,when Kd=1,level of IPCS_1 is increased but when the value of Kd is rose to 1.01,there is switch in the level of IPCS_2 because the dissociation between IPCS_1 and regulatory binding site is increased, this leads the circuit to toggle and level of IPCS_2 is raised. Similar fluctuations in protein level is observed when Kd values at regular intervals

Fig. 4: Simulation results for IPCS_1 by change in the kd values of IPCS_1

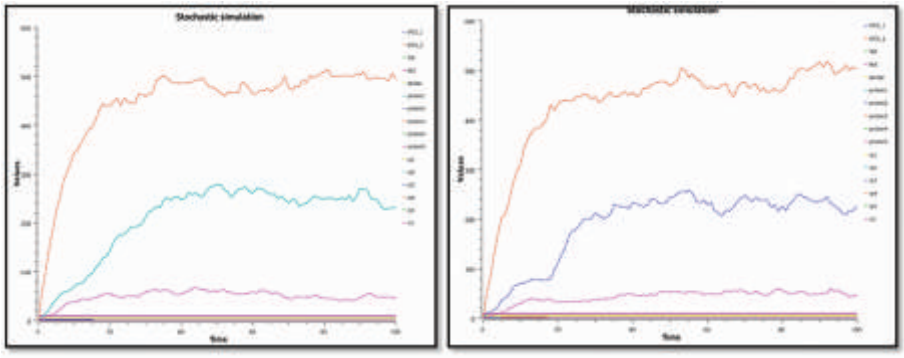


a) Kd=1 **b)** Kd=1.01

Legend : IPCS_1 : IPCS_2 : LacI : Tetr : LamdaR :

are altered. In Fig 5, when Kd=1.01, the level of IPCS_2 is increased but when the value of Kd rose to 1.01, a switch in the level of IPCS_1 is observed because the dissociation between IPCS_2 and repressor is increased. This leads the circuit to flip and level of IPCS_1 is raised. Similar perturbations in the levels of IPCS_1 and IPCS_2 is observed when there are changes in Kd at regular intervals. Above results shows the bistable behavior between IPCS_1 and IPCS_2 of the genetic ciruit and coupling between IPCS_1 of toggle switch and LacI of the repressilator is also supported.

Fig. 5: Simulations results for the circuit by change in the Kd values of IPCS_2



a) Kd=1.01 **b)** Kd=1.02

Legend : IPCS_1 : IPCS_2 : LacI : Tetr : LamdaR :

Digital circuit representation of the Synthetic Genetic Circuit : Digital circuits give an insight into the logic gates implemented in the circuit design for the functioning of the circuit. The circuit consists of two genes coding for two repressors. The two repressors mutually repress each other such that a high concentration of one protein inhibits the transcription of the other gene. Any signal that causes the breakdown of the existing repressor protein molecules or increases the transcription of the other repressor protein would cause the flip. A Toggle Switch has two IMPLIES gates connected in such a way that the output of one represses the other. Two IMPLIES gates mutually connected with each other where the ouptut signal of one IMPLIES gate is input signal for the opposite IMPLIES gate. The repressilator is constructed by integrating an odd number of NOT gates in a

circular fashion such that the output of the last gate is the input of the first one. Three NOT gates are connected in a cyclic manner in which the output signal of first gate is the input signal for the second gate,output signal for the second gate is input signal for the third gate and the output signal for the last gate is input signal for the first gate.

Truth table of logic gates for the genetic circuit of IPCS is represented in Table 2. Table 3 features the simplified representation of the truth table through Karnaugh map.

Table 2. Truth Table for the genetic circuit. Input A and B are the input signal of genetic toggle switch and C is the input signal of the repressilator.

Input(A)	Input (B)	Input (C)	output
0	0	0	1
0	0	1	0
0	1	0	1
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	0

Table 3. Karnaugh map of the truth table of the genetic circuit constructed

			AB		
		00	01	11	10
C	0	1	1	1	0
	1	0	0	0	0

The values of **C** are written on the rows of the Karnaugh map, whereas the values of **A** and **B** lie on its columns. By re-grouping or mapping input-output relations of the specification, it allows a conversion into a Boolean formula that is usually composed of several logical terms. More specifically, the method permits to derive two different descriptions of every digital circuit that, are called as POS (Product Of Sums) and SOP (Sum Of Products). Table 3 features the Karnaugh map representation of the IPCS circuit. Digital circuit implements the logic gates deciphered for the genetic circuit. Digital circuit shows the logic gate for the toggle switch and repressilator. The circuit scheme in figure 7 gives the logic design as to how the input signals affect the output of the circuit.

SOP= (|A*C) + (|A * |B) POS=|A* (|B+ C)

Fig 6. a) Logic gates for the genetic toggle switch b) Logic gates for the repressilator

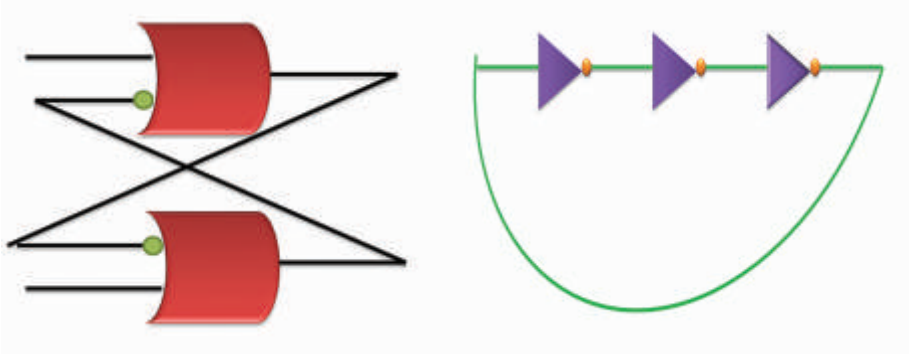
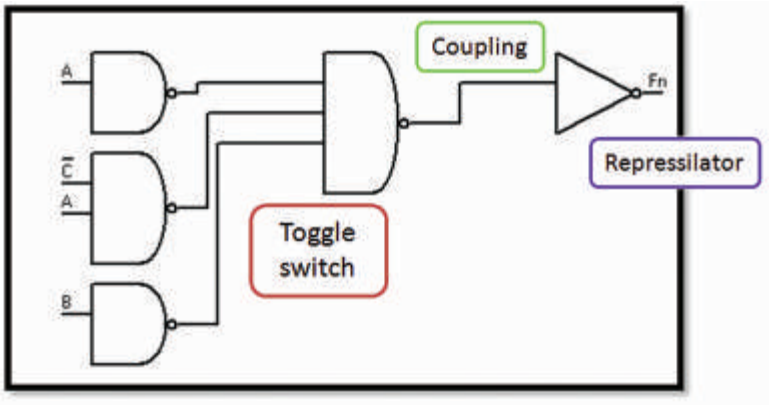


Fig. 7: Conversion of a truth table into a digital circuit via the Karnaugh map method.



Genetic circuit model validation

Model validation was done by using Qualitative and Quantitative approaches; qualitative by using Boolean method and quantitative by Bayesian method with the aid of ODE (Ordinary Differential Equations).

Network Inference

Gene regulatory network was constructed for the genetic circuit using Bioconductor packages and the model was simulated using COPASI. Time series data was generated and used for qualitative and quantitative network modeling. Circuit model is asymptotically stable hence Linear network construction was followed. Quantitative network modeling performed by GRENITS gave the probability of each regulator in the regulatory network circuit. Posterior probability was derived by using Monte Carlo Markov Simulation (MCMC) with default parameters. Simulation generated two markov chains that resulted into link probability of the network generated. Results includes probability matrix for each gene in the regulatory network. Analysis plot and convergence plot was generated for the network circuit. The Convergence plots contain plots associated to the adequate convergence of the Markov chains. This is crucial for further analysis as, if convergence has not been reached, the results are not trustworthy. Analysis plot contains the link probability of each regulator with other in the circuit. Network inference was made for 10 and 100 time series. Inferred network showed the regulatory mechanism in the circuit.

In Fig 8, inferred network displays the regulation between IPCS_1 and IPCS_2 which suggests the switching behavior of the toggle switch. Network inference is made at 10 seconds. As there is no regulation between LacI and IPCS_1 it shows no coupling between genetic toggle switch and repressilator which results into repression of IPCS_2 by IPCS_1 (regulation indicated by edge in the network) lack of coupling, IPCS_1 is ON and IPCS_2 is OFF. In Fig 9, as there is regulation between LacI and IPCS_1 it shows coupling between genetic toggle switch and repressilator which results into repression of IPCS_1 by LacI. Network inference was made at 100 seconds. Coupling between IPCS_1 and LacI leads to IPCS_1 in OFF state and IPCS_2 in ON state. Thus, the probability derived justifies the associated parameters that has accounted for the designability of the IPCS genetic circuit.

Fig. 8: At 10 seconds Inferred network of the genetic circuit for IPCS

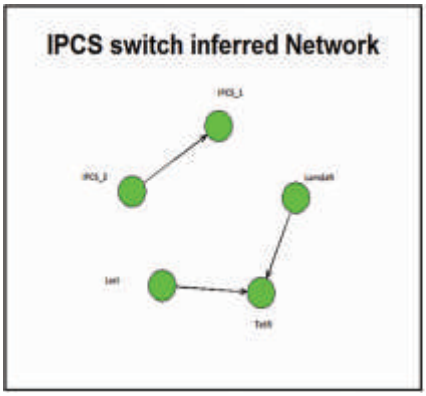


Fig. 9: Inferred network of the genetic for IPCS at 100 seconds

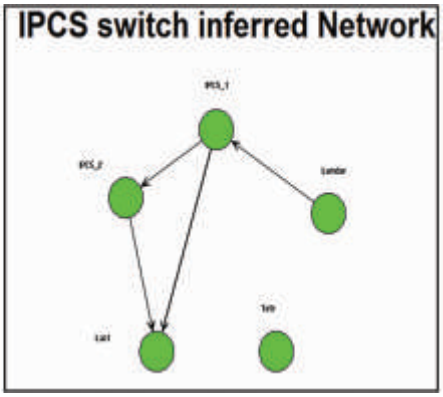


Fig. 10: Robustness plot of the genetic circuit

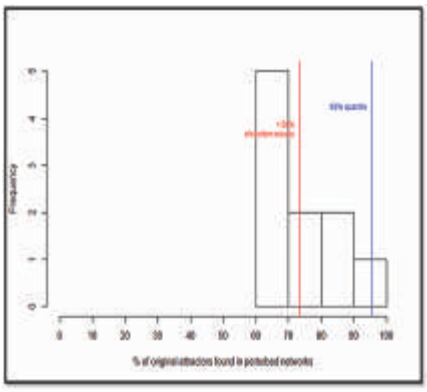
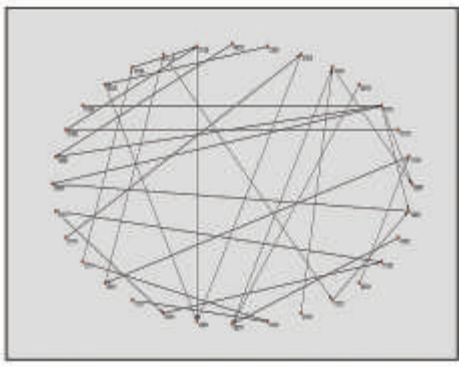


Fig. 11: State graph of the transition of states



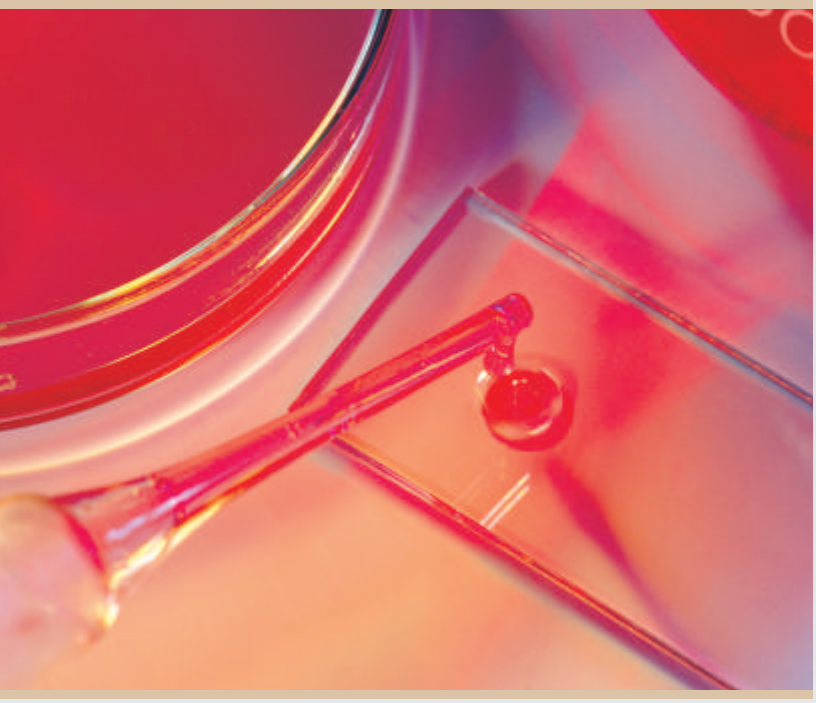
Robustness

Figure 10 represent the robustness plot for the genetic circuit and the percentage obtained is greater than 50 % which confirms the robustness of the constructed IPCS genetic circuit. Network for the genetic circuit was exported to Pajek for the visualization of the transition states in the network (Figure 11). Thus, the robustness plot of the circuit and the attractors obtained for the genetic circuit justified the design of the IPCS circuit. Genetic circuit designed for IPCS subjected to simulation and validated by both qualitative and quantitative approaches revealed the bistability of the genetic circuit and also the hypothesis laid down. The methodology incorporated specifies those regions where the probability of observing the desired behavior is appreciable. This may allow a more comparative assessment of different design proposals for other protozoan parasites, especially when dynamics are expected or desired to exhibit elements of stochasticity. The Bayesian and Boolean model approaches automatically strikes a balance of not only dealing between the systems' abilities to generate the desired behavior effectively but also robustly.

Future Work

1. To assess the selection and reliability of the constructed IPCS genetic circuit and development of a more complex hierarchial design platform wherein more diverse regulatory elements would be considered involving riboregulatory and signaling elements.
2. Further, library of models of new regulatory elements may help to analyse the evolvability of such models.

Research Report



Cancer Biology

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Identification of discrete molecular classes in ovarian cancer

Background

Epithelial to mesenchymal transition (EMT) - based signature profiling of tumors has recently identified invasive, aggressive disease in several cancers. The process involves cooperation with niche factors through autocrine-paracrine signaling and culminates in a transcriptional program driven by E12/47, Twist1, ZEB1, ZEB2, Snail1 (Snail), Snail2 (Slug), FOXC2, etc. (collectively termed as EMT-TFs). We have earlier shown a mechanistic association of EMT with ovarian cancer metastases. Further, since aggressive metastasis itself is a major cause of mortality in high-grade serous ovarian adenocarcinoma; we hypothesized that expression patterns of classical markers would be a useful tool to stratify tumors.

Aims and objectives

Molecular classification of high-grade serous ovarian cancer based on metastases-associated and metastases-independent gene-signature based clustering.

Work Achieved

Identification of a 39-gene metastases based signature in Serous ovarian carcinoma

In our previous work, we have identified the interaction of EMT-Transcription Factors (EMT-TFs) as a regulatory network in Serous ovarian carcinoma. Since these various EMT-TFs seemed to be capable of trans-repressing and trans-activating a common set of epithelial and mesenchymal markers, we extended these comparisons by determining the effects of *TWIST1*, *SNAI1* and *SNAI2* on global targets within cells. Through integrated ChIP-on-chip and expression analyses 99 common targets were thus identified. Exploring expression of known receptors and targets of LPA-LSL signaling pathways in the same datasets revealed a strong correlation between the receptors *S1PR1*, *S1PR4*, *LPAR4*, *LPAR5*, *LPAR6* and additionally identified 61 other coregulated genes. Hierarchial clustering, median absolute deviation (MAD) analyses and Pearson correlation between these 160 EMT-LPA-LSL genes led us to derive a core set of 39 genes involved either in EMT or LPA-LSL signaling. Applying this signature to the tumor samples led to resolution of 3 classes as seen in Fig.1.

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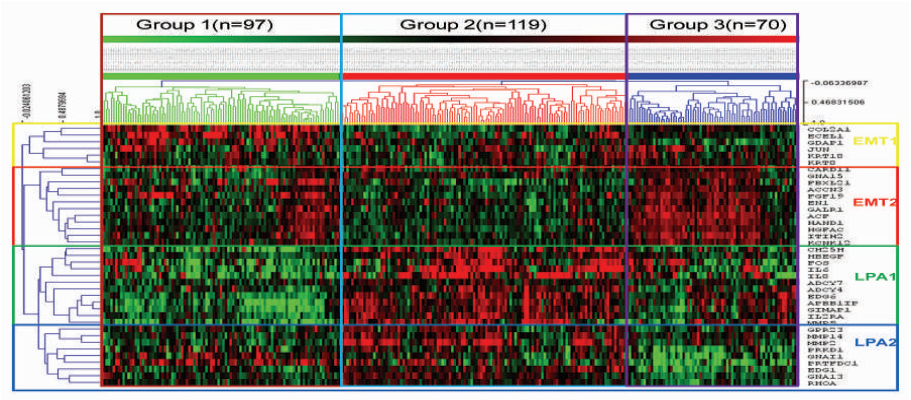
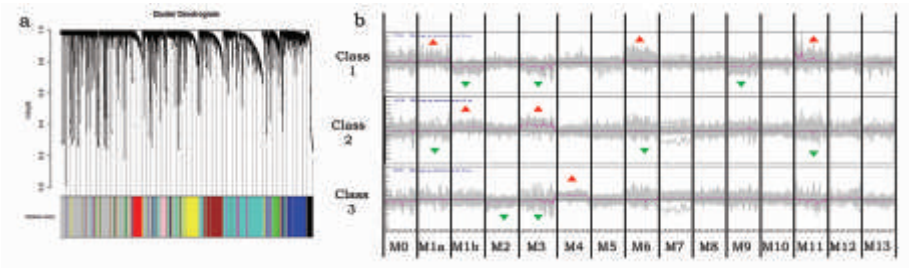
Fig.1: Heat map representing differential expression of 39 metastases-associated gene signature in the three tumour classes.



Weighted Correlation Network Analysis (WGCNA) also led to a similar resolution of three tumor classes

Concurrently, we evaluated a metastases-independent WGCNA classification scheme that determines Pearson correlation coefficients across all possible pairs of genes through generation of an adjacency matrix and organizes intensity data to identify unique functional modules of highly correlating genes. Applying such a matrix at power 6 (weighted correlation=correlation^{6}) generated 13 exclusive *Modules* (Fig. 2a), each comprising genes with high inter-connectedness and an expression pattern distinct from other module genes as revealed in the Topological Overlap Matrix (TOM) plot. K-means clustering of module genes led to the resolution of 3 tumor classes. Class comparison between the metastases- and WGCNA- based classifiers suggested similar sample distribution between classes. K-means clustering was applied to tumor samples common in both schemes to identify class-specific functional module associations. This resolved three robust classes viz. Class 1(n=79), Class2 (n=93) and Class 3 (n=55) besides identifying enrichment of modules M1a,M6,M11 in Class1; M1b,M3,M9 in Class2 and M2, M4 in Class3 respectively (Fig. 2b).

Fig. 2: **a.** Gene dendrogram and module colors. The color row underneath the dendrogram shows the module assignment determined by the Dynamic Tree Cut. **b.** k-mean clustering using Top 50 genes and 359 samples



Module gene-gene network interactions in two differential classes

The gene expression patterns of modules M1a-M6-M11 (Class1) and M1b-M3-M9 (Class2) tumors were observed to be anti-correlative (Fig. 3a). We hypothesized that they could instruct class-defining features. To further affirm overlap of gene sets and exclusive functionalities in the tumor classes, we evaluated node-node and node-interactor networks in ARACNe in the enriched modules. Within a tumor class, each module displayed strongly co-regulated nodes with their own hubs of influence but

lacked direct node-node interactions between modules. This is in agreement with the strong, exclusive intra-modular correlations suggested by WGCNA, while inter-modular connectivity is restricted to few hub components linking nodes across modules (Figs.3b), that could possibly identify regulatory feature(s).

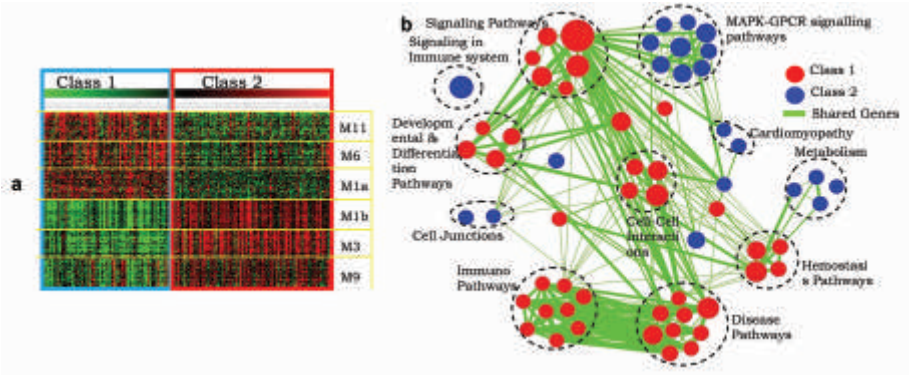


Fig. 3: **a.** Heatmap of enriched module genes in the two tumor classes. **b.** GSEA based functional identities for tumor classes visualized as an Enrichment Map Network; each network node of Class1 and Class2 (red and blue respectively) represents enriched pathway ($p<0.05$; FDR cut-off <0.25). The connecting green line between two pathways (nodes) indicates shared genes; line thickness represents number of shared genes.

In conclusion, we achieved gene expression pattern-based tumor classification through (i) metastases-associated gene-signature based clustering, and (ii) metastases-independent identification of correlating modules of genes with similar expression. This analysis suggests EMT as a differential function between the tumor groups; further, molecular and systems networks based resolution of class-specific cooperative tumor-associated biological functions holds the promise of novel and unexpected pathway associations.

Future Work

1. Development of experimental models representative of the two tumor classes.
2. *In vitro* and *in vivo* validation of specific functionalities of the tumor classes in these model systems.

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Cancer, Chemotherapy, and Metabolic disorders

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 important. 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 understanding the interrelationship between cancer and chemotherapeutic drugs but also will improve sensitivity and specificity of the treatment. Also, metabolic disorders diabetes and obesity alter the risk of developing variety of cancers, and deciphering common molecular events will facilitate in innovative avenues of pharmaceutical investigation.

Recent studies have suggested that diabetes and obesity alter the risk of developing variety of cancers, and the associations are biologically plausible. Study by American Cancer Society states that 14% of all cancer deaths in men and 20% of all cancer deaths in women from range of cancer types can be ascribed to excess body weight. Data from the National Health and Nutrition Examination Survey (NHANES) shows increased prevalence of overweight and obese adults in US population with a similar trend in children. Traditionally, cancers that are associated with obesity are breast, colon, pancreas, liver, cervix, stomach and kidney.

Over the past few years several reports have emerged highlighting a possible link between obesity and melanomas. Solar radiation has been identified as a principal causal factor for melanoma. However, the role of changing lifestyle patterns associated with obesity may also contribute to the development and progression of melanoma. Though, the mechanisms by which obesity facilitates carcinogenesis have been elucidated for several cancer types, epidemiological studies suggest that they may not be similar for all cancer types. Surprisingly, the effects of diet induced obesity on melanoma occurrence and progression are yet to be detailed. Moreover, the mechanisms or factors that

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contribute towards increased melanoma progression in obese condition remain inconclusive and poorly understood.

We explored the effects of diet induced obesity on occurrence and progression of melanoma in male C57BL/6J mice and probed into underlying mechanisms. We demonstrate that in high fat diet (HFD)-fed mice, melanoma progression was significantly increased in comparison to their counterparts fed on regular diet. Factors contributing to this phenomenon must involve increased expression and activation of survival molecules or oncogenes. Therefore, we investigated the status of several important signalling intermediates involved in tumorigenesis. Very interestingly, we observed that in tumors from HFD mice caveolin-1 (Cav-1) and fatty acid synthase (FASN) expression and pAkt levels were increased significantly which associate with rapid progression of melanoma.

Aims and objectives

1. To investigate the consequences of diet induced obesity on the progression of melanoma *in-vivo*.
2. To explore the molecular events *in-vitro* and their status in tumors.

Work achieved

High fat diet causes significant weight gain in wild type C57BL/6J mice and alters serum lipids and adipokines

Mice with normal and obese phenotype were generated by manipulating their caloric intake. Thirty male C57BL/6J mice 4-5 weeks of age were divided into two groups. Group one referred to as control group was fed on normal diet (ND), whereas group two mice fed on high fat diet supplemented with groundnut and dried coconut were termed as

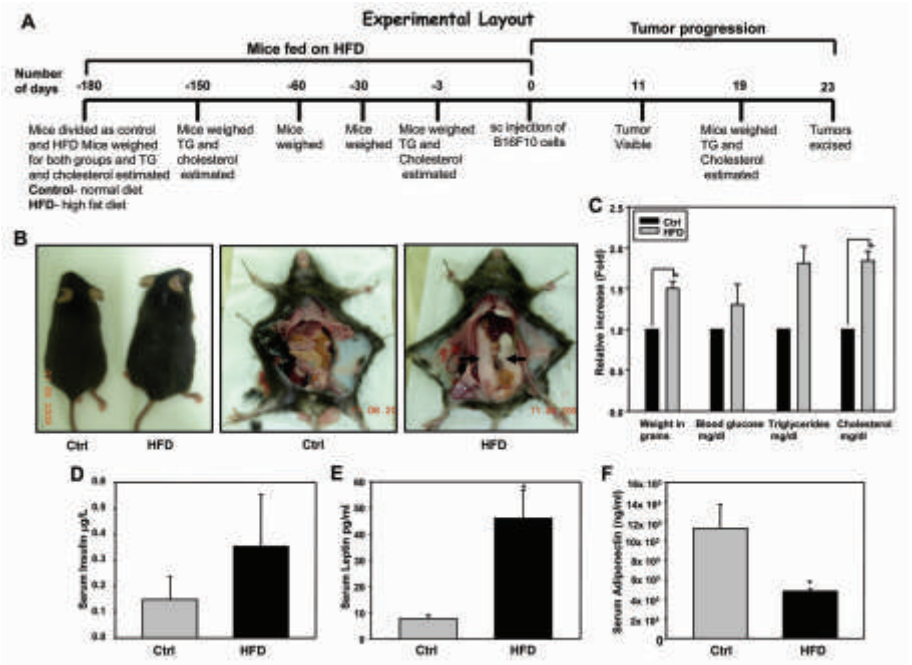


Fig.1: : A) Experimental layout for studying the effects of diet induced obesity on initiation and progression of melanoma. B) Pictures of control and HFD male C57BL/6J mice showing increase in body size and deposition of fat in the peritoneal cavity. Black arrows indicate the adipose tissue deposits. No fat deposition in control mice was detected. C) Relative fold levels of weight (g), blood glucose (mg/dl), triglycerides (mg/dl) and cholesterol (mg/dl) in control and HFD mice. Levels of D) serum insulin (µg/L), E) serum leptin (pg/µl) and F) serum adiponectin (ng/ml) in control and HFD fed mice. *P < 0.05

HFD mice. All animal experiments have been performed following the requirement of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and after obtaining permission of the Institute's Animal Care and Use Committee (IACUC). Mice maintained on control or HFD were monitored at regular intervals for weight gain as per the experimental plan given in Fig. 1A, After 25 weeks, mice from control and HFD group showed a significant difference in the body weight (Fig. 1B). Average weight of mice from control group was 18.9±2.8 as compared to 26.4±3.9 in HFD mice ($P<0.05$, Fig. 1C). Increased body weight was also accompanied by increase in serum triglyceride and a significant increase in total cholesterol ($P<0.05$). Serum triglycerides and cholesterol increased by approximately 1.8 fold and also, blood glucose levels increased 1.3 fold as compared to the control mice (Fig. 1C). Moreover, HFD mice showed a noticeable increase in serum insulin as well as leptin levels and decrease in serum adiponectin levels. Though, there was 2.4 fold increase in serum insulin levels in HFD mice, it was insignificant ($P= 0.098$) (Fig. 1D). Interestingly, in HFD mice, leptin levels increased significantly ($P<0.01$) by 6 fold and adiponectin levels decreased by 2.3 fold as compared to control mice ($P<0.05$) (Fig. 1E and 1F).

Melanoma progresses rapidly in mice fed on high fat diet

To test whether diet induced obesity has any impact on the growth and progression of melanoma, tumor growth rates were compared between control and HFD mice. When a weight difference of 8-10 g was attained between the control and HFD group, tumors were induced by subcutaneous (sc) injection of 2×10^5 B16F10 cells and mice were observed for the initiation and progression of melanoma tumors. Tumors in both control as well as HFD mice were detected after 11 days of injecting cells. Although, there was no noticeable difference in the time of initiation of tumor formation between the two groups, tumors in HFD mice progressed rapidly as compared to control mice (Fig. 2A). The average tumor weight and tumor volume in HFD mice was 3.52 g and 1920 mm^3 as compared to 0.92 g and 924 mm^3 respectively in control mice, (Fig. 2B, a and b). This

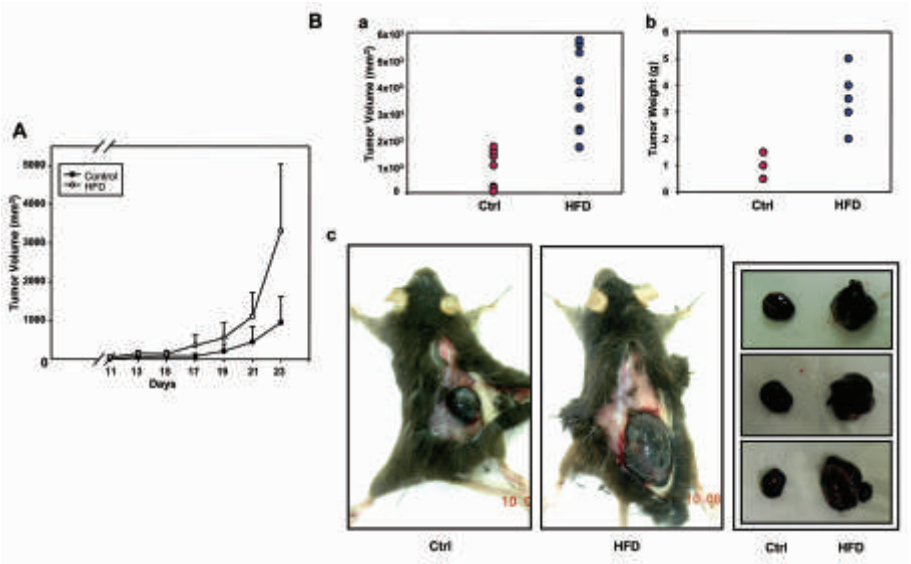


Fig. 2: **A)** Tumor initiation and progression in control and HFD mice. **B)** Final weight (a) and volume (b) of tumors in control and HFD mice at termination of the experiment. **C)** Representative pictures of tumors from control and HFD mice in situ and after being excised. For control and HFD groups n=8. *P< 0.05

clearly indicates that diet induced obesity favors progression of melanoma in male C57BL/6J mice (Fig. 2B c).

Increased caveolin-1 expression is involved in the proliferation of melanoma cells

In several tumor types the involvement of JAK-STAT pathways in obesity enhanced tumorigenesis has been reported. However, the underlying mechanisms still remain obscure for melanoma. We checked for the activation and expression of several molecules that have been implicated in increased tumor progression under obese conditions. No changes in activation status of STAT-3 and ERK were detected. Also, no changes in the expression levels of ß-catenin and PPAR-y were detected. Very interestingly we detected strikingly increased expression of caveolin-1 (Cav-1) as well as its Tyr-14 phosphorylated form in tumors from HFD mice (Fig. 3A). Also, we detected increased level of Cav-1 mRNA in tumors from HFD mice (Fig. 3A). Cav-1 belongs to a family of scaffolding proteins necessary for the formation of 50–100 nm plasma membrane invaginations, named caveolae. Cav-1 can either act as a tumor promoter or a tumor suppressor depending on the tumor type. To confirm the significance of Cav-1 in melanoma, B16F10 (murine) and A375 (human) cells were treated with increasing concentrations of methyl ß-cyclodextrin (MCD), an inhibitor of Cav-1. In both cell lines MCD in a dose dependent manner decreased cell survival and at 10 mM MCD concentration less than 20% cells survived (Fig. 3B, a and b). To ascertain that the increased Cav-1/pCav-1 levels are a direct consequence of altered serum composition in HFD mice, we cultured B16F10 cells in 5% serum obtained from control and HFD mice and performed immunofluorescence analysis for Cav-1 and other molecules. Increased levels of Cav-1 as well as its Tyr-14 phosphorylated form were detected in B16F10 cells cultured in serum from HFD mice (Fig. 3C). Significance of Cav-1 was re-verified by

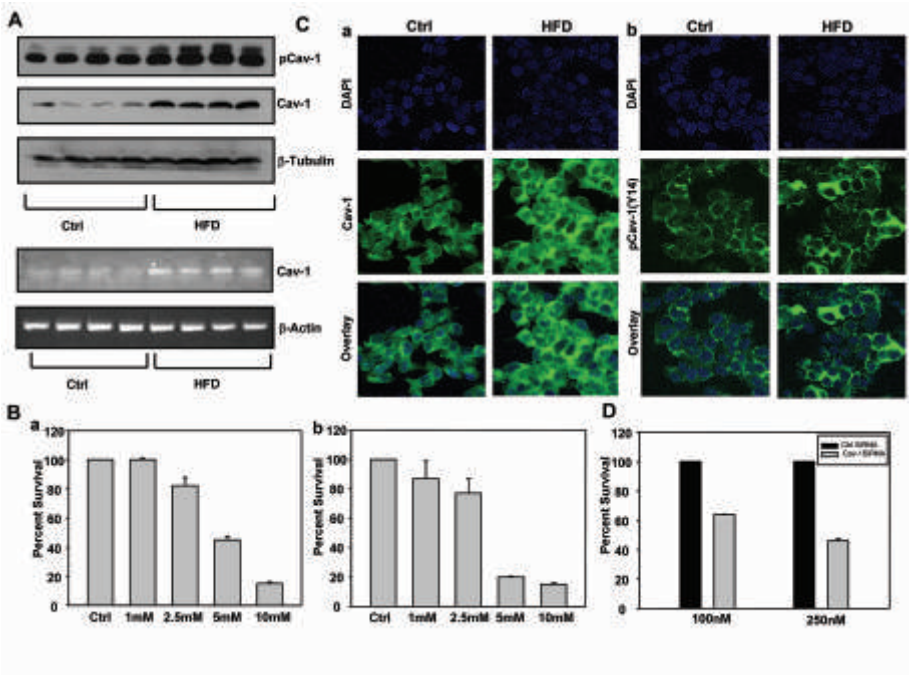


Fig. 3: A) Protein level and phosphorylation at tyrosine 14 residue of Cav-1 as well as Cav-1 mRNA level in representative tumor samples from control and HFD mice. B) Dose dependent reduction in cell survival in B16F10 (a) mouse melanoma cells and A375 (b) human melanoma cells in the presence of MCD, an agent known to cause Cav-1 depletion as assessed by MTT assay. C) Effect of serum from control and HFD mice on Cav-1 (a) and pCav-1(Y14) (b) expression in B16F10 melanoma cells. Cells were cultured in 5% mouse serum for 12 days and then immuno-stained for the expression of pCav and Cav-1. Cells show an increased expression of pCav and Cav-1 when cultured in serum from HFD mice. D) Inhibition of cell growth in the presence of Cav-1 specific SiRNA as assessed by MTT assay.

utilizing its specific SiRNA. Silencing Cav-1 by SiRNA inhibited growth of A375 cells (Fig. 3D). Put together, these results clearly indicate that over expression and activation of Cav-1 is likely to be involved in the growth of melanoma tumors in HFD mice.

Melanoma from HFD mice have increased expression of FASN which interacts with Cav-1

Fatty acid synthase (FASN) is an enzyme required for de novo synthesis of fatty acids in animals. It has been implicated in survival and proliferation of melanoma cells and we observed that its expression in melanoma tumors from HFD mice is enhanced in comparison to mice on normal diet (Fig. 4A). Also, FASN expression increased in B16F10 cells cultured in serum from HFD mice (Fig. 4B). To confirm the role of FASN in proliferation of melanoma, B16F10 and A375 cells were treated with inhibitors cerulenin and orlistat. In the presence of FASN inhibitors growth of cells was retarded in a dose dependent manner (Fig. 4C, a, b, c and d). In B16F10 cells cultured in 2% FBS in presence of orlistat cell growth was reduced by 60% (Fig. 4C c). Cerulenin in a dose dependent manner reduced the survival of B16F10 cells cultured in 10% FBS (Fig. 4C a). In A375 cells, both cerulenin and orlistat reduced the survival by approximately 50% (Fig. 4C, b and d). Silencing of FASN gene with the corresponding SiRNA decreased A375 cells survival by approximately 50% (Fig. 4D). Thus, both Cav-1 and FASN are overexpressed in melanoma tumors from HFD mice (Fig. 3A and 4A). Recently, it has been demonstrated that Cav-1 interacts with FASN in the membrane of prostate cancer cells. To explore this in B16F10 and A375 melanoma cells, immunoprecipitation was carried out using Cav-1 specific antibody. Cav-1 and FASN were detected in the immune complex by immunoblotting. IgG heavy chain served

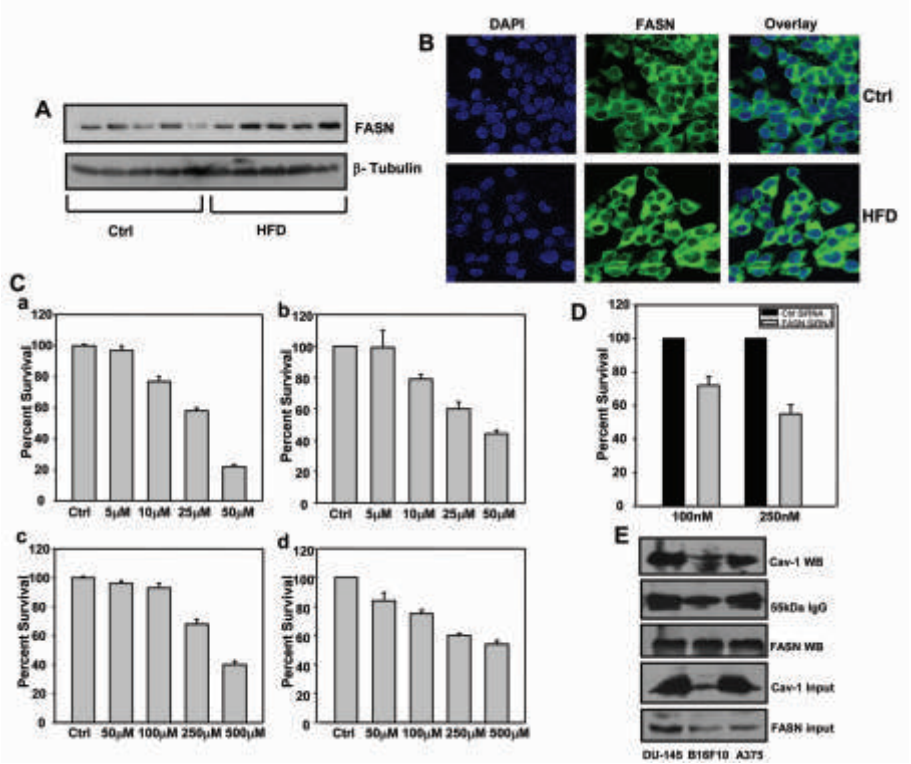
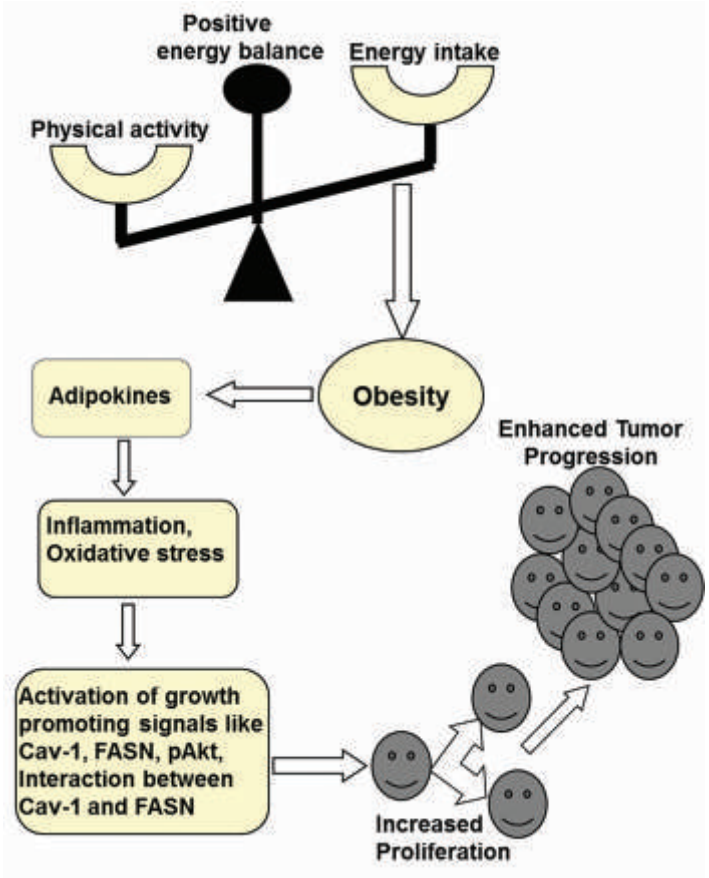


Fig. 4: **A)** Expression levels of FASN in representative tumor samples from control and HFD mice. **B)** Effect of serum from control and HFD mice on FASN expression in B16F10 melanoma cells. **C)** Dose-dependent reduction in cell growth in B16F10, mouse melanoma cells and A375, human melanoma cells in the presence of cerulenin and orlistat, known inhibitors of FASN, as assessed by MTT assay. (a) Dose-dependent inhibition of growth in B16F10 cells in the presence of cerulenin, (b) Dose-dependent inhibition of growth in A375 cells in the presence of cerulenin, (c) Dose-dependent inhibition of growth in B16F10 cells cultured in 2% fetal bovine serum in the presence of orlistat, (d) Dose-dependent inhibition of growth in A375 cells cultured in normal condition of 10% fetal bovine serum in the presence of orlistat **D)** Inhibition of growth in the presence of FASN specific SiRNA as assessed by MTT assay. **E)** Co-immunoprecipitation of Cav-1 and FASN in B16F10 mouse melanoma cells and A375, human melanoma cells. DU145, human prostate cancer cells were used as a positive control. Immunoprecipitation was carried out using Cav-1 specific antibody. Cav-1 and FASN were detected in the immune complex by immunoblotting. IgG heavy chain served as loading control.

as loading control. As shown in Fig. 4E, we detected FASN in the immunocomplex suggesting a functional interaction between Cav-1 and FASN. To the best of our knowledge, this is the first report demonstrating direct interaction between Cav-1 and FASN in melanoma cells.

To conclude, in the present study we provide firm evidence that diet induced obesity causes increased melanoma progression in C57BL/6J mice (Fig. 5). We demonstrate that obesity induced metabolic alterations directly affect the transcriptional regulation of Cav-1, protein expression of Cav-1 and FASN, both being important mediators of proliferation in melanoma cells. We for the first time, provide evidence that Cav-1 and FASN interact in melanoma cells. Also, FASN and Cav-1 coordinately regulate each other which have an impact on activation status of Akt and growth of melanoma cells. Thus, further investigation of molecular interplay between Cav-1, FASN and Akt activation as well as therapeutic intervention of these pathways may be helpful in developing newer approaches for preventing rapid progression of melanoma in obese individuals.



Graphical representation of the molecular changes caused by diet-induced obesity:
When energy intake outweighs its consumption, obesity develops. This causes secretion of adipokines and changes in the inflammatory status, thereby inducing oxidative stress. Obesity-related alterations bring about an increased expression of FASN and Cav-1, and an increased activation of Akt, which together contributes to enhanced progression of melanoma.

Future work

1. Effect of adipose secreted factors on cancer cells.
2. The status of tumor cell growth by regulating obese phenotype.

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Understanding the F-box proteins functioning as tumor suppressors

Background

Normal cells maintain their homeostasis through precise co-ordination of transcription, translational, post translational modifications and selected protein's degradation. Cell cycle progression is regulated by the sequential activation and inactivation of cyclins, cyclin dependent kinases and cyclin dependent kinase inhibitors. In eukaryotes, most of the cell cycle dependent proteins and signalling proteins are degraded by proteasome mediated pathway. Proteasome-mediated degradation of proteins involves polyubiquitylation by ubiquitin ligases. Ubiquitin ligases are classified into two main classes based on their structural domains: the RING-finger domain proteins and HECT-domain proteins. SCF (SKP1-CUL1-F-box) is RING-finger type E3 ubiquitin ligase. The SCF E3 ubiquitin ligase is the largest and the best characterized RING-finger type E3 ubiquitin ligase. F-box protein is the only variable component of SCF complex. Phosphorylated substrates are essential for the SCF-mediated proteasomal degradation. Phosphorylated substrates are recognized by F-box protein and determine the substrate specificity for SCF complex.

The members of F-box protein family have a conserved 50-60 amino acid motif known as F-box motif, at their N-terminal region. They have been further classified into 3 classes based on the substrate interaction motif located in their C-terminal region. F-box proteins containing leucine rich repeats (LLRs) are known as FBXL, those containing WD repeats as FBXW, and those lacking all known protein interaction domains known as FBXO. In addition to proteasomal protein degradation, F-box proteins have also been associated with cellular functions such as signal transduction and regulation of the cell cycle progression. In the human genome, 69 F-box proteins have been identified. However functions and targets of most of the F-box proteins are yet to be explored. Recent studies show that SCF E3 ubiquitin ligases could be novel targets for cancer chemo-therapeutics and many proteasome inhibiting drugs are under clinical trials.

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Previous studies have shown that F-box proteins play an important role in the regulation of cell cycle progression, signal transduction and cancer progression (Craig KL, Tyers M, 1999, *Prog. Biophys. Mol. Biol.* 72: 299–328, Nakayama KI and Nakayama K, 2006, *Nature Review Cancer,* 6: 369-381, Frescas D and Pagano M, 2008, *Nature Review Cancer,* 8: 438-449). Till today, only 9 F-box proteins have been characterized in detail and they can function either as oncoprotein or tumor suppressor or both (Nakayama KI and Nakayama K, 2006, *Nature Review Cancer,* 6: 369-381). We do not know the function of the majority of F-box proteins, their cellular targets, and their expression profiles in various cancers. We aim to perform a SCF E3 ubiquitin ligase screen to identify the F-box proteins functioning as tumor suppressors.

Kumar *et al.* (2005) have shown that FBXO31 is a putative tumor suppressor and arrests the cells at G1 phase of the cell cycle. Recently, we showed that FBXO31 functions as a tumor suppressor by regulating cyclin D1 *via* proteasomal degradation pathway (Santra *et al.* Nature 2009). Further, we have established that FBXO31 is an important checkpoint protein during genotoxic stress. It is stabilized by phosphorylation at serine 278 position mediated by ATM upon genotoxic stress. However, we do not know whether FBXO31 has any effect on other phases of cell cycle and what are its other targets.

Our long term goal is to identify F-box proteins functioning as tumor suppressors and identification of their substrate(s) to elucidate their molecular mechanism of tumor suppression. In order to identify new F-box proteins functioning as tumor suppressors, we will over express the candidate F-box genes in cancer cell lines and follow their effect on growth profile (*in vitro*) and confirm their tumor suppressive activity in nude mice (*in vivo*). Promising candidate genes will be studied in detail to understand their mechanism of action. This study will be helpful to understand cancer progression in a better way and may lead to the identification of novel targets for cancer therapy. We are also interested in investigating the role of FBXO31 in cell cycle regulation.

Aims and Objectives

1. Elucidation of the role of FBXO31 in the cell cycle regulation.
2. SCF E3 ubiquitin ligase screen to identify F-box proteins functioning as tumor suppressor.
3. Elucidation of tumor suppressive function of candidate F-box proteins.

Research Findings

Elucidation of the role of FBXO31 in the cell cycle regulation

Our previous study showed that FBXO31 arrests the cell cycle at G1 phase via proteasomal degradation of cyclin D1 (Nature 2009). It is the only F-box protein known to be stabilized under various genotoxic stress conditions and mediates rapid cyclin D1 degradation. Kinetics of rapid down regulation of cyclin D1 completely matches with the stabilization kinetics of FBXO31 under various genotoxic stresses, suggesting that FBXO31 is an important checkpoint regulator in DNA damage pathway. Previous studies

have established that FBXO31 has a role in G1 phase regulation. In the present study, we aim to investigate the role of FBXO31 in various other stages of the cell cycle. We have used HEK293T cells for this study. We generated the scrambled shRNA (NS) and FBXO31 knockdown 293T cells (FBXO31KD). Both NS and FBXO31KD cells were synchronized by treating with 0.5 mM hydroxy urea for 22 hours. Cells were collected at different time intervals after release from hydroxy urea synchronization. FACS analysis suggested that FBXO31KD cells follow relatively slower kinetics in the progression from S-phase (after 3 hours of hydroxy urea release) to G2/M and G2/M to G1 (after 9 hours of hydroxyl urea release) as compared to NS cells (Figure 1). We repeated the same experiment in COS7 cell line and similar results were observed suggesting that FBXO31KD cells are defective in the cell cycle progression. This may be due to alteration in the activation of cell cycle regulatory cyclins, cyclin dependent kinases (CDK) and cyclin dependent kinase inhibitory proteins (CDKi).

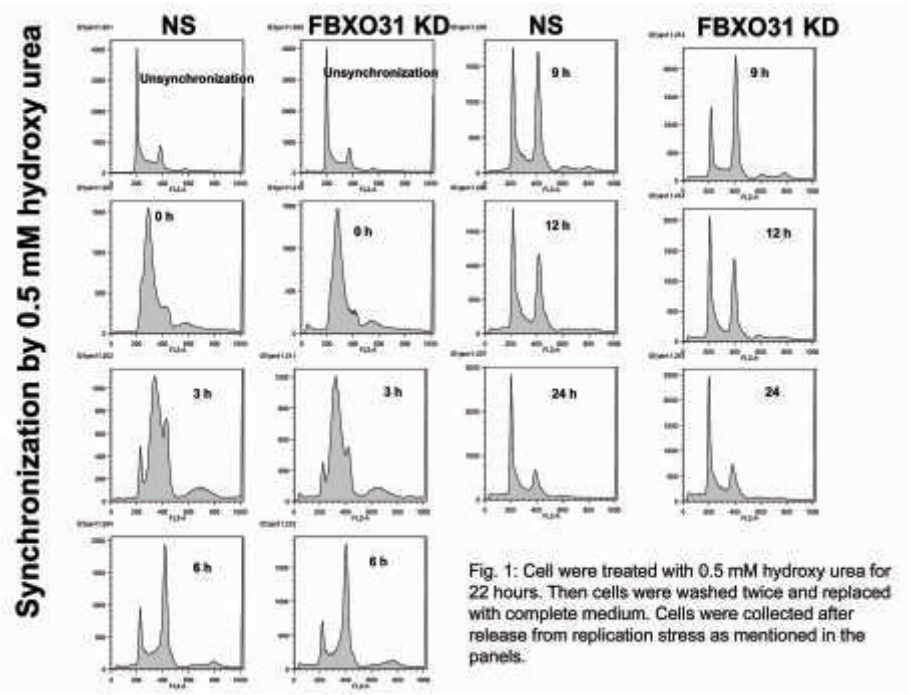
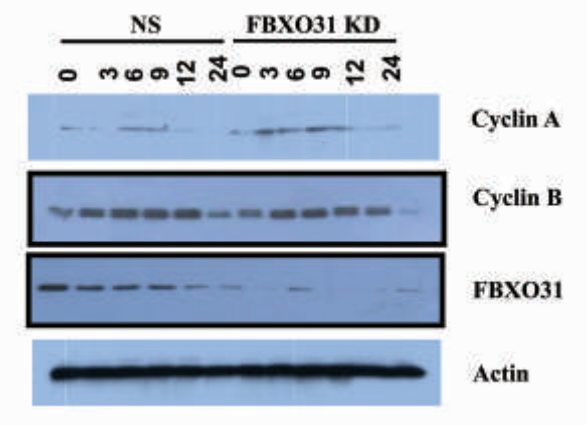


Fig. 1: Cells were treated with 0.5 mM hydroxy urea for 22 hours. Then cells were washed twice and replaced with complete medium. Cells were collected after release from replication stress as mentioned in the panels.

To understand why FBXO31KD cells are defective in cell cycle progression, we measured the levels of cyclins involved in different stages of cell cycle by immunoblotting. Results showed that cyclin A turnover in FBXO31KD cells are different from that of NS control (Figure 2). Cyclin A is stabilized in FBXO31KD cells as compared to NS cells. However, we did not see any difference in the transcription level of cyclin A in the FBXO31 KD cells as compared to NS 293T cells. These results suggest that FBXO31 may be involved in cyclin A turnover. Similar results were also observed in COS7 cells. Further, we will check the level of CDKs and CDKis. To understand the role of FBXO31 in cyclin A turnover, we will perform co-immunoprecipitation, immunofluorescence and proteasomal degradation.

Fig. 2: Cellular level of G2/M specific cyclins. Cells were synchronized by 0.5 mM hydroxy urea for 22 hours. Then cells are released and collected at mentioned time points.

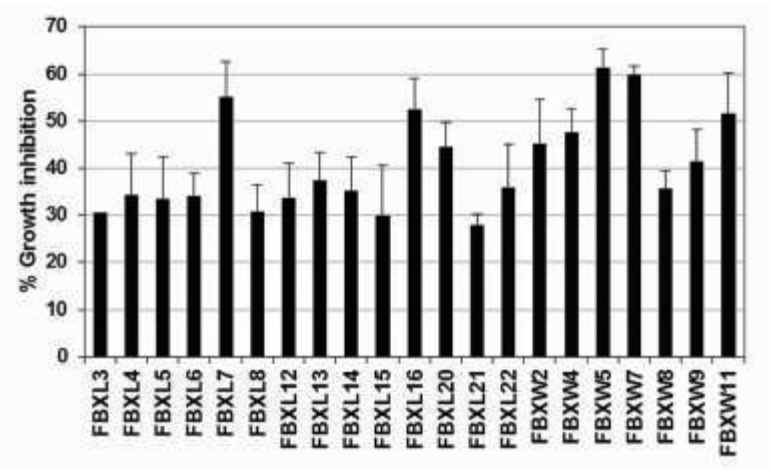


SCF E3 ubiquitin ligase screen to identify F-box proteins functioning as tumor suppressor

In the human genome, 69 F-box proteins have been identified. However, only 9 F-box proteins have been studied in detail. But most of the other F-box proteins are still to be characterized. Previous studies showed that F-box proteins can function as tumor suppressor, oncogene or both. For example, SKP2 functions as an oncogene, FBXW7 functions as a tumor suppressor while FBXW1 functions as both oncogene as well as tumor suppressor. We are interested in identifying the F-box proteins functioning as tumor suppressors.

We ectopically expressed 24 F-box genes in the MCF7 breast cancer cell line. After 48 hours of transfection, we measured the cell viability using MTT assay (Figure 3). Vector transfected cells were considered as a control. Results showed that many F-box genes can function as anti-proliferative genes (Figure 3). F-box protein FBXL7, FBXL16, FBXW5, FBXW7 and FBXW11 suppressed the growth of MCF7 cells >50 %. Interestingly, FBXW7 is known to be a tumor suppressor. We are in the process of further validation of this data using alamarblue assay and colony formation assay.

Fig. 3: Growth suppression activity of F-box genes. We transfected the F-box genes in MCF7 cells. Cell viability was measured using MTT assay after 48 hours of transfection.



Future Work

1. Detailed studies will be done in the coming year to understand how and why cyclin A turnover is altered in FBXO31KD cells. Mass spectrometry will be performed for identification of the interacting partner(s) of FBXO31.
2. Screening and validation of those F-box genes which can function as tumor suppressors.
3. Elucidation of the molecular mechanism of tumor suppressive activity of validated F-box gene(s).

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

Background

Glioblastoma multiforme is the most common, aggressive and lethal primary malignant brain tumor. Malignant gliomas display extensive infiltration into the surrounding brain tissue making them resistant to the existing therapeutic strategies. Inflammatory cytokines such as TNF-α, Interleukin-1, VEGF and other chemokines secreted by monocytes/ macrophages in the vicinity of the tumor regulate invasion and angiogenesis aided by the secretion of high levels of proteolytic enzymes such as Matrix Metalloproteinases (MMPs). TNF-α is an activator of the major survival pathways–NF-κB and PI3K/Akt. The mammalian Target of Rapamycin (mTOR) functions downstream of PI3K/Akt pathway by forming two distinct complexes - mTORC1 and mTORC2 - to regulate cell growth, proliferation and survival. Importantly, activation of Akt/mTOR pathway confers high resistance to varied range of cancer therapy and is poor prognostic factor for several cancers. The mTORC1 formed by mTOR with Raptor (regulatory associated protein of mTOR), mLST8 and PRAS40 regulates translation through its targets S6Kinase, 4E-BP1 and eIF-4E. Raptor has been proposed as scaffolding protein to recruit substrates for mTOR and is indispensable for phosphorylation of S6K and 4E-BP1. Treatment with Rapamycin reduces Raptor's binding to mTOR resulting in inhibition of mTORC1 activity and its functions. The mTORC2 formed by the association of mTOR with Rictor (Rapamycin-insensitive companion of mTOR), mLST8 and mSIN1 is important in the regulation of actin cytoskeleton and phosphorylation of Akt (PKB) at the S473 residue. Physical structure and the physiological functions of mTORC2 are distinct from mTORC1. Recent reports provide evidence that prolonged exposure to Rapamycin affects the phosphorylation of Akt (PKB) at S473 residue and thus affects mTORC2 which was earlier considered Rapamycin-insensitive.

Aims and Objectives

1. Identification of the downstream targets of Akt and NF-κB pathways in TNF-α-mediated responses in gliomas.
2. To understand the mechanism(s) that contributes to invasiveness in these tumors with focus on the role of mTORC2 component –Rictor.

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3. To study crosstalk between the two m-TOR complexes (m-TORC1 and m-TORC2) pathways using inhibitors in context with tumor micro-environmental factors such as TNFα, growth factors and MMPs.

Research findings

Recent work from our group demonstrated that loss of expression of Rictor by small interfering RNA (siRNA) induced MMP-9 activity and enhanced invasiveness via activation of Raf-1/MEK/ERK pathway in human glioma cells and primary cultures. Since ERK functions up-stream of NF-κB in many cell systems and our earlier studies showed that silencing of Rictor upregulated MMP-9 and p21 that are target genes of NF-κB, the current study aimed to explore the role of Rictor in NF-κB regulation. The results showed that knockdown of Rictor by specific siRNA resulted in enhanced expression of nuclear p65 and increase in DNA-binding ability of NF-κB in human glioma cell lines (Fig.1) and primary cultures derived from GBM tumors. The activation was associated with upregulation of NF-κB target genes that play a crucial role in adhesion, invasion and

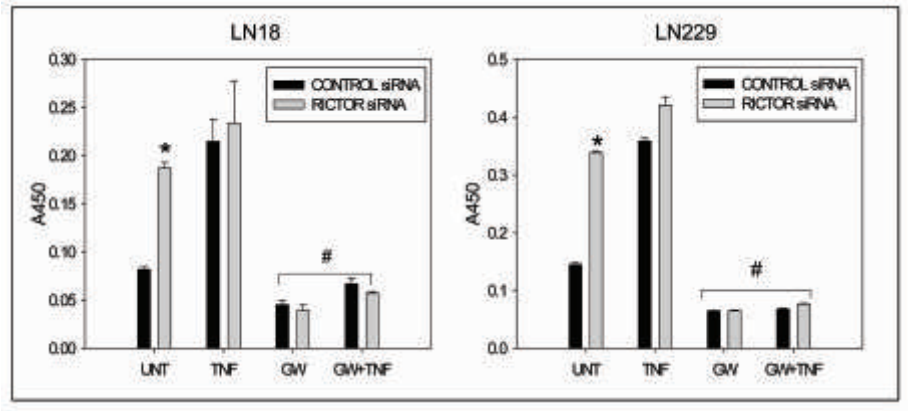

Fig. 1: Upregulation of DNA-binding of NF-κB mediated by Raf-1/ERK pathway. LN18 and LN229 cells were transfected with control or Rictor siRNA followed by treatment with TNF-a (10ng/ml) in the presence or absence of GW5074 (10mM) for 12h. The nuclear extracts were assayed for NF-κB activation by No Shift transcription factor assay. Data is represented as mean absorbance values +/- SEM of three similar experiments performed in triplicates. *p<0.05 control vs Rictor siRNA transfected, #p<0.05 in absence vs presence of GW5074.

inflammation (Fig.2). While human glioma cell lines, LN-18 and LN-229 predominantly expressed IKKβ, primary cultures derived from glioma tumor displayed comparable levels of IKKα and IKKβ. Interestingly, rictor ablation enhanced the levels of IKKβ but not IKKα in glioma cell lines and primary cultures. In rictor-silenced cells, siRNA-mediated

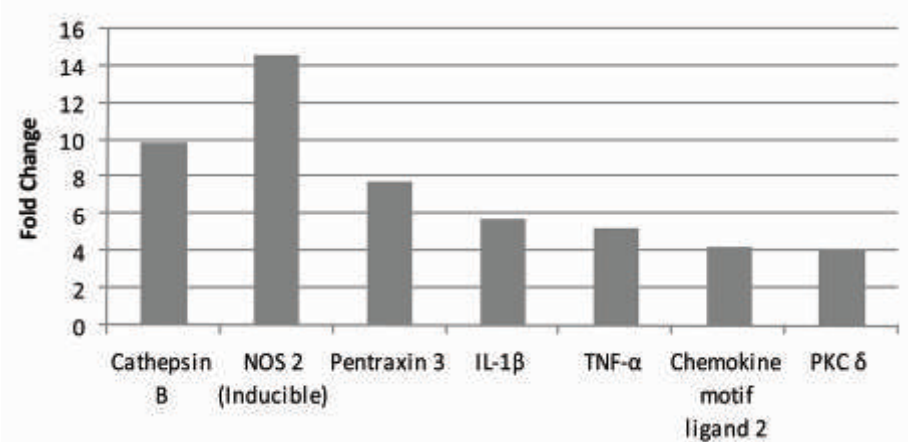

Fig. 2: NF-κB targets analyzed for gene expression studies in cells transfected with Rictor siRNA by qPCR. Fold change is with reference to control transfected cells.

knockdown of IKKβ reduced pp65 levels indicating that regulation of IKKβ was crucial for activation of NF-κB. The findings also indicated that presence or absence of Rictor had no consequence on TNF-α-induced NF-κB. Mechanistically, inhibitors of Raf-1 kinase (GW5074) or MEK/ERK (PD98059 and U0126) suppressed IKKβ expression, blocked translocation of p65 into the nucleus (Fig.3) and its DNA-binding activity suggesting the involvement of Raf-1 kinase mediated via the MEK/ERK pathway in NF-κB activation. We demonstrate here that Rictor negatively regulates p65 activation and transcription of NF-κB target genes important in invasion by enhancing expression of IKKβ. The data also reveal the involvement of Raf-1/ERK pathway in the regulation of IKK β and NF-κB activation.

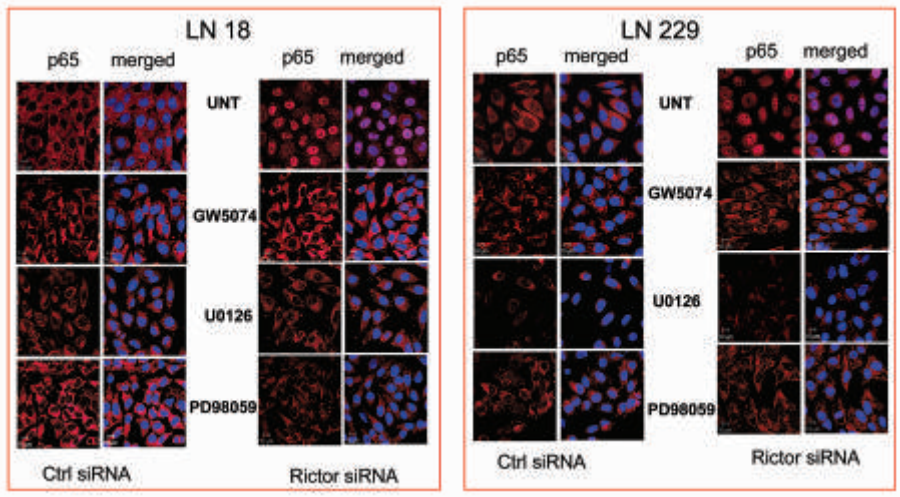

Fig. 3: Activation of NF-κB in Rictor-knockdown cells through Raf-1/MEK/ERK pathway. Human glioma cells transfected withcontrol and Rictor siRNA were treated with GW5074 (10mM), U0126 (20mM) or PD98059 (40mM) for 12h and analyzed for expression of Rictor and p65 by immunofluorescence using Cy-3 conjugated secondary antibodies and DAPI as nuclear stain.

Conclusions: The findings suggest an undescribed role for Rictor in regulating invasion by MMP-9 activty via Raf 1-MEK-ERK involving NFκB signaling in gliomas.

Future work

Upregulation of mTORC1 activity provides the cells with growth advantages in multiple biological processes including proliferation and survival. While it was earlier believed to be distinct from mTORC2 signaling, recently it was shown that prolonged exposure to Rapamycin affects the phosphorylation of Akt (PKB) at S473 residue and thus affects mTORC2 which was considered Rapamycin-insensitive. Based on the data from mTORC2 signaling, the group aims to study crosstalk between the m-TORC1 and m-TORC2 pathways in context with tumor micro-environmental factors such as TNFα, growth factors and MMPs.

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

Background

RNA molecules which do not encode for any proteins are termed as non-coding RNAs (NCR). They play pivotal roles in normal growth regulation and homeostasis. We have identified in our lab, a pair of non-coding, over-lapping transcripts- Ginir (sense transcript) and Giniras (anti-sense transcript) which do not have a protein coding potential. Studies in our lab have demonstrated roles of Ginir in cell transformation, wherein over-expression of Ginir in a range of diploid fibroblast cell-lines have led to transformation *in vitro* and induction of tumors *in vivo*. The potential of Ginir to form tumors is an outcome of its key role in inducing DNA damage, the function from which it derives its name - "Ginir"- Genomic INstability Inducing RNA. By BLAST analyses, Ginir was found to be localized on q arm of mouse X chromosome. Sequence analyses of human genome indicated homology of Ginir with mouse Ginir sequences but its exact location on human chromosome is not yet known. The RNA was detected as a long 1.9 Kb transcript in mouse by Northern Blot analyses. However, a 612 bp cDNA clone was found competent to functionally manifest its transforming potential *in vitro* as well as *in vivo* in both mouse and human cell systems. The identification of targets through which the oncogenic functions of Ginir are manifested is a focus of the present study as this would entail a mechanistic insight into the role of Ginir in development, cell growth, differentiation, regeneration and cancer.

Aims and Objectives:

1. To determine localization of Ginir on mouse and human chromosomes and investigate its expression and function in mouse embryos.
2. To analyse the specific pathways effected by Ginir for elucidating the biological functions followed by validation and functional studies by knock-in and knock-down experiments.
3. To identify the targets for Ginir and Giniras to unravel the molecular signalling mechanisms regulated by them during cellular growth

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Research Findings

A vast majority of non-coding RNAs are transcribed in a developmentally regulated manner. We have investigated the role of non-coding RNAs – Ginir and Giniras during mouse embryo development and obtained a temporal variation in their expression pattern. We detected significantly higher levels of Ginir in 14.5 dpc of mouse embryonic development (Fig.1A, a). A detailed tissue specific expression analyses for Ginir and Giniras transcripts by Orientation specific PCR have yielded key data regarding their functions. Amongst, the various organs analyzed from 14.5 dpc embryos, there was no significant expression of either sense or anti-sense transcripts in heart and lungs. Interestingly tissues like liver, tail and limbs with higher tissue regeneration potential and morphogenesis showed predominant Ginir expression (Fig.1A, b). Brain, where significant neurogenesis occurs during development and is known to possess a stem cell pool in the SVZ region of the brain showed similar extent of expression of the Sense (Ginir) and anti-sense (Giniras) transcripts. To further this study we used whole mount sections of 14.5 days old mouse embryos and stained them with Ginir and Giniras specific LNA probes and performed localization studies using *in situ* RNA-FISH. Data showed that both Ginir and Giniras transcripts showed co-localization that was restricted to forebrain and midbrain of 14.5 dpc brain whereas liver showed significantly higher expression of Ginir (Fig. 1B,a). Few areas of forebrain (dorsal pallium and mediam pallium) and midbrain (Colliculus superior, inferior calliculus, alar plate of M1, roof plate of M1) showed highly specific staining for both the transcripts (Fig. 1B, b). These areas show extensive neurogenesis in 14.5 dpc-15.5 dpc embryos, signifying that expression of Ginir may be associated with neural development. Interestingly, predominant expressions of these transcripts were seen in the cytoplasmic regions of these organs (Fig.1C). Investigating various diploid cell-lines of mouse and human origin by RNA-FISH confirmed the

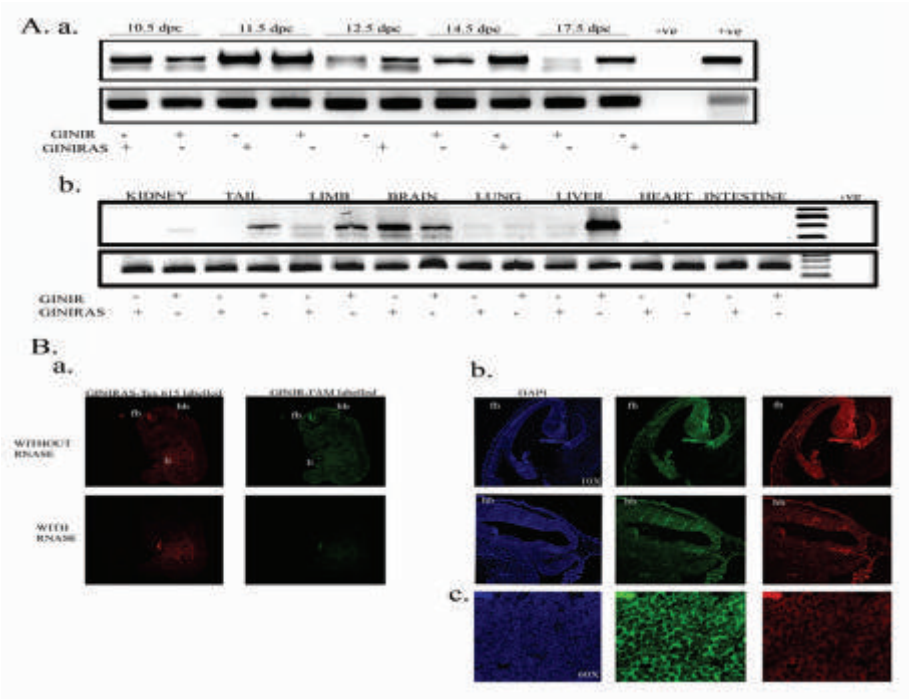


Fig. 1: Expression and localization of Ginir / Giniras. A) RT-PCR analyses of mouse embryos (a) and organs excised from 14.5 dpc. mouse embryos (b). B) LNA-FISH analyses of Ginir (green) and Giniras (red) in 14.5 dpc mouse embryo at 4X (a), 10X (b) and 60X (c) magnifications . Nuclei are stained with DAPI (10X).

cytoplasmic localization of Ginir/Giniras transcripts. Localization studies of embryonic fibroblasts done with DNA-FISH indicated the presence of Ginir on the mouse chromosomes, though banding studies need to be performed to confirm their exact chromosome specific localization.

Our earlier studies in the lab showed that Ginir over-expression led to induction of high levels of genomic instability. Detailed analyses of the molecular pathways activated in Ginir expressing mouse and human cells have demonstrated 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). Interestingly, the ATM, NBS1, and BRCA1 proteins are considered to be part of a mega-dalton sized protein complex referred to as BASC (BRCA1- associated genome surveillance complex) that also includes numerous other DNA repair and replication factors. We found over-activation of all the molecules of the BASC complex in Ginir cells along with greater density of stalled replication forks. The defects in firing of the replication forks was visualized by formation of large number of DNA damage foci co-expressing ATM with γH2AX1 and RPA32 with PCNA, further signifying that Ginir overexpression led to inappropriate G1-S transition and/or progression through the S-phase (Fig. 2A). Interestingly, the Ginir cells also showed very high expression of Brca1 at the RNA levels. Most notably, multi-fold upregulation of both *Igf2* and IGF2R, minor satellite elements, LINE1 elements and IAP expression was induced by Ginir as demonstrated by us in quantitative RT-PCR experiments (Fig. 2B). The detection of amplified satellite transcripts occurring due to their transcriptional de-repression was specifically induced by Ginir and not Giniras. There are reports stating that satellite transcripts in the presence of wild type BRCA1 partially phenocopies BRCA1 loss

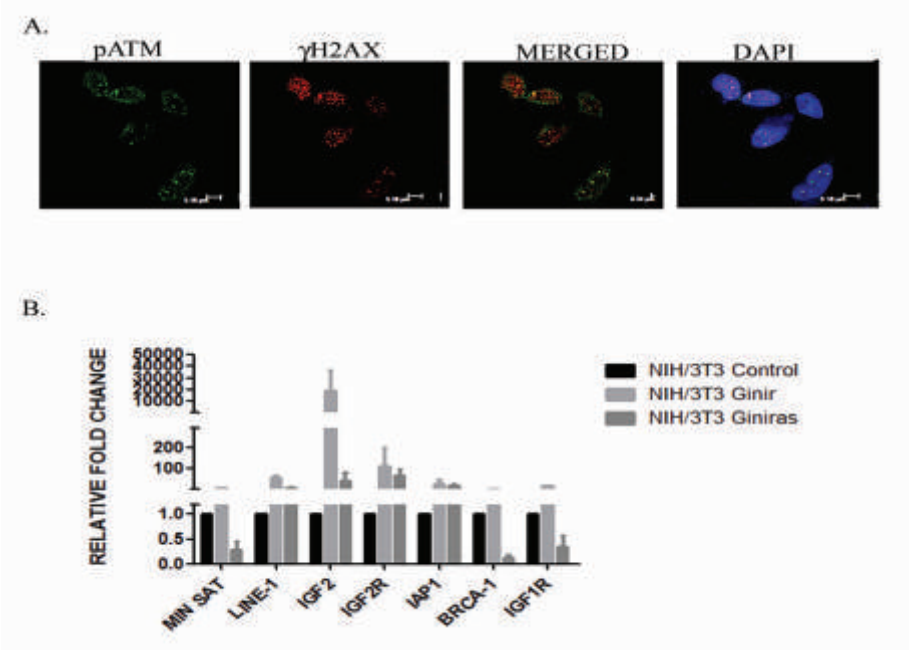
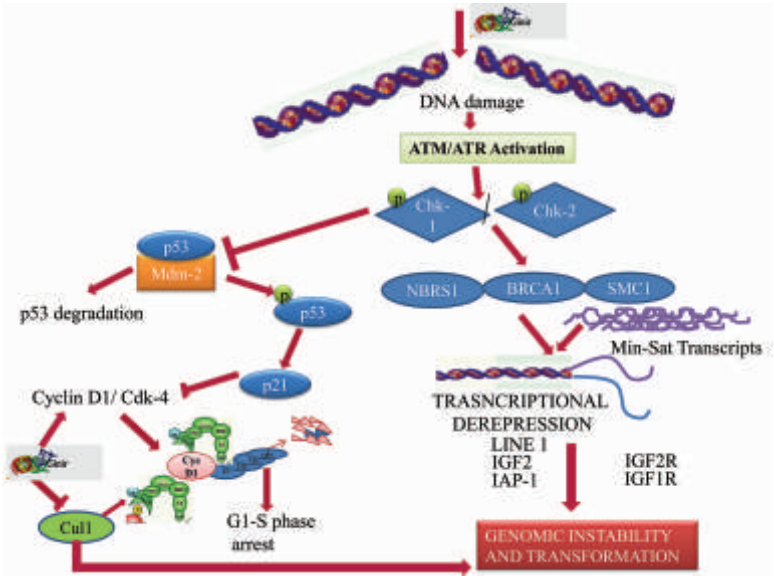


Fig. 2: Genomic Instability induced by Ginir in NIH/3T3 cells. **A)** Co-localization of pATM (green) with γ H2AX(red). Nuclei are stained with DAPI(blue). **B)** q-RT PCR analyses of expression of satellite elements and other genes involved in genomic surveillance in NIH/3T3 Ginir . Data represents Means ± SEM compared to NIH3T3 cells

Fig. 3: Mechanisms of Action of Ginir

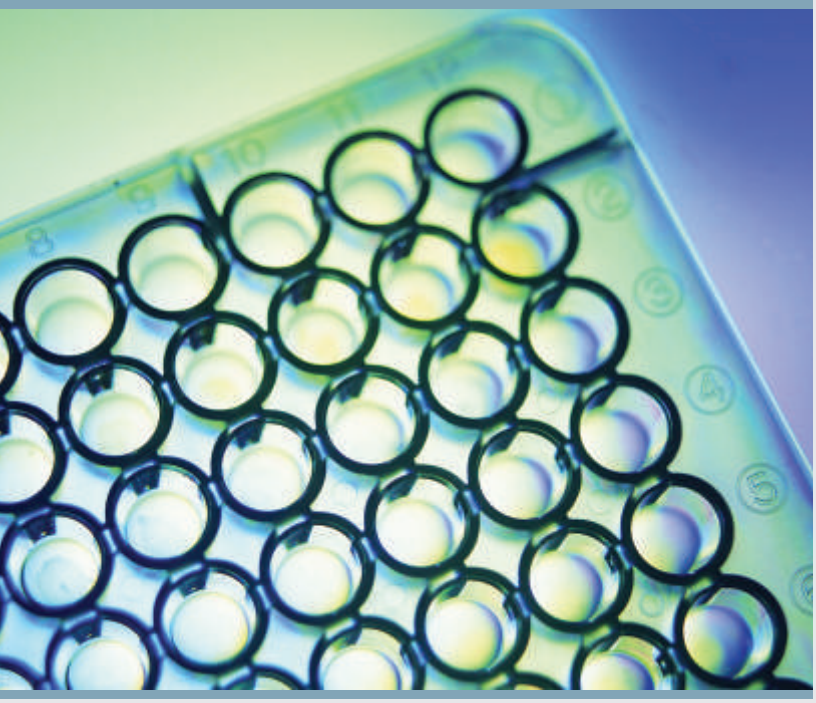


including centrosome amplification and γH2Ax foci formation, thereby affecting integrity of centromeric heterochromatin. Centrosome amplification, mitotic spindle defects visualized through over-expression of Pericentrin, cell cycle check point defects and over-activated DNA damage response were found consequential to Ginir action in a range of Ginir over-expressing cells. Comprehensively analyzing our data we propose a model of action for Ginir that is depicted schematically in Fig. 3. Although it is presently unclear how these processes are elicited at a mechanistic level it is tempting to speculate from our recent and previous data that defects in ubiquitination pathways as well as derepression of satellite transcripts are causal in Ginir mediated tumorigenesis. Although the pathological significance of these findings is unknown, our work suggests that mechanisms of derepression of microsatellite repeats could contribute to the evolution of the cancer cell through the induction of genomic instability.

Future Work

1) Study the role of Ginir in cellular reprogramming of various cell systems including human adult dermal fibroblasts.
2) Investigate the role of Ginir / Giniras in normal cellular homestasis during development and differentiation in mouse and human systems.

Research Report



Cell Biology

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Nup358 interacts with Dishevelled and aPKC to regulate neuronal polarity

Background

Cell polarity is fundamental to many aspects of growth, development and homeostasis of multicellular organisms. A set of genes that were identified to be responsible for the asymmetric zygotic division of *Caenorhabditis elegans*, namely, *par1* to *par6* and an atypical protein kinase C, were subsequently found to be highly conserved players regulating the process of polarization from worms to mammals. The polarity proteins distribute to specific subcellular locations in diverse cellular contexts such as in one-cell embryo of *C. elegans*, *Drosophila* neuroblasts, polarized epithelial cells, migrating cells and differentiating neurons, and play a crucial role in generating and maintaining polarity.

The Par3-Par6-aPKC complex (Par polarity complex), a sub set of the Par proteins, specifically localizes to the anterior end of the dividing *C. elegans* one-cell embryos, apical side of polarized epithelia and leading edge of migrating cells. Such an asymmetric distribution of these key players is important in generating distinct cellular domains and achieving polarization, mostly through regulation of downstream effectors by aPKC-mediated phosphorylation. The key events modulated by Par proteins involve cytoskeleton arrangement and membrane trafficking.

In epithelial cells, Cdc42 determines the localization of Par complex to the tight junctions through interaction with Par6 and activation of aPKC. Activated Cdc42 also recruits the Par complex to the leading edges of migrating cells. It is believed that this results in the spatial inactivation of GSK3ß, which promotes the interaction between microtubules and the plus end binding protein adenomatous polyposis coli (APC), leading to subsequent cortical capture and stabilization of microtubules in the direction of migration.

Neuronal cells are one of the highly polarized cell types. The Par complex has been reported to be present at the tip of the growing axon, and the complex is transported to the location through direct interaction of Par3 with the KIF3A subunit of the Kinesin-2 complex and APC. In a recent study, it was shown that the non-canonical Wnt signaling

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induces Dvl-mediated activation of aPKC to regulate neuronal polarization. Such a conserved function of Wnt has also been identified during directed migration of astrocytes.

We previously reported that the nucleoporin Nup358 interacts with APC, and regulates the process of polarized cell migration. We wished to investigate whether Nup358 plays a conserved role in other contexts of cell polarization and to unravel the molecular mechanism involved. To address these questions, we chose the well-established model of axon-dendrite polarization of cultured rat hippocampal neurons.

Aims and Objectives

1. Does Nup358 play a role in neuronal polarization?
2. What is the mechanism of Nup358 function in neuronal polarization?

Work Achieved

Nup358 interacts with Dishevelled

We previously had reported that Nup358 interacts with the Wnt signaling component APC. Immunoprecipitation analysis of HEK293T cells transfected with HA-tagged Dvl1 suggested a physical interaction between Nup358 and Dvl *in vivo*. Consistent with the interaction, GFP-tagged Nup358 and HA-tagged Dvl1 were found to co-localize into cytoplasmic puncta. These data suggest that Nup358 and Dvl exist in a complex *in vivo*.

Next, the region of Nup358 important for interaction with Dvl was mapped using cells expressing HA-Dvl1 and GFP-tagged fragments of Nup358; namely, N terminus (spanning 1-900 amino acids, BPN), middle region (901-2219 amino acids, BPM) or C terminus (2220-3224 amino acids, BPC). Immunoprecipitation results suggested that Dvl1 associated specifically with the N-terminal region (BPN) of Nup358. In agreement with the interaction data, both Dvl1 and BPN were found to be co-localized in the cytoplasmic puncta. However, independent expression of BPN results in its localization to interphase microtubules. Co-immunoprecipitation experiments further suggested that the middle region of Dvl1, encompassing the PDZ domain, was involved in the interaction with Nup358.

Nup358 associates with the members of Par polarity complex

As previous studies in migrating astrocytes and hippocampal neurons have suggested that Dvl acts through regulation of aPKC, we were interested to investigate if any physical association and/or functional connection exists between Nup358 and the components of Par polarity complex. Immunoprecipitation of endogenous Nup358 and western analysis of the immunoprecipitates with PKCζ antibodies, clearly suggested that Nup358 and aPKC interact with each other *in vivo*. Also, we observed that the kinase activity of PKCζ was not essential for the interaction with Nup358, since similar extent of Nup358 interaction was observed with wild type as well as a kinase dead (KD) PKCζ mutant (K281M). Nup358 was also found to interact with ectopically expressed HA-Par3 and myc-Par6, indicating that this nucleoporin associates with Par complex *in vivo*.

We further identified the domains of PKCζ and Nup358 important for the interaction with each other. The results suggested that the C-terminal region of PKCζ, consisting of the catalytic domain, and N-terminal region of Nup358 are involved in the interaction.

Downregulation of Nup358 leads to generation of multiple axons

To validate the functional significance of Nup358 interaction with Dvl1 and Par proteins in cell polarization, we chose cultured hippocampal neurons. During axon-dendrite differentiation, the neurons proceed through several distinct stages. After plating of hippocampal neuronal cells *in vitro*, within 10-36 h, the cells develop short neurites of almost equal lengths. Out of these, one neurite grows exponentially and forms an axon within 36–72 h.

To determine the role of Nup358 in the process of axon specification *in vitro*, we resorted to siRNA-mediated depletion of Nup358 in hippocampal neurons isolated from E18 rat embryos. Nup358 siRNA or control siRNA was transfected into hippocampal neurons along with pBetaActin-eGFP using Amaxa nucleofector, and cultured for 72 h. Axon-dendrite differentiation was assessed by immunostaining for the axonal marker Tau-1. In control siRNA transfected cells, majority of the transfected cells produced single axons. However, upon Nup358 depletion, a significantly higher number of GFP-positive (transfected) cells developed multiple axons (more than one axon), as compared to the control siRNA treated cells (Fig. 1). This result suggests that Nup358 plays an important role in neuronal polarization.

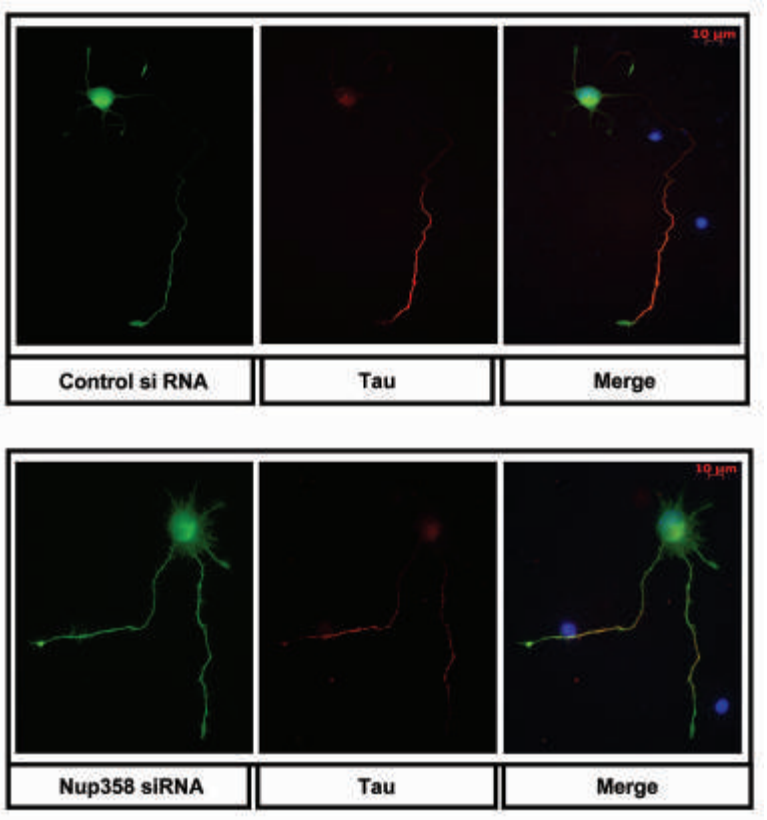

Fig. 1: Nup358 depletion causes impairment of neuronal polarity. E18 hippocampal neurons were transfected with control (Control siRNA) or Nup358 siRNA (Nup358 siRNA) along with pBetaActin-eGFP as transfection control, and were immunostained after culturing for 72 h *in vitro*. The transfected neurons were identified by GFP expression (green). The effect of Nup358 depletion on axon formation was analysed using anti-Tau-1 antibodies (red). DNA was stained with Hoechst 33342 dye (blue). Scale bar, 10 µm.

Overexpression of BPN leads to inhibition of axon-dendrite differentiation and partially reverts Dvl-induced multiple axon formation

Further studies were performed to determine the effect of overexpression of different fragments of Nup358 in the establishment of neuronal polarity. We observed that a significantly higher number of the cells transfected with BPN did not develop axons, whereas, overexpression of BPM and BPC did not affect the axon-dendrite polarization.

Previous studies have shown that ectopic expression of Dvl1 results in the formation of multiple axons, whereas, its down regulation leads to no axon formation. Therefore, we wanted to test whether Dvl and Nup358 are part of the same polarity cascade, and if yes, which one acts upstream of the other. Co-transfection studies indicated that Dvl-induced multiple axons phenotype could be significantly interfered by BPN, whereas BPN-induced abrogation of axon formation almost remained unaffected in the presence of overexpressed Dvl (Fig. 2). Based on these results, we conclude that Nup358 acts downstream of Dvl in the molecular cascade responsible for the generation of polarity in neurons.

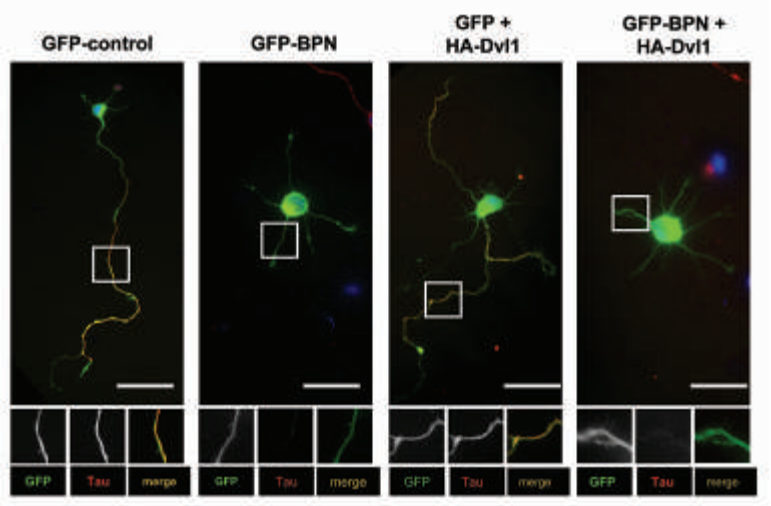

Fig. 2: Dvl induced multiple axon formation is interfered by overexpression of the N-terminal region of Nup358. Hippocampal neurons were co-transfected with indicated constructs and pBetaActin-eGFP (green). Tau-1 staining (red) was performed to study the effect on axon formation. DNA was stained with Hoechst 33342 dye (blue). Scale bar, 25 µm.

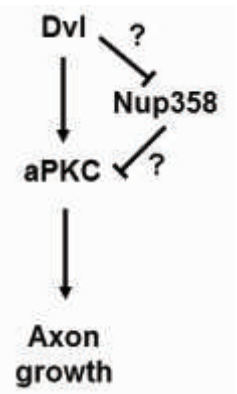

Fig. 3: A working model for the function of Nup358 in neuronal polarization. During the initial stages of single axon formation, Dvl-mediated activation of PKCζ occurs at the nascent axon. Differentiation of other neurites into axons could be inhibited by Nup358-mediated interference of aPKC activity. It is possible that in the developing axon, Dvl could inhibit the function of Nup358.

Our findings showed that Nup358 interacts with Dvl and aPKC, and is indispensable for the process of axon specification. Removal of Nup358 from developing neurons leads to improper differentiation and generation of multiple axons, while overexpression of a Nup358 fragment important for interaction with Dvl and aPKC leads to abrogation of neuronal polarization (Fig. 3).

Future Work

Studying the molecular mechanism involved in Nup358's function in neuronal polarity

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Identification of molecular mechanisms involved in the HT1080 tumor angiogenesis

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Background

Development of a large tumour involves multiple processes, amongst which the tumour angiogenesis plays a critical role. Tumour angiogenesis primarily involves at least three distinct processes: formation of new blood vessels by the host-derived endothelial cells in response to the secretion of angiogenic factors by the tumour cells - the most accepted model, the formation of lymphatic vessels from the pre-existing lymphatic vessels - a process termed as lymphangiogenesis and the formation of vascular channels lined exclusively by the tumour cells mimicking endothelial cells - a process called as vasculogenic mimicry . Since the molecular mechanisms involved in the development of angiogenesis vary with tumour type, elucidation of the process employed by a particular type of tumour forms the first line of investigation followed by the determination of the molecular mechanisms involved in the process. This is a crucial aspect in the development of anti-angiogenic strategies, as the de-differentiating tumour cells undergoing a process of vasculogenic mimicry may not necessarily acquire sensitivity to angiogenic inhibitors.

As the tumors grow in size, the hypoxia sets in. As small as 1-2 mm size tumors show signs of hypoxia and need angiogenesis for further growth. Although hypoxia is toxic to normal cells, the tumour cells undergo adaptive changes that allow them to survive and even proliferate in a hypoxic environment. Over the past decade, the data generated by many groups have indicated that hypoxic microenvironments contribute to the cancer progression by activating adaptive transcriptional programs that promote cell survival, motility, and tumour angiogenesis. Under hypoxic conditions, a signaling pathway involving a key oxygen response regulator, termed as HIF-1α (Hypoxia Inducible Factor), is switched on. HIF1α in turn induces the transcription of genes like PECAM, VE-Cadherin, VEGF and other proteins that stimulate the formation of blood vessels, which supply nutrients to the tumour cells.

Vascular Endothelial Growth Factor (VEGF) is a cytokine released from the cells that promotes the neo-vessel formation and morphogenesis. VEGF, also designated as VEGF-A, belongs to a family of growth factors with a predominant endothelial target-specificity.

It acts as a mitogen as well as a chemo-attractant for the endothelial cells (EC), thereby acting as an angiogenesis-promoting factor *in vivo*.

NRP-1 (Neuropilin 1) is a co-receptor of VEGFR-2 and it specifically increases the binding of $VEGF_{165}$, a splice variant of VEGF-A playing an important role in tumour growth and angiogenesis. NRP-1 interacts with VEGFR-2, and increases the $VEGF_{165}$-mediated chemotaxis of the endothelial cells. Growing evidence supports a critical role of these receptors in the tumour progression. NRP-1 expression is found to be up-regulated in multiple tumour types and correlates with tumour progression and/or prognosis in a tumour-specific manner. NRP-1 may either mediate its effects on the tumour progression indirectly by promoting tumour angiogenesis or directly by affecting the biology of the tumour cells themselves.

Since VEGF is considered as a critical factor for tumour angiogenesis, an attempt has been made to silence the VEGF by siRNA mediated approach to control the tumour growth in an experimental model system. A complete abrogation of VEGF secretion slowed down the tumour growth but did not prevent the tumour formation by the human fibrosarcoma-derived HT1080 cells, indicating the involvement of other mechanisms in the tumour growth.

Although the HT1080 cells are used as a model system in several studies, the crucial molecular events involved in their angiogenic behavior have not been identified.

Aims and Objectives

1. To elucidate the effect of hypoxia on the angiogenic and tumorigenic properties of HT1080 cells.
2. To elucidate the molecular mechanisms involved in the hypoxia-mediated aggressive angiogenic tumor formation by HT1080 cells.

Research Findings

HT1080 cells show enhanced proliferation under hypoxia

In order to elucidate the mechanistic aspects of the hypoxia-mediated activation of angiogenic program in the HT1080 cells *in vitro*, it was necessary to ascertain whether these cells can survive and grow under hypoxia. The growth rate of the HT1080 cells under normoxia vs. hypoxia was determined. As seen in the Figure 1A, the HT1080 cells incubated under hypoxic conditions (1% oxygen) showed an enhanced growth compared to the cells incubated under normoxia. The enhanced proliferation became evident by 48 hours and peaked at 72 hours, indicating that the processes needed to combat the hypoxia got fully activated by 48 hours of incubation under hypoxia. Since $VEGF_{165}$ is known to act as a growth-promoting cytokine under hypoxic conditions, we quantified the $VEGF_{165}$ mRNA in these cells by performing real time PCR experiments. We observed a 9.14 folds up-regulation of $VEGF_{165}$ mRNA in the cells cultured under hypoxic condition as compared to those cultured under normoxic conditions (*** $p<0.001$; Figure 1B). Immuno-fluorescence staining of the cells with an antibody to $VEGF_{165}$ also showed a significant up-regulation of this cytokine under hypoxia at protein level (Figure 1C). These

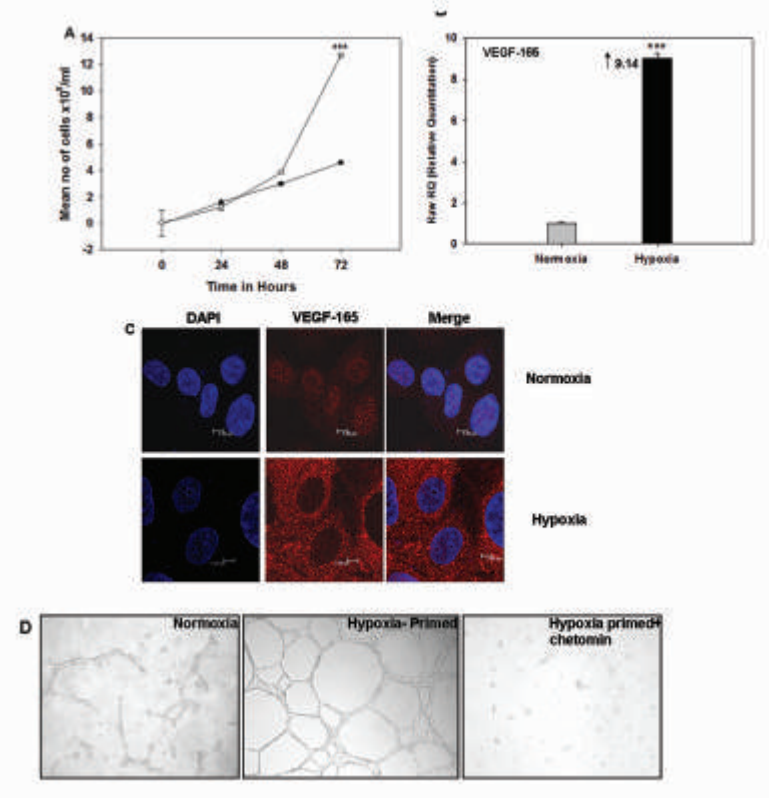

Fig. 1: HT1080 cells combat hypoxia by up-regulation of growth-promoting cytokine, $VEGF_{165}$

A. Growth kinetics of the HT1080 cells was studied under normoxia and hypoxia. The proliferation rate is higher in hypoxic condition as compared to normoxic condition. **B.** Quantitative PCR experiments were performed on the HT1080 cells incubated under normoxia and hypoxia for quantification of $VEGF_{165}$ mRNA. The hypoxic cells showed ~ 9 folds higher expression of $VEGF_{165}$ mRNA compared to the normoxic ones (6 hours time point, N =3, *** P<0.001). **C.** Confocal microscopy analysis of the cells incubated under normoxia vs. hypoxia (48 hours) using an antibody to $VEGF_{165}$ shows that the hypoxic cells secret this growth-promoting cytokine at high levels. Nuclei are demarcated by DAPI (Blue) **D.** Consistent with the high level of $VEGF_{165}$ at gene and protein levels, the hypoxia-primed HT1080 cells undergo a robust tubule formation on growth factor-reduced matrigel (middle panel) compared to the normoxic cells (left hand panel). The tubule formation was sensitive to the presence of chetomin in the medium (right hand panel) indicating that the HIF1-α-mediated transcription is involved in the process. (Original magnification: 40 X)

data showed that the HT1080 cells cultured under hypoxia (hypoxia-primed) for 48 hours form a suitable model to study the hypoxia-mediated molecular events involved in tumour angiogenesis and growth.

Hypoxia-primed cells show enhanced tubulogenesis on matrigel in a HIF-1α-dependent manner

Tubule formation on matrigel is an excellent *in vitro* correlate of angiogenesis. Hypoxia-mediated activation of HIF-1α has been shown to drive tubule formation on matrigel. We, therefore, examined whether hypoxia-priming of the HT1080 cells leads to an enhanced tubulogenesis on matrigel. Hypoxia- or normoxia-primed HT1080 cells were seeded on a polymerized growth factor-reduced matrigel (BD). Tubule formation was monitored at regular intervals and the images were captured. It was observed that the hypoxia-primed cells started forming the tubules at a much earlier time point compared to their normoxic counterpart (3 hrs vs. 6 hrs respectively) and formed a denser network of tubes (Figure 1D - middle panel).

Most of the hypoxia-induced processes are dependent on the HIF-1α-mediated transcription, but several HIF-1α independent effects are also known. It was, therefore, necessary to examine whether the enhanced tubule-formation by the hypoxia-primed cells was a HIF1α-dependent or HIF1α-independent process. Chetomin is a well-known pharmacological inhibitor of HIF-1α that blocks the binding of p300 subunit with the HRE region of HIF-1α, thereby blocking the transcription of downstream genes. Incorporation of Chetomin (100nM) in the incubation medium resulted in a complete abrogation of

tubule formation by the cells cultured under hypoxic conditions (Figure 1D, right panel), confirming the role of HIF-1α mediated transcription in angiogenesis.

HT1080 cells exhibit vasculogenic mimicry *in vivo*

Many types of cells form tubules on matrigel. It is, however, necessary to show that they form functional blood vessels *in vivo*. In order to ascertain that the HT1080/WT tumour cells themselves acquired a functional endothelial phenotype and participated in the angiogenic process *in vivo*, we employed a strategy of using HT1080 cells that were stably transfected with eGFP-N1 plasmid, so that we could clearly visualize them in the tumor mass. These cells were subcutaneously injected in NOD/SCID mice and both cryosections and paraffin sections were prepared from them. A visual inspection of a cryosection under confocal microscope clearly showed that the GFP^+ cells formed vessel-like structures running crisscross in the tumor mass (Figure 2A).

We then performed a double IHC experiment with the paraffin sections using antibodies to GFP and PECAM. As seen in the Figure 2 A, the entire tumor mass was formed by GFP^+ cells (brown nuclei). The GFP^+ cells themselves expressed PECAM on their surface (violet borders, Figure 2B inset), strongly supporting our contention that the HT1080 cells themselves had acquired the endothelial cell phenotype. It was important to note that the PECAM staining was very prominent in the GFP^+ cells that participated in formation of the tube-like structures. The tube-like structures formed by the double positive cells were seen to harbor red blood cells indicating that they closely resembled the functional blood vessels (Figure 2C). The data confirmed that the HT1080 cells themselves contributed to the tumor angiogenesis by undergoing the process of vasculogenic mimicry.

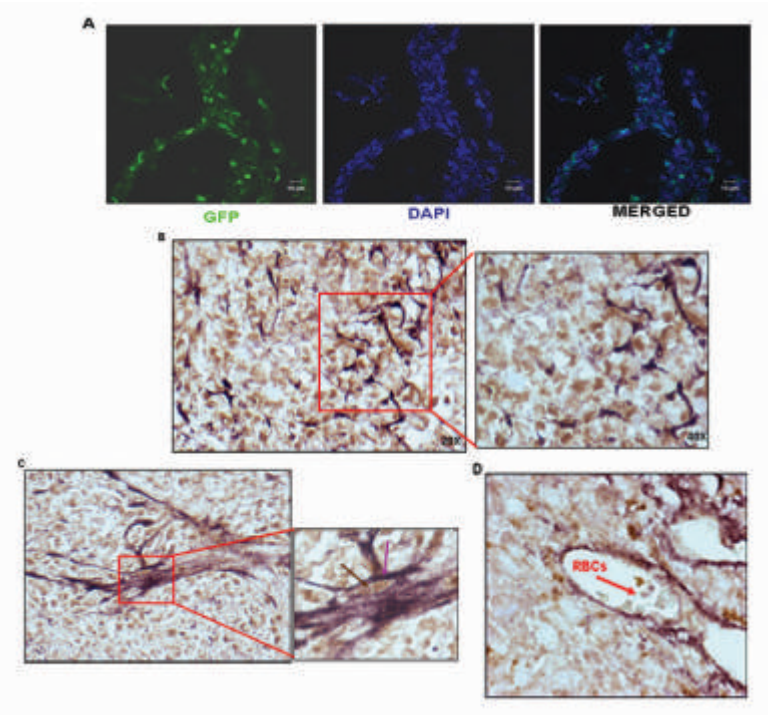

Fig. 2: HT1080 tumour cells employ vascular mimicry to combat *in situ* hypoxia

A. Visualization of GFP^+ tumour cells in the cryosections of the tumours formed by HT1080/WT/GFP cells showed that the tumour cells themselves form vessels that run criss-cross in the tumour mass. DAPI was used to demarcate the nuclei (bar - 10μm) **B.** Double IHC experiments performed on the paraffin sections of the tumours formed by HT1080-GFP cells with anti-GFP and anti-PECAM antibodies show that the entire tumour mass was filled with GFP^+ tumour cells with the vessels formed by the double positive cells. The intensity of PECAM staining was maximal in the cells forming tubes. (Original magnification 200X). A part of the image has been magnified to show the details (original magnification 400 X). **C.** A vessel formed by the double positive cells is shown (original magnification 200X). The inset clearly shows that the cells forming the vessels have brown nuclei (GFP - indicated by a brown arrow) and violet border (PECAM - indicated by a violet arrow) (original magnification 630 X). **D.** A cross section of the blood vessel formed by GFP-PECAM double positive cells is depicted. Presence of red blood cells in the lumen is marked by a red arrow (original magnification 630X).

Hypoxia up-regulates angiogenic program in the HT1080 cells

NRP-1 has been identified as a receptor for $VEGF_{165}$ and is known to play an important role in the angiogenic process. We, therefore, performed immuno-fluorescence staining of the cells grown under hypoxia and normoxia using an antibody specific to NRP-1. The hypoxic cells showed a clear up-regulation of NRP-1 at the cell membrane (Figure 3 A a, b ** $p<0.01$). As expected, the nuclear stabilization of HIF-1α was also seen in the hypoxic cells (Figure 3A c,d, ***$p<0.001$).

Real time PCR experiments performed to quantify the NRP-1 mRNA showed that the expression of NRP-1 was 2.4 folds higher in the hypoxic cells compared to the normoxic cells (Figure 3B a, *$p<0.05$). The level of HIF-1α mRNA was also found to be up-regulated (1.7 folds high) by the hypoxia (Figure 3 B b, ***$p<0.001$). The up-regulation of NRP-1 and HIF-1α under hypoxia was further confirmed by performing western blot experiments on the hypoxia-treated cells. As seen in the Figure 3C a, the expression of both these molecules was highly up-regulated under hypoxia at translational levels as well (1.36 folds for HIF-1α and 3.2 folds for NRP-1, Figure 3 C b, c).

The up-regulation of NRP-1 and $VEGF_{165}$ mRNA by hypoxia was found to be sensitive to the presence of chetomin in the medium (Figure 3 D a,b) confirming that, in the HT1080 cells expression of NRP-1 and its ligand, $VEGF_{165}$, by hypoxia is dependent on the HIF-1α-mediated transcription.

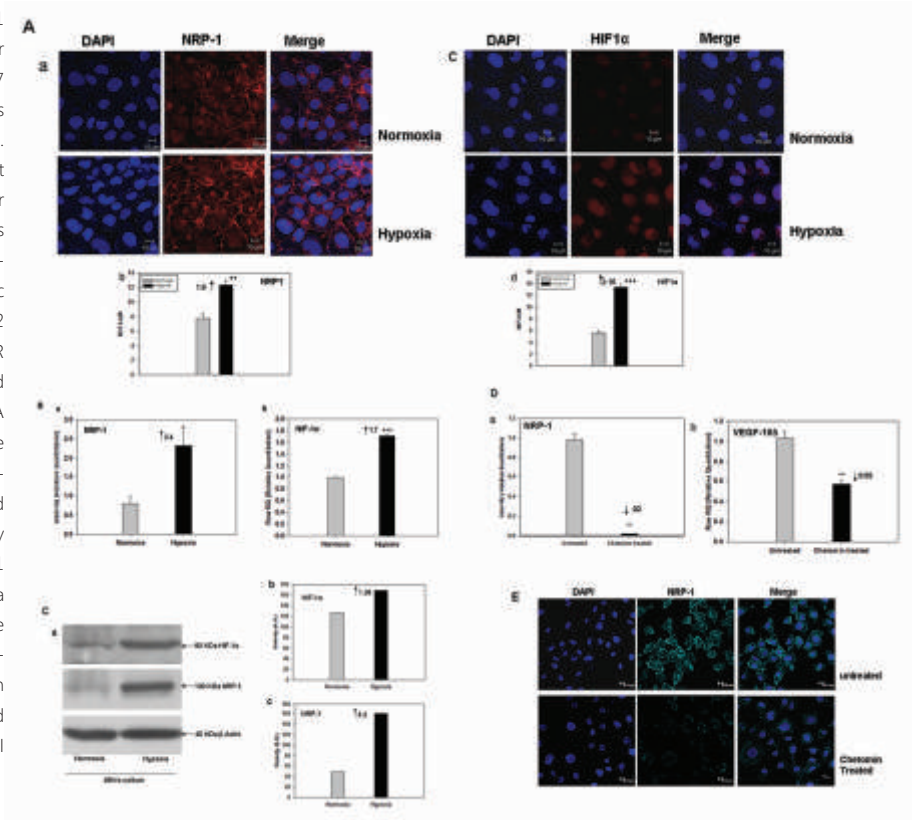

Fig. 3: Hypoxia up-regulates angiogenic program in HT1080 cells

A. Confocal microscopy analyses show that the hypoxic cells exhibit up-regulation of angiogenic molecules like NRP-1 **(a)** and nuclear stabilization of HIF-1α **(c)**. Nuclei are demarcated by DAPI (Blue). Mean fluorescence intensity (M.F.I.) of the cells was measured by Image J software (NIH) at membrane (for NRP-1) and in the nuclear region (for HIF-1α). The M.F.I. of 30 randomly selected cells was used to calculate mean ± S.E.M. The analyses have been graphically depicted (b and d for NRP-1 and HIF-1α respectively) *** $p< .001$ and ** $p< 0.01$. **B.** Quantitative PCR analyses for NRP-1 **(a)** and HIF-1α **(b)** mRNA show 2.4 and 1.7 folds up-regulation of these genes in the cells incubated under hypoxia compared to normoxia. (N=3; ***$p< .001$ and ** $p<.01$). **C.** Western blot experiments performed on the cells grown under normoxia vs. hypoxia show that the protein levels of both HIF-1α (**a** –upper panel, **b**) and NRP-1 (**a**-middle panel, **c**) are up-regulated in the hypoxic cells compared to the normoxic ones (1.36 and 3.2 folds respectively). **D.** Results of quantitative PCR experiments show that the hypoxia-induced expression of NRP-1 **(a)** and $VEFG_{165}$ **(b)** mRNA was sensitive to the presence of chetomin in the medium, indicating that these genes are down-stream events in the HIF-1α-mediated transcription process. **E.** Confocal microscopy analysis shows that the expression of NRP-1 protein (Cyan) is highly up-regulated by hypoxia (upper panel). Presence of chetomin in the medium abrogated the expression of NRP-1(lower panel), suggesting that NRP-1 expression critically depends on the HIF-1α -mediated transcription. Nuclei are demarcated by DAPI (Blue).

The figure 3E illustrates the results obtained in an immuno-fluorescence experiment performed on the cells incubated under hypoxia with or without the addition of chetomin. Addition of chetomin in the medium abrogated the expression of the NRP-1 protein, confirming the above mentioned real time PCR data. These data showed that hypoxia up-regulates the expression of two important angiogenic markers, NRP-1 and $VEGF_{165}$, in HT1080 cells in a HIF-1α-dependent manner.

NRP-1 controls the hypoxia-induced angiogenic properties of HT1080 cells

In order to validate the role of NRP-1 in the hypoxia-induced angiogenic behavior of the HT1080 cells, we developed stable clones of these cells expressing NRP-1-specific shRNAs (HT/shNRP). After confirmation of the down-regulation of NRP1 by real time PCR and western blot analysis these clones were used in further experiments.

PECAM, $VEGF_{165}$ and VEGFR-2 are some of the important signature molecules involved in the tumour angiogenesis. We examined whether hypoxia regulates the expression of these angiogenic markers through NRP1 by performing PCR experiments to quantify the mRNA levels of these genes in the HT/shNRP cells grown under hypoxia. A quantitative PCR analysis for PECAM and $VEGF_{165}$ mRNA and a semi-quantitative PCR analysis for VEGFR-2 mRNA showed that hypoxia failed to up-regulate the expression of these genes in the HT/shNRP cells (data not shown). The data clearly showed that expression of these angiogenic markers was a down-stream event of the HIF-1α-NRP1 axis in the HT1080 cells.

Therefore, it became imperative to determine whether silencing of NRP-1 also affects the hypoxia-mediated enhancement of tubule formation by the HT1080 cells. The HT1080/WT and HT/shNRP cells were primed with hypoxia/normoxia for 48 hours and were then seeded on polymerized growth factor-reduced matrigel. The tubule formation was monitored at regular time intervals and the images were captured. It was observed that the hypoxia-primed HT1080/WT cells showed a higher degree of tubule formation, whereas, HT/shNRP cells failed to undergo tubulogenesis even after hypoxia-priming (data not shown). These data clearly showed that NRP-1 acts as a pivotal determinant of the *in vitro* angiogenic properties of the HT1080 cells.

NRP-1 plays a crucial role in tumor growth and angiogenesis *in vivo*

In order to examine whether silencing of NRP1 affects the tumor formation *in vivo*, HT1080/WT and HT/shNRP1 cells were injected subcutaneously in the flanks of the NOD/SCID mice and the tumour formation was monitored regularly. In case of the HT/shNRP cells, the tumour formation was completely abrogated (0/9 mice injected, data not shown) clearly indicating that NRP1 is a critical molecular event required for the tumor formation by HT1080 cells.

Future Work

Effect of hypoxia on the angiogenic properties of various cancer cell lines will be investigated.

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Stat3 may be dispensable for Wnt mediated ES cells maintenance

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. While BMP acts in concert with LIF, it remains unclear whether there exists any synergy between LIF-Stat3 and Wnt pathways during the maintenance of ES cells. Our earlier findings indicated the expression of undifferentiated ES cells markers, Oct4 and Nanog, in the differentiating EBs, in response to Wnt activation during ES cells differentiation into cardiomyocytes. Hence, we extended our investigation to decipher whether Wnt signaling would help maintaining the unlimited self-renewal and pluripotency in ES cells and accordingly, to delineate the mechanistic basis whether it would work independent of or in concert with LIF-Stat3 pathway.

Aims and Objectives

1. The maintenance of ES cells in undifferentiated state 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

While studying the influence of Wnt during cardiomyogenic differentiation from ES cells *in vitro*, the expression of undifferentiated ES cell markers, OCT4 and nanog, in the differentiating EBs in response to Wnt activation led us to presume that Wnt probably would help in the maintenance of ES cells in undifferentiated state. Accordingly, ES cells were maintained with or without LIF and in the presence or absence of Wnt signaling

Participants

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activators/inhibitors and were monitored for their stemness and the retention of pluripotency. The occurrence of Wnt dependent maintenance of ES cells under the –LIF condition prompted us to investigate the interrelation between both these pathways, whether they act in concert or independent of each other in maintaining ES cells. Accordingly, to verify LIF influence on canonical Wnt activation, TCF dependent luciferase activity was monitored at various time points post-transfection. However, there was no difference in TCF dependent luciferase activity upon LIF treatment at any of the time points tested suggesting LIF does not influence the Wnt activity. Next, we examined whether Wnt signaling could regulate Stat3, an obligatory down-stream target of the LIF pathway. The expression of Stat3 was analyzed in cells maintained with either LIF or Wnt activated conditions using either BIO or rWnt3a. Surprisingly, both BIO and rWnt3a maintained ES cells displayed reduced *Stat3* expression compared to those maintained with LIF (Fig. 1A). The expression of *c-Myc*, a Stat3 downstream molecule, also showed similar pattern as that of Stat3 (Fig. 1B). The translational profile of Stat3 also corroborated well with their transcripts where a significant reduction in the expression level of the active S^{727} phosphorylated Stat3 and total Stat3 protein was noted in both BIO and rWnt3a maintained ES cells (Fig. 1C). This suggested that, Wnt might maintain ES cells in culture despite low Stat3 activity and it might be doing so by inhibiting some of the differentiation promoting signals. Accordingly, to decipher the underlying molecular mechanism the activated ERK1/2 levels were analyzed, since the increase in ERK1/2 activation leads to the differentiation of ES cells, while its inhibition facilitates ES cells maintenance. Incidentally, the levels of total ERK1/2 remained similar to the LIF maintained ES cells irrespective of

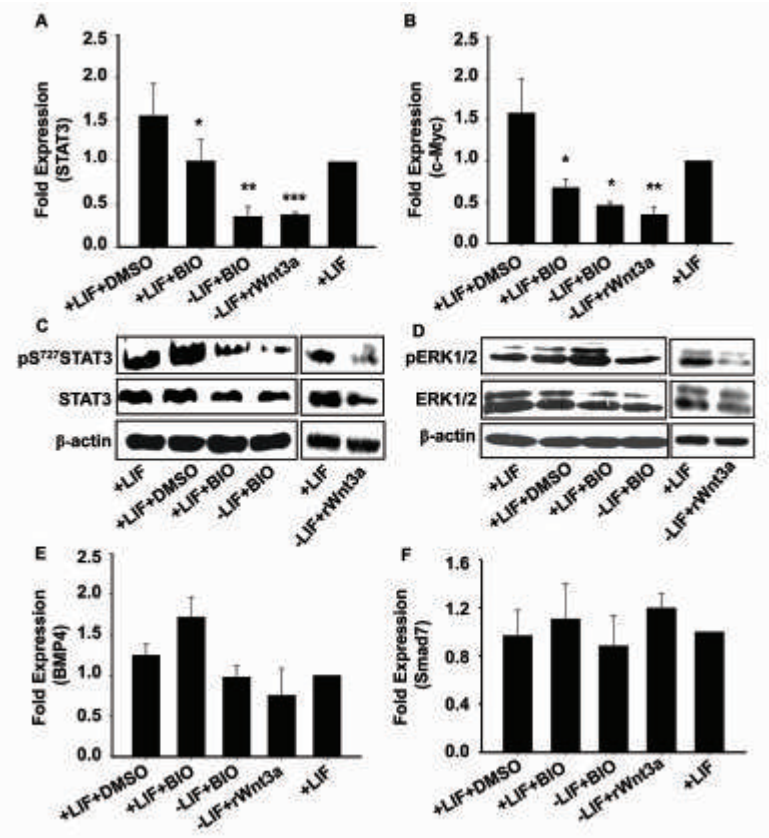

Fig. 1: Quantification of *Stat3* **(A)** and *cMyc* **(B)** transcripts in ES cells maintained either with BIO or rWnt3a under the +LIF and -LIF conditions. C, D: Detection and quantification of both total and activated Stat3 **(C)** and ERK1/2 **(D)** proteins in ES cells maintained with either BIO or rWnt3a under the indicated +LIF and –LIF conditions. E, F: Quantification of *BMP4* **(E)** and *Smad7* **(F)** transcripts in ES cells maintained with either BIO or rWnt3a under the +LIF and –LIF conditions.

Wnt activation. However, pERK1/2 levels remained low in BIO or rWnt3a maintained cells compared to the controls (Fig. 1D). Thus, Wnt could maintain ES cells with either comparable or lower ERK1/2 levels and with lower Stat3 unlike LIF. Together our data suggested that, the LIF-Stat3 and Wnt/β-catenin signaling though are sufficient in maintaining the self-renewal of ES cells individually, both might be operating independent of each other. To further authenticate the Stat3 independent action of Wnt, we generated shRNA mediated Stat3 knockdown ES cells. Stat3 knockdown affected the overall growth rate of ES cells irrespective of the presence of LIF or Wnt activator in the medium. Further investigation is ongoing to assess the role of Stat3 during ES cells maintenance and differentiation.

Further, we sought to address the involvement of BMP signaling during Wnt dependent ES cells maintenance in long term culture, since. BMP signaling has been reported to act in concert with LIF-Stat3 during ES cells maintenance and we have shown a positive correlation between Wnt and BMP during cardiac fate decision. Transcriptional analysis revealed similar expression level of *Bmp4* as well as *Smad7*, a BMP signaling feed-back inhibitor (Fig. 1E, F), in ES cells maintained with either BIO or LIF. However, a combined exposure of LIF and BIO showed a comparatively higher *Bmp4* expression thereby suggesting that, BMP might as well be a player acting in concert with Wnt during ES cells maintenance similar to that seen with LIF. To further attribute the role of BMP signaling in the maintenance of ESCs, cells were grown with LIF or BIO along with BMP inhibitors, chordin or BMP4 neutralizing antibody. However, inhibition of BMP neither affected Wnt mediated ES cells maintenance nor was there any appreciable alteration in the number Oct4 positive cells and also in the expression of *Oct4* and *Nanog* transcripts in them. Considering that serum containing medium (SCM) could have serum factors including BMP and that might have influenced the overall outcome, ES cells were further maintained in serum replacement medium (SRM) with either LIF or BIO and were characterized. LIF alone was able to maintain ES cells colony morphology in both SCM and SRM. However, BIO could maintain it only in the presence of serum as the colonies attained flattened morphology in SRM. Interestingly though, ES cells maintained with BIO in SRM could be passaged every other day, but with a slower growth rate and with reduced *Nanog* expression compared to that of LIF maintained ones. BMP supplementation to the medium could marginally enhance the growth rate of ES cells in SRM; hence, attributing BMP in serum likely to be one of the plausible factors to help in ES cells maintenance. However, the expression of both *Oct4* and Nanog in BIO maintained ES cells in SRM was not at par with the LIF maintained ones irrespective of the supplementation of BMP, even though, the number of $Oct4^{+}$ cells remained comparable in both the groups. Interestingly, there was further decrease in *Nanog* expression with BMP inhibition in SRM in BIO maintained cultures compared to the LIF maintained ones. Collectively, it may be comprehended that BMP along with yet to be identified serum factors might act in synergy with Wnt during ESCs maintenance.

Future Work

Further investigation will be undertaken to ascertain the role of Stat3 during both Wnt dependent and independent ES cells maintenance and identify the plausible serum factors contribution during the same.

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Studies on expansion, cryopreservation and differentiation of hematopoietic stem cells

Background

A. Generation of MSCs from cord blood and their differentiation to neural cells

The bone marrow (BM) is a major source of mesenchymal stem cells (MSCs); however, umbilical cord blood and cord tissues are now being explored as the alternative sources. Procuring these sources poses less ethical concerns and involves no invasive procedure. The MSCs so obtained can be used for transplantation purposes and can be differentiated into various cell types. Neurologic diseases such as Parkinson's disease, spinal cord injury, Alzheimer's disease, etc. pose a concern as no proper therapy exists for it. Isolation of neural stem cells from brain is very difficult and will involve use of embryonic stem cells which are associated with many ethical issues. Human umbilical cord tissue derived mesenchymal stem cells can be induced to form neural cells *in vitro*. The research in this area has recently begun and can be exploited for therapeutic applications in neurologic diseases. Thus in the present project we propose to generate MSCs from umbilical cord tissues and differentiate them to neural cells which can have applications in clinics in future.

B. Functional characterization of in vitro generated dendritic cells

Earlier a method of large scale generation of DCs from Cord blood source was standardized in our laboratory. But for these DCs to be used as anticancer vaccines the prerequisite is that they should exhibit CTL activity both *in vitro* and *in vivo*. Further the commonly used source of DCs for cellular vaccine preparation is from monocytes of PBL. Thus a comparison of DCs from both the sources is essential to find out whether the UCB derived DCs by our method have same potency as PBL derived ones. The present project was initiated keeping this aim in mind.

C. Ex vivo expansion of HSCs

Our earlier study revealed a novel application of two apoptotic protease inhibitors in HSPC functions and suggested that the regulation of these proteases may help in overcoming the major homing defects prevalent in the expansion cultures thereby

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generating a more competent graft in transplant settings. The expansion system used was suspension cultures with cytokines and with or without antiapoptotic agents.

In the present project we are evaluating the effect of antiapoptotic agents on expansion of HSCs using a co culture system of MSCs and HSCs.

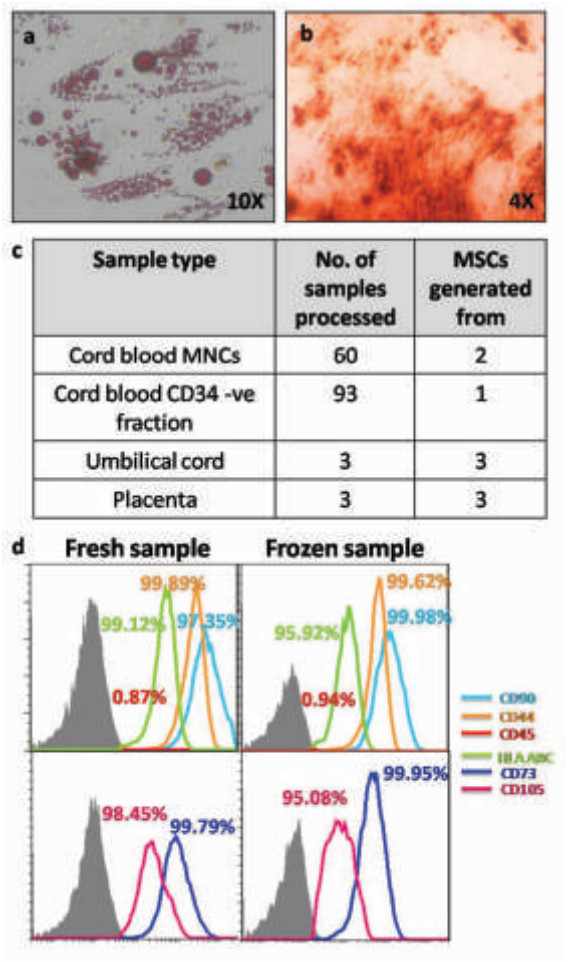
Aims and Objectives

1. Attempting to generate mesenchymal stem cells from various tissues like umbilical cord blood, cord and placenta and their characterization and differentiation to neural cells.
2. Comparing functional properties of DCs generated from cord blood versus peripheral blood.
3. Use of antiapoptotic agents in expansion of HSCs in stromal based cultures.

Work Achieved

MSC generation

MSCs generated from various cord tissues were further characterized by differentiation to adipocytes (Fig.1a) and osteoblasts (Fig.1b). The frequency of obtaining MSCs from



Sample type	No. of samples processed	MSCs generated from
Cord blood MNCs	60	2
Cord blood CD34 -ve fraction	93	1
Umbilical cord	3	3
Placenta	3	3

Fig. 1: Multilineage differentiation ability of MSCs shown by **a)** adipogenic) and **b)** osteogenic differentiation; **c)** Frequency of obtaining MSCs from cord tissue and related sources; **d)** Comparative marker expression profile before and after cryopreservation of MSCs derived from cord.

different sources is also depicted in Fig. 1c. The MSCs were cryopreserved and revived and the frozen samples were found to express no change in marker profile when compared with fresh MSCs (Fig. 1d).

DC generation

So far, the data show that PBL / UCB derived DCs are similar in morphology and phenotype (Fig.2a) and MLR. (Fig.2b). We have also standardized the *in vitro* CTL assay and the data clearly demonstrates that both CB derived as well as PBL derived DCs can activate T cells, which in turn kill the breast cancer cell line MCF-7. For performing these assays we use HLA A2 positive samples. Our data show that even CTL assays from the two sources are comparable (Fig.2c).

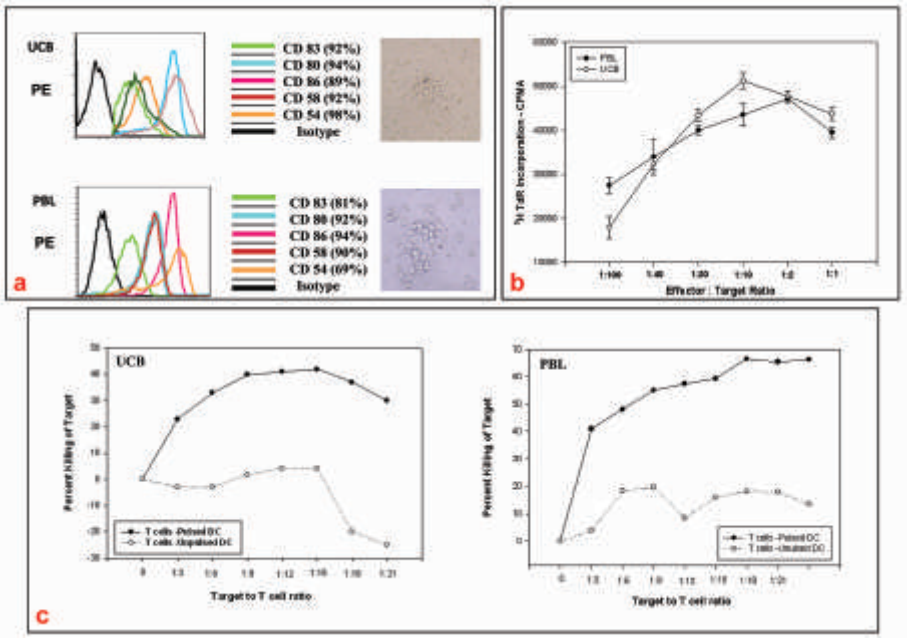
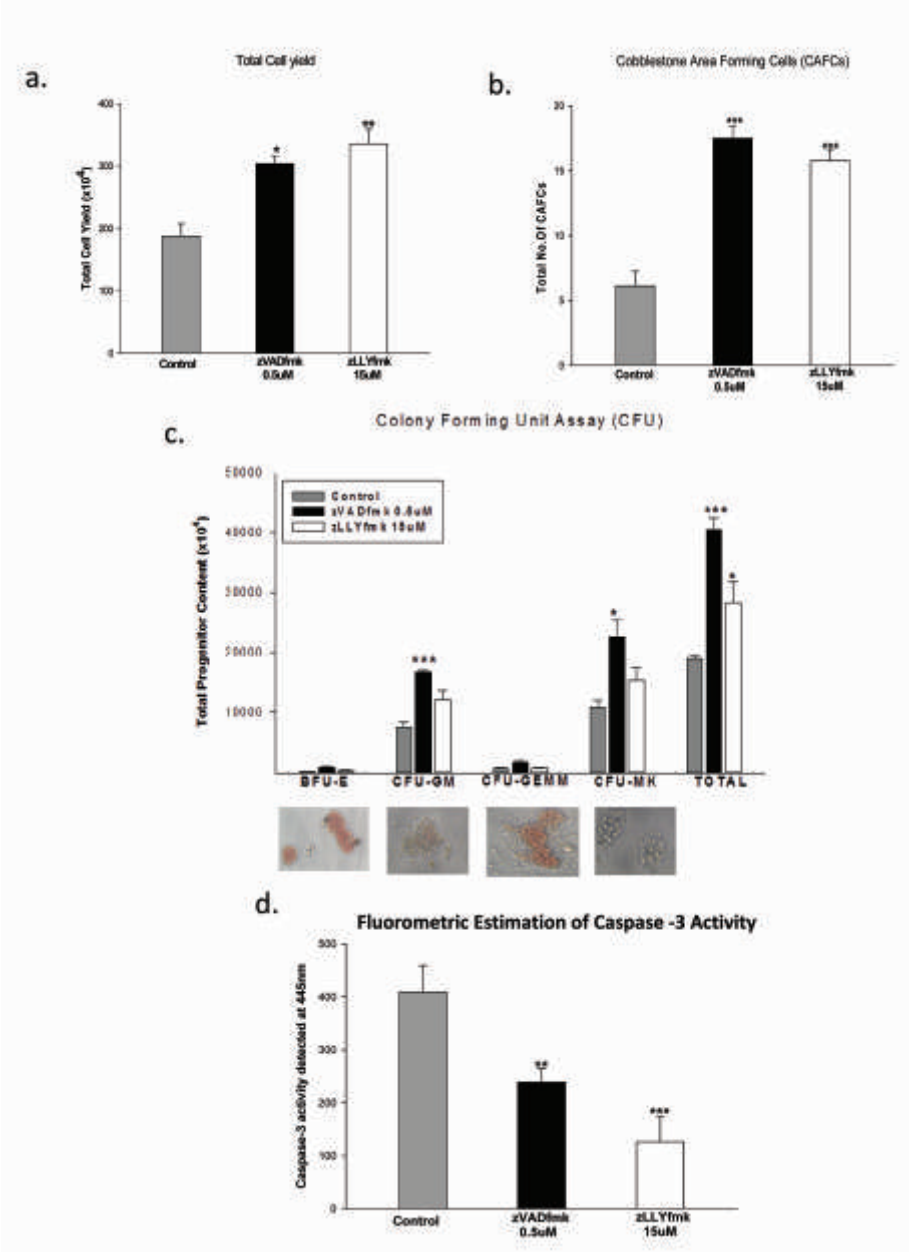


Fig. 2: Generation of Dendritic cells from UCB and Peripheral blood samples : **a)** FACS profile (overlays) of CD markers expression **b)** MLR assay. **c)** CTL assay of the effector cells on the target cell (MCF-7).

Ex vivo expansion of HSCs

In our earlier studies we have clearly shown that inclusion of antiapoptotic agents in cytokine containing suspension cultures results in improved expansion of umbilical cord blood derived CD34+ cells. Now we are trying to understand how the caspase and calpain inhibitors influence the stromal mediated expansion of UCB CD34+ cells. These studies are being carried out using a coculture system of MSCs and cord blood derived CD34+ cells. The preliminary data shows that there is improved cell yield (Fig.3 a), cobble stone areas (Fig.3 b), CFU content (Fig.3 c) and reduction in Caspase activity in sets expanded with antiapoptotic agents (Fig.3 d).

Fig. 3: UCB CD 34+ cells were co-cultured with human umbilical cord derived MSCs in the presence of growth factors and with or without apoptotic inhibitors for 10 days : **a)** Viable count of Total nucleated Cells (TNC). **b)** Total number of the cobblestone area forming cells. **c)** Total progenitor content as assessed by colony forming assay. **d)** Fluorometric estimation of Caspase-3 activity (p-value≤0.001***;p-value≤0.01**;p-value ≤0.05*)



Future Work

1. Optimizing generation , characterization and differentiation of neurons from cord derived MSCs
2. Standardizing In vivo CTL assays using DCs
3. Comparison of expansion in suspension cultures vs stromal cultures

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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 ß 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 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. In recent time, microRNAs (miRNAs) have been shown to regulate a number of pancreatic events like development of pancreatic islets, ß cell differentiation, insulin secretion, insulin resistance and diabetes.

miRNAs are short single stranded RNAs of ~22nt that can bind to the complementary sequence and control the translation of several genes. Normally miRNA targets the 3'UTRs and inhibits the translation or degrade the target mRNA in an Ago2 (Argonaute 2) dependant manner. However, recent studies have shown that miRNA can upregulate the translation as well by targeting the 3'UTR. Bioinformatics studies have suggested the presence of large number of potential target sites in the 5'UTR region and coding region of mRNA along with the 3'UTR. 5'UTR targeted miRNA mediated translation regulation has been shown in case of miR-10a and target ribosomal mRNA. Thus, the regulation of translation by miRNA is not determined by the position of the microribonucleoprotein (miRNP) complex on the target mRNA. MiRNA can also upregulate the translation by targeting the 5'UTR as well as the 3'UTR of the target mRNA with or without the

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involvement of Ago2, a key player of RISC (RNA-induced Silencing Complex). The mechanism of miRNA mediated upregulation in translation is not fully understood. Insulin mRNA has some putative target sites for specific miRNA in the 5'UTR, and some of them have been shown to be expressed in pancreatic islets.

In our previous study, we have shown that a 12bp 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. In the present study, we show that mouse miR-196b can specifically target the 5'UTR of mIns2 mRNA for translation regulation.

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

Insulin mRNA expressed in mouse pancreas consists of a pool of different 5'UTR containing transcripts. The variations in the 5'UTR is due to the two non allelic genes as well as alternative splicing resulting in at least three different 5'UTR containing insulin mRNA. Recently 5'UTR of mRNA has been reported to be the target of miRNA mediated regulation, hence we explored the possibility that any one of the insulin mRNA 5'UTR isoform could be a target for miRNA mediated regulation. miRNAs that can target the insulin 5'UTR were screened by Micro Inspector web tool (http://bioinfo.uni-plovdiv.bg/microinspector/). The miRNAs with high seed sequence complementarity (at least 5 base pair out of 7 in nucleotide position 2-7 of the miRNA) and a free energy of -22 kCal/Mole were selected for further analysis. Expression of these miRNA were analysed in mouse embryonic pancreas by RT-PCR method using stem-loop primer based cDNA synthesis. Specific PCR products corresponding to miR-196b and miR-375 were detected in e14.5 day mouse embryonic pancreas (Fig. 1A). Although miR-196, miR-338-3p, miR-223 and miR-370 have been reported to be expressed in adult mouse pancreas, we were unable to detect it in our experimental conditions consistently (Fig. 1B.). Further, RT-PCR of mouse insulin2 transcript from e14.5 day embryonic pancreas shows the expression of both mIns2 and mIns2-S isoforms (Fig. 1C).

Mouse miR-196b upregulates the expression of mIns2 5'UTR containing reporter gene

Translation regulation ability of these miRNAs was tested using a Luciferase reporter system. Insulin 5'UTR corresponding to mouse insulin1, mIns2 and mIns2-S were cloned as the 5'UTR of Luciferase reporter gene. Double stranded oligonucleotides corresponding to the miRNAs were cloned in pSuper vector and were transfected into HEK cells. The luciferase reporter plasmids, along with the control renilla plasmid were

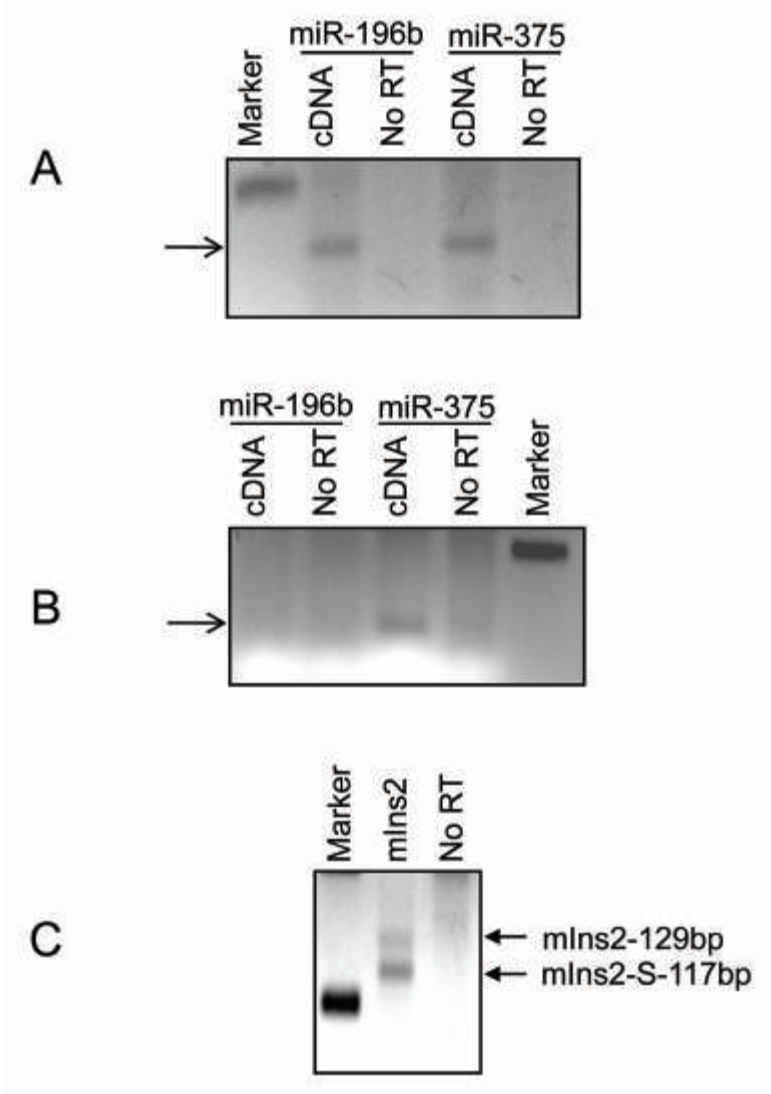

Fig. 1: Expression of miR-196b and mIns2 transcript in mouse embryonic pancreas; **(A-B)** The cDNA for miR-196b was prepared by miR-196b-RT stem-loop primer and amplified with primer 18 and 20. The cDNA for miR-375 was prepared by miR-375-RT stem-loop primer and amplified from e14.5 day pancreas. **(A)** or adult pancreas. **(B)** The PCR product was resolved on a 3.5% agarose gel and samples as mentioned. **(C)** The insulin2 RT-PCR with primer 24 and 25 from e14.5 day mouse embryonic pancreas.

transfected, and after 48hr the reporter Luciferase activity was measured. Expression of miR196b resulted in increased levels of relative Luciferase activity in constructs containing the target mIns2 5'UTR by about 70% while it had no effect on the other 5'UTR luciferase constructs in HEK293T cells (Fig 2A). The over expression of miR-196b by co-transfection of miR-196b pSUPER and mIns2-luciferase construct in ßTC6 cells did not show any up-regulation which could be simply because of its low transfection efficiency. Expression of other miRNAs (miR370 and miR338-5p) did not have any effect on the normalised Luciferase expression levels even for the constructs containing the target miRNA sequence. The miR196b target site is just at the exon-exon junction of the insulin2 mRNA which makes it very specific to this mIns2 isoform and not the mIns2-S splice variant which lacks the miR-196b seed sequence.

To confirm the effects of the miR196b, we synthesized the duplex miRNA and co-transfected it with the mIns2-5'UTR-luciferse construct in HEK293T. A ~240% increase in

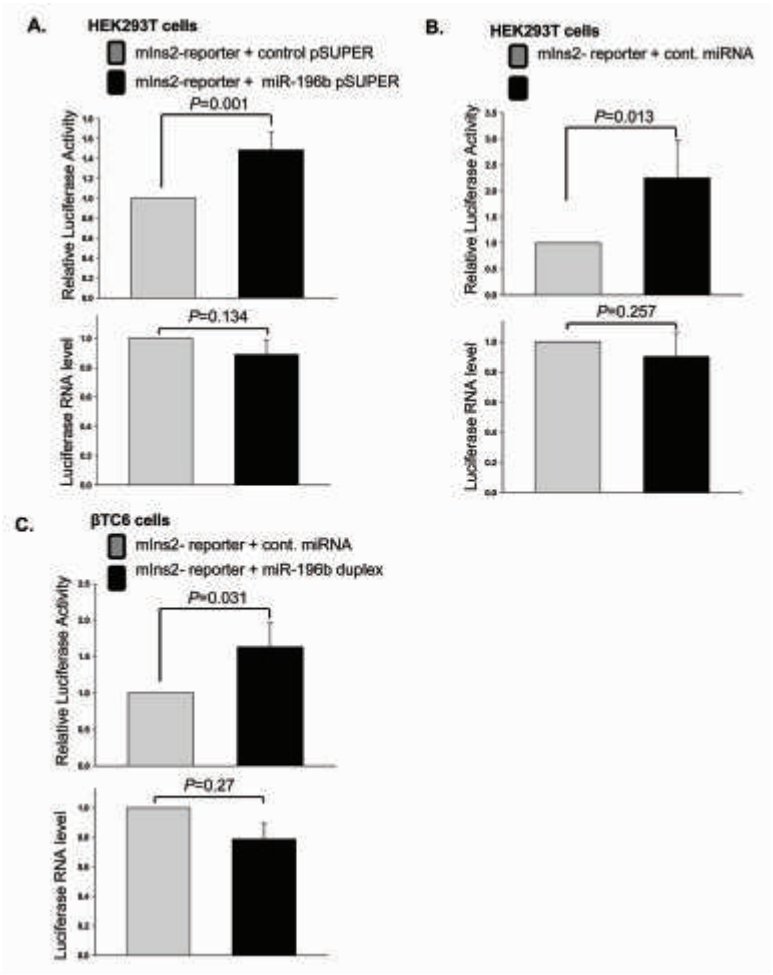

Fig. 2: In vivo role of miR-196b in mIns2-reporter expression. **(A)** HEK293T cells were co-transfected with mIns2 reporter construct and miR-196b pSUPER/cont miR pSUPER. 48hrs later, firefly and Renilla luciferase activities were measured. The fold change in translation efficiency is shown for the mIns2 reporter and the translatable mRNA level is shown below the bars for respective samples. **(B-C)** The miR-196b duplex/cont miRNA was transfected along with mIns2 reporter construct and Renilla luciferase as internal control in HEK293T. **(B)** or ßTC6 **(C)** or. 48hrs later, firefly and Renilla luciferase activities were measured. The fold change in translation efficiency is shown for the mIns2 reporter as the luciferase mRNA level is shown below the bars for respective samples.

luciferase expression was observed when compared to control miRNA without significant change in the mRNA level (Fig. 2B). Similarly in ßTC6 cells, an insulin producing cell line, we observed an increase in the Ins2 5'UTR containing luciferase translation compared to the control miRNA transfection (Fig. 2C). The control mIns2-S luciferase reporter did not show any significant change in the expression with miR-196b transfection in ßTC6 cells. These data suggest that the underlying mechanism of the miR196b mediated translation regulation of the insulin2 5'UTR containing mRNA is not restricted to only insulin producing cells.

The specificity of the miR196b mediated translation regulation was shown by the inhibition of the miRNA effect by antisense transcript. The Luc reporter plasmid was co-transfected with miR-196b or cont. miRNA and 2'O-methylated antisense inhibitor of miR196b in HEK293T cells. miR-196b inhibitor blocks the miR-196 mediated activation of mIns2-5'UTR-Luciferase translation. (Fig. 3A.). The control reporter without the miR196b target site shows no significant change in expression with the inhibitor (Fig. 3B). Similar results were obtained with ßTC6 cells (Fig. 3C). These results show that mouse miR-196b regulates the translation of the mouse mIns2 mRNA by targeting its 5'UTR.

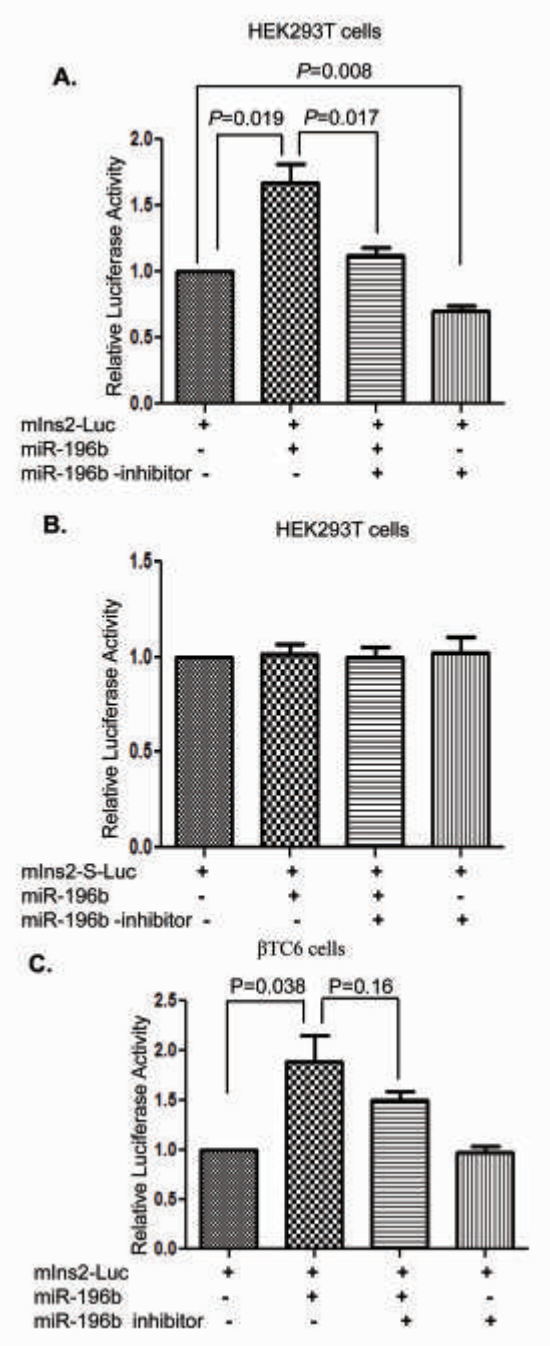

Fig. 3: Translation regulation of mIns2-reporter by miR-196b can be abolished by the inhibitor. **(A-B)** Anti sense inhibitor against miR-196b was introduced into HEK293T cells transfected with reporter and the miRNA-pSuper as mentioned in the figure. After 48 hrs the relative luciferase activity in mIns2 **(A)** or mIns2-S **(B)** reporter transfected cells. **(C)** miR-196b inhibitor and miR-196b duplex/control miRNA was transfected into ßTC6 cells followed by mIns2-luciferase reporter transfections. After 48 hrs the relative luciferase activity was measured.

Since miR196b targets the 5'UTR and activates translation, we tested whether Ago2 is required for this miRNA mediated translation regulation. We knocked down the Ago2 with specific siRNA in HEK293T cells and transfected the reporter containing mIns2 5'UTR along with miR-196b. The Ago2-siRNA reduced the Ago2 expression by almost 70% and the miR196b mediated translation activation was also completely inhibited while the control siRNA did not show any significant alterations in the expression levels (Fig. 4A).

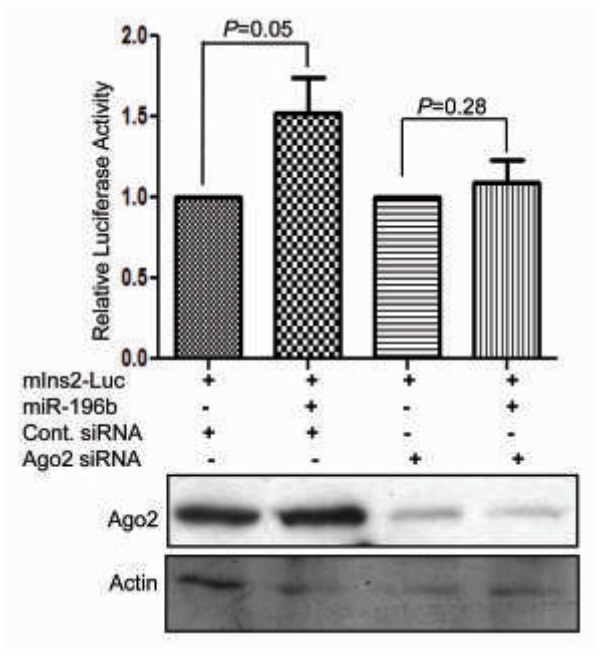

Fig. 4: miR-196b require RISC to up-regulate the target mRNA translation. Luciferase reporter with miR-196b/cont pSUPER vector introduced into control- or Ago2-siRNA transfected HEK293T cells and the fold change in luciferase expression over control is shown in the Y-axis upon miR-196 over expression. All graphs show the error bar with S.D. of at least three independent experiments. The lower panel shows the Ago2 silencing by the Immunoblotting and the lanes as mentioned in the figure.

The physiological relevance of this regulation is still unclear as mIns2 isoform contributes only about 13 % of total insulin mRNA and also it is poorly translated when compared to the mouse Insulin1 or the mIns2-S isoform. Thus under normal physiological conditions the protein product from this mRNA isoform is less than 10% of the total insulin. But this regulation could be important in embryonic development where it can significantly increase the insulin production from few insulin mRNA transcripts. In summary, the present study demonstrates for the first time that specific miRNA can target insulin mRNA and can regulate its translation. We believe that miR-196b interaction with insulin mRNA disrupts the interaction of specific factors to the mRNA thereby resulting in translation activation.

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

Background

Based on morbidity, mortality, economic burden, and emotional hardship, cancer may be considered the most onerous health problem afflicting people worldwide. Currently, over 22.4 million people in the world are suffering from cancer. Approximately 10.1 million new cases are diagnosed with cancer annually, and more than 6.2 million die of the disease in the year 2000. This represents an increase of around 19% in incidence and 18% in mortality since 1990. An important aim of cancer research is to find therapeutic compounds having high specificity for cancerous cells/tumor and fewer side effects than the presently used cytostatic/cytotoxic agents.

Numerous plant-derived compounds used in cancer chemotherapy include vinblastine, vincristine, camptothecin derivatives, etoposide derived from epipodophyllotoxin, and paclitaxel (taxol®). However most of these compounds exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non-tumor cells, and some of them failed in earlier clinical studies. Another most widely used metal-based drug at present against selected types of cancers is cisplatin, but use of cisplatin in curative therapy was associated with some serious clinical problems, such as severe normal tissue toxicity and resistance to the treatment. These side effects limit their use as chemotherapeutic agents despite their high efficacy in treating target malignant cells. Consequently, new therapies and treatment strategies for this disease are necessary for treating patients with this disease. Therefore, the search for alternative drugs that are both effective in the treatment of cancers as well as non-toxic to normal tissue is an important research line.

Terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Natural triterpenoids, such as oleanolic acid and ursolic acid, are compounds with anti-tumorigenic and anti-inflammatory properties. Synthetic triterpenoid derivatives such as 2-Cyano-3, 13 dioxooleana-1,9(11)-dien-28-oic acid

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(CDDO) and its derivative 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole (CDDO-Im) also have anti-tumor activity.

Recently, we reported an isolation and characterization of a new compound *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. The structure of AECHL-1 with its mass fragments by NMR spectroscopy is shown in Figure 1. This compound possesses anti-cancer activity against a variety of cancer cell lines of different origin, including B16F10, MDA-MB-231, MCF-7, and PC3 cells between 12-48h with minimum growth inhibition in normal HEK 293. The antitumor effect of AECHL-1 was comparable with that of the conventional antitumor drugs paclitaxel and cisplatin. AECHL-1-induced growth inhibition was associated with S/G_2-M arrests in MDA-MB-231, MCF-7, and PC3 cells and a G_1 arrest in B16F10 cells. We observed microtubule disruption in MCF-7 cells treated with AECHL-1 in vitro. Compared with control, subcutaneous injection of AECHL-1 to the sites of tumor of mouse melanoma B16F10 implanted in C57BL/6 mice and human breast cancer MCF-7 cells in athymic nude mice resulted in significant decrease in tumor volume. In B16F10 tumors, AECHL-1 at 50 µg/mouse/day dose for 15 days resulted in increased expression of tumor suppressor proteins P53/p21, reduction in the expression of the oncogene c-Myc, and downregulation of cyclin D1 and cdk4. Additionally, AECHL-1 treatment resulted in the phosphorylation of p53 at serine 15 in B16F10 tumors, which seems to exhibit p53-dependent growth inhibitory responses (Lavhale et. al. *PLoS One*. 2009;4(4):e5365).

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. Understanding the mechanism of action of AECHL-1 governing its bioactivity is essential for the development of developing further 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. 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.

Also, invasion and metastasis, the hallmark of malignant tumor, is 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, how to prevent tumor metastasis has been the biggest challenge in cancer chemotherapy. In recent years, many natural products have been found to have 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-

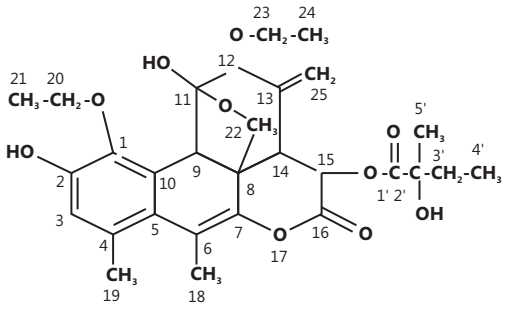
κB activation, induce apoptosis, inhibit signal transducer, and activate transcription and angiogenesis. Several triterpenoids, including ursolic and oleanolic acid, betulinic acid, celastrol, pristimerin, lupeol, and avicins possess antitumor and anti-angiogenic properties. AECHL-1 is also a tri-terpenoid and the anti-angiogenic activity of it has not been investigated.

Aims and Objectives

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

Work achieved

Our previous work showed that AECHL-1 treatment for 12 to 48 hr inhibited cell proliferation and induced death in B16F10, MDA-MB-231, MCF-7, and PC3 cells with minimum growth inhibition in normal HEK 293. The antitumor effect of AECHL-1 was comparable with that of the conventional antitumor drugs paclitaxel and cisplatin. AECHL-1-induced growth inhibition was associated with S/G_2-M arrests in MDA-MB-231, MCF-7, and PC3 cells and a G_1 arrest in B16F10 cells. We observed microtubule disruption in MCF-7 cells treated with AECHL-1 *in vitro*. Compared with control, subcutaneous injection of AECHL-1 to the sites of tumor of mouse melanoma B16F10 implanted in C57BL/6 mice and human breast cancer MCF-7 cells in athymic nude mice resulted in significant decrease in tumor volume. In B16F10 tumors, AECHL-1 at 50 µg/mouse/day dose for 15 days resulted in increased expression of tumor suppressor proteins p53/p21, reduction in the expression of the oncogene c-Myc, and downregulation of cyclin D1 and cdk4. Additionally, AECHL-1 treatment resulted in the phosphorylation of p53 at serine 15 in B16F10 tumors, which seems to exhibit p53-dependent growth inhibitory responses.



AECHL [m/z : 543 C $_{29}H_{36}O_{10}$]

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

Since cytotoxicity activity of AECHL-1 was found to be highly effective against hormone dependent breast cancer cells MCF-7, the present study was conducted to investigate its underlying mechanisms. Flow cytometry TUNEL assay was carried out to confirm the

induction of apoptosis by AECHL-1 in MCF-7 cells. After 12, 24, 36 and 48 h exposure of AECHL-1, approximate 30% and 50% of MCF-7 cells were stained as TUNEL positive, respectively (Fig. 2).

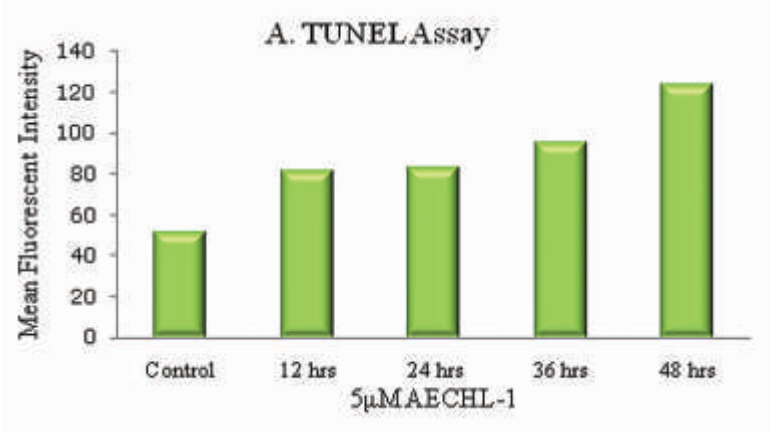


Fig. 2: Time dependent measurement of apoptosis in MCF-7 cells induced by 5 μM AECHL-1 assessed by TUNEL assay.

With mitochondria being an important mediator of apoptosis, and Ca^{2+} playing a crucial role in mitochondrial function as well as being an important inducer of apoptosis via mitochondria, we decided to study the effect of AECHL-1 on the levels of mitochondrial Ca^{2+} load. Flow cytometry analyses using a mitochondria specific calcium probe Rhod-2AM showed that AECHL-1 increased the mitochondrial calcium between 16-20h (Fig. 3A). The results described above suggested that the increase in mitochondrial calcium overload may be a consistent biochemical event resulting from AECHL-1 treatment. To further evaluate the role of increased mitochondrial calcium in AECHL-1-induced apoptosis, we used the cell-permeable calcium chelator 1, 2-bis(2-aminophenoxy) ethane-N,N,N′,N′ - tetraacetic acid tetrakis acetoxymethyl ester (BAPTA-AM) to test if chelation of cytosolic Ca^{2+} would alter the amount of drug-induced cell death. As illustrated in Fig. 3B, exposure of MCF-7 cells to AECHL-1 (5μM) resulted in a loss of 40% of cells within 24 hours. Addition of 20 μM BAPTA-AM, which by itself did not alter cell

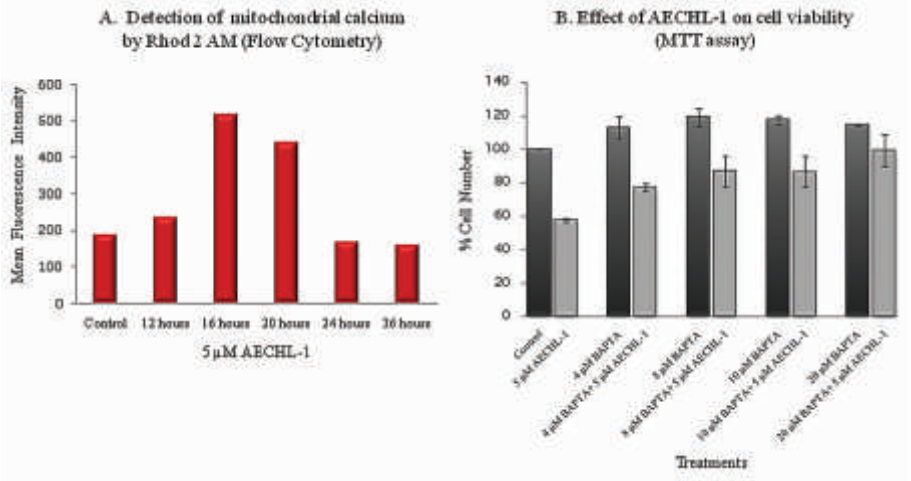


Fig. 3: Role of mitochondrial calcium in AECHL-1 treated MCF-7 cells. (A) Mitochondrial calcium in 5μM AECHL-1 treated MCF-7 cells was determined using Rhod 2 AM (2μM) by flow cytometry. (B) Effect of calcium chelator BAPTA on cell viability of 5μM AECHL-1 treated MCF-7 cells was determined by MTT assay.

viability at 24 hours, substantially suppressed AECHL-1-induced cell death as assessed by MTT assay, resulting in a loss of only 5-10% of the cells. These data suggest that an increase of mitochondrial calcium may be a critical event in mediating AECHL-1-induced apoptosis.

Increase in the mitochondrial Ca^{2+}, is known to increase the reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels which play an important role in the process of apoptosis in many cell types. Increased caspase-dependent apoptosis, ROS generation and mitochondrial damage are phenomena, which can be frequently observed altogether in cells subjected to anticancer drugs treatment, that is, accumulation of ROS inside the cell often signalizes apoptosis or terminal differentiation. Generation of ROS and RNS in cells was monitored during the treatment with AECHL-1 for different time periods. A significant increase in the fluorescence of DHE (probe for cytosolic superoxide) up to 48h, an increase in the fluorescence of MitoSox Red (probe for mitochondrial superoxide) and an increase in the fluorescence of DHR-123 (probe for mitochondrial peroxynitrite) was observed at 24h (Fig. 4A-C). However, DCF-DA (probe for H_2O_2) and DAF-2DA (probe for NO.) failed to increase after AECHL-1 treatment.

Also MCF-7 cells treated with 5μM AECHL-1 for 24 hrs showed a decrease in GSH levels as assessed using mBCI (a probe for cellular GSH content), indicating a decrease in cellular anti-oxidant levels after AECHL-1 treatment (Fig. 4D).

Whether the ROS produced by AECHL-1 treatment played any direct role in apoptosis was checked using anti-oxidants NAC and it was found that the cell viability after AECHL-1 treatment as assessed by MTT was not restored proving that the ROS produced was not responsible for apoptosis (Fig. 4E).

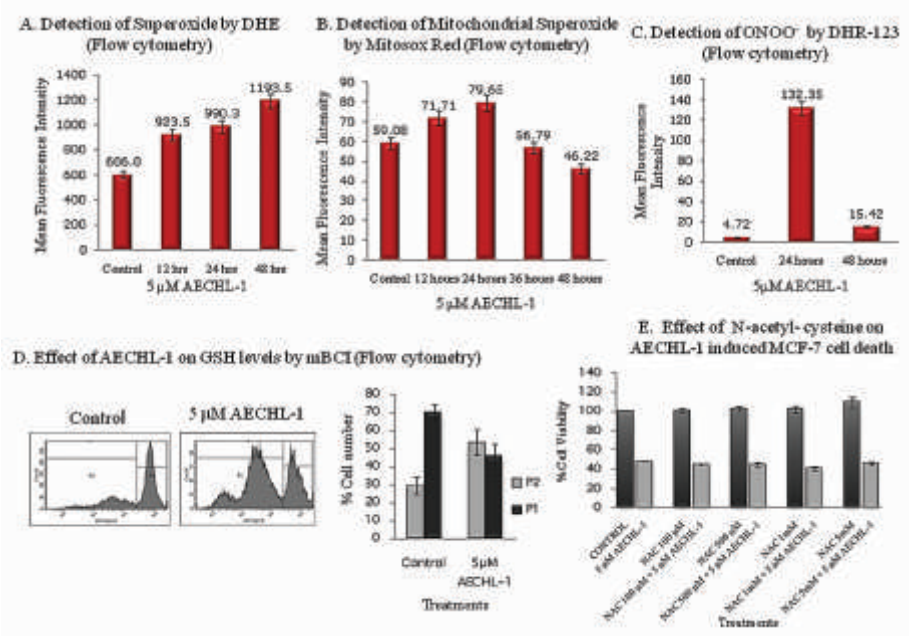


Fig. 4: Effect of AECHL-1 on induction of oxidative and nitrosative stress in MCF-7 cells. **(A)** The intracellular ROS levels were measured by flow cytometry in terms of O_2^- using 3μM DHE, **(B)** mitochondrial superoxide levels were measured using 5μM Mitosox Redby flow cytometry, **(C)** The intracellular RNS levels were measured in terms of $OONO^-$ using 5μM DHR-123 by flow cytometry. **(D)**The effect of AECHL-1 on GSH levels by flow cytometry was done usingm BCI. **(E)** Effect of the antioxidant N-acetyl- cysteine on AECHL-1 induced cell death was assessed using MTT assay.

It is known that increase in ROS levels disrupt the mitochondrial membrane potential (MMP) to execute the apoptosis, the third experiment was carried out to find out whether AECHL-1 treatment caused any changes in MMP. As shown in Fig. 5, incubation of MCF-7 cells with 5 µM AECHL-1 disrupted the MMP in a time dependent manner as determined using JC-1.

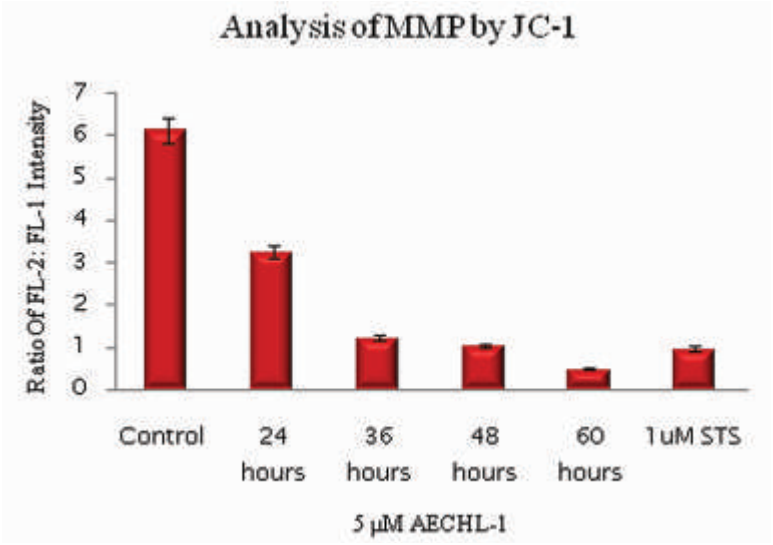


Fig. 5: Analysis of mitochondrial membrane potential of AECHL-1 treated MCF-7 cells by flow cytometry was done using the cationic dye JC-1.

Since disruption of MMP leads to induction of apoptosis by intrinsic pathway via caspase induction, in the next experiment the effect of caspase inhibitors such as caspase 2 inhibitor, caspase 9 inhibitor, caspase inhibitor I and pan caspase inhibitor viz. Z-VAD-FMK on cell viability by MTT as well as caspase activation after AECHL-1 treatment was measured by Flow cytometry and it was found that treatment of MCF-7 cells with AECHL-1 did not induce the activation of caspases nor did the caspase inhibitors revert AECHL-1 induced apoptosis, indicating that AECHL-1induced apoptosis to be caspase independent (Fig. 6A and B). Also if AECHL-1 follows the caspase independent pathway mediated by AIF (Apoptosis Inducing Factor) is still to be studied. Thus AECHL-1 induced apoptosis was found to be Ca^{2+} dependent but independent of caspase activation.

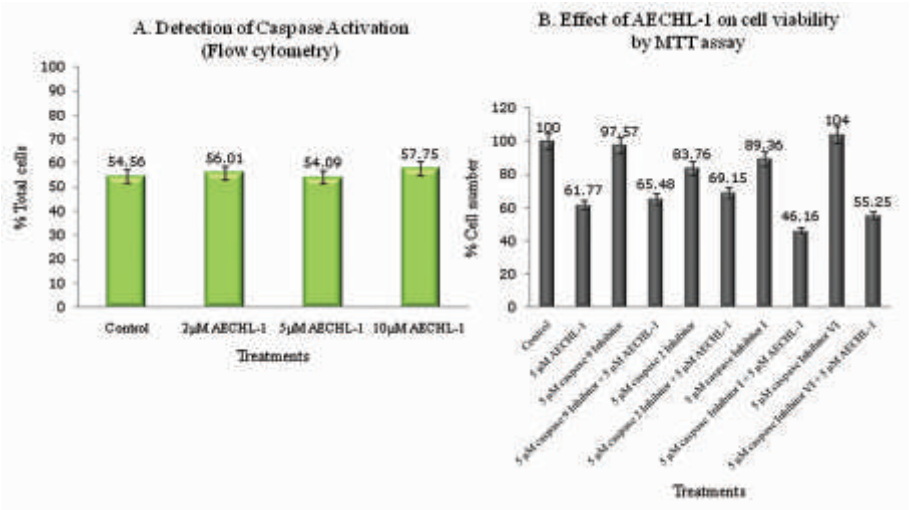


Fig. 6: Detection of caspase activation. **(A)** Activation of caspases in AECHL-1 induced MCF-7 cell death was assessed using a caspase activation kit. **(B)** Effect of caspase inhibitors such as, caspase 9 inhibitor, caspase 2 inhibitor, caspase inhibitor I and VI on AECHL-1 induced MCF-7 cell death was performed using MTT assay.

Further to evaluate the anti-angiogenic activity of AECHL-1, MTT assay was carried out in HUVECS (5000/well) cultured in 96-well plates and treated with different concentrations of AECHL-1 (0–30μM) for 48 hr at 37°C.A concentration dependent decrease in HUVEC survival was seen. After 48 hrs 25μM AECHL-1 induced maximum cell death (Fig. 7A). Also a dose dependent decrease in the proliferation of HUVECs as determined by (^{3H}) thymidine incorporation was observed in response to increased concentrations of AECHL-1 (Fig. 7B). In order to evaluate the total percentage of apoptotic cells induced by AECHL-1 Annexin V-conjugated FITC and propidium iodide (PI) stain was used. The total percentage of apoptotic cells increased up to 1.3% at 5 μM and 3% at 10μM from 0.62% in the control population (Fig. 7C).

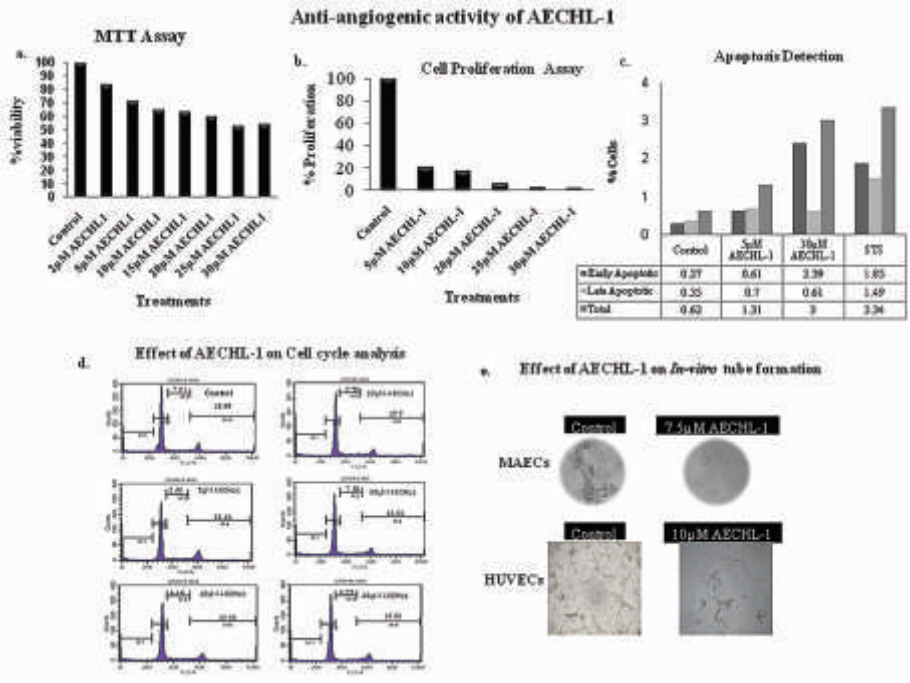


Fig. 7: Anti-angiogenic activity of AECHL-1. HUVECS were treated with different concentrations of AECHL-1 for 24-48hr **a)** cell viability was determined by MTT assay. **b)** Cell proliferation by (3H) thymidine incorporation **c)** Apoptosis by Annexin V FITC/PI staining. **d)** Cell cycle analysis by PI staining. **e)** Inhibition of tube formation by HUVECS and MAECS on matrigel.

In order to determine the phase of cell cycle at which AECHL-1 exerts its growth inhibitory effect, exponentially growing HUVECS were treated with different concentrations of AECHL-1 for 24 hrs and analyzed by flow cytometry (Fig. 7D). It was observed that 5μM AECHL-1 induced a G_2M arrest, as there was an increase in the G_2M population (23.1-32.5%) with a concomitant decrease in the G1 population (72.8-63.1%).

The classical in vitro tube formation assay on matrigel was carried out on MAECs (Mouse Aortic Endothelial Cells) and HUVECs. AECHL-1 was seen to inhibit in-vitro tube formation in MAEC as well as HUVECs at concentrations of 7.5μM and 10μM respectively (Fig. 7E).

Future work

We will continue to investigate the anti-angiogenic efficacy / potency and molecular mechanism of the novel anti-cancer compound AECHL-1 using in vivo models.

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Studies on the role of IL-3 in regulation of osteoblast differentiation

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Background

In bone microenvironment interactions between T cells, osteoclasts, and osteoblasts are very crucial in maintaining the bone homeostasis. IL-3, a cytokine secreted by T helper cells is known to stimulate the proliferation, differentiation and survival of hematopoietic stem cells. Previously, we have demonstrated that IL-3 is a potent inhibitor of osteoclast formation and bone resorption in both mouse and human hematopoietic cells. IL-3 also prevents the development of inflammatory arthritis, and protects cartilage and bone destruction in mice. However, the role of IL-3 on osteoblast differentiation and bone formation is not yet delineated. IL-3 exerts its biological effects by binding to its specific receptor IL-3Rα. Expression of IL-3Rα on human mesenchymal stem cells (MSCs), pre-osteoblasts and mature osteoblasts indicated that these cells could be the target for IL-3 action. Recently, we demonstrated that IL-3 significantly increases the osteoblast differentiation and mineralized bone matrix synthesis in human bone marrow-derived MSCs. IL-3 increases the expression of early and late markers of osteoblast differentiation and the specific transcription factors Runx-2 and osterix. We also found that IL-3 increases osteoblast differentiation by regulating the expression of bone morphogenetic protein-2. In further studies, we investigated the role of IL-3 in migration and would healing abilities of human bone marrow-derived MSCs and its characteristics.

Aims and Objectives

1. To investigate the role of IL-3 in migration and wound healing abilities of MSCs.
2. To study the effect of IL-3 on characteristics of MSCs.

Work Achieved

IL-3 enhances the migration and wound healing abilities of human MSCs

Healing of bone damage or regeneration of new bone requires recruitment of MSCs with increased potential for osteoblast differentiation and bone formation. The chemotactic molecules that induce the migration of MSCs to appropriate microenvironment are important for induction of bone formation. Since IL-3 enhances the osteoblast

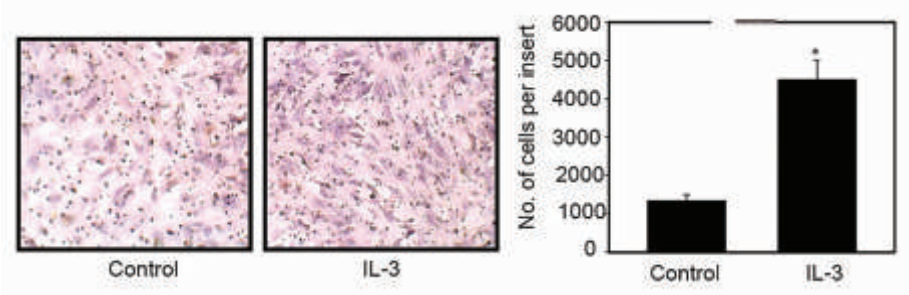

Fig. 1: Effect of IL-3 on migration of human MSCs. Human MSCs (2 x 10^5 cells in 200 µl/insert were added to the upper chamber and IL-3 in 500 µl was added to the lower chamber of cell culture inserts. After 18 hours cells from the upper side of the filters were removed and cells at the lower side of the filter were stained with hematoxylene. Cells from some inserts were also recovered from lower side of filter by trypsinization and counted. Data are expressed as number of migrated cells per insert. *$p \leq 0.05$. Similar results were obtained in four independent experiments.

differentiation we checked whether IL-3 induces in vitro migration of MSCs using transwell migration chamber assay. Human MSCs were added to the upper chamber of cell culture insert followed by the addition of IL-3 into the lower chamber. After 18 hours cells recovered from the lower side of the filter were counted. The spontaneous migration capacity of MSCs in the presence of medium alone was low and it was significantly increased in the presence of IL-3 (Fig.1)

The migration ability of human MSCs was further confirmed by examining the effect of IL-3 on motility of MSCs using *in vitro* wound healing assay that mimics cell migration during *in vivo* wound healing. Human MSCs were seeded in tissue culture plates and after 80-90% confluency wounds were created on monolayer of MSCs. The wounded cells were incubated for 20 hours in the presence or absence of IL-3. As compared to control, many IL-3 treated MSCs migrated from the edge of the wound (Fig. 2). These results suggest that IL-3 induces migration and wound healing abilities of MSCs, which would be beneficial for the increased homing of MSCs towards the wound. Although, we did not analyze whether IL-3 induces migration of MSCs in vivo, our results suggest that IL-3 may play a dual role in inducing differentiation of MSCs into osteoblasts as well as migration of MSCs towards the site of bone damage.

Effect of IL-3 on characteristics of MSCs

It has been reported that IL-3 indirectly inhibits osteoblast differentiation in multiple myeloma patients through increased $CD45^+$ cell population. Therefore, the effect of IL-3

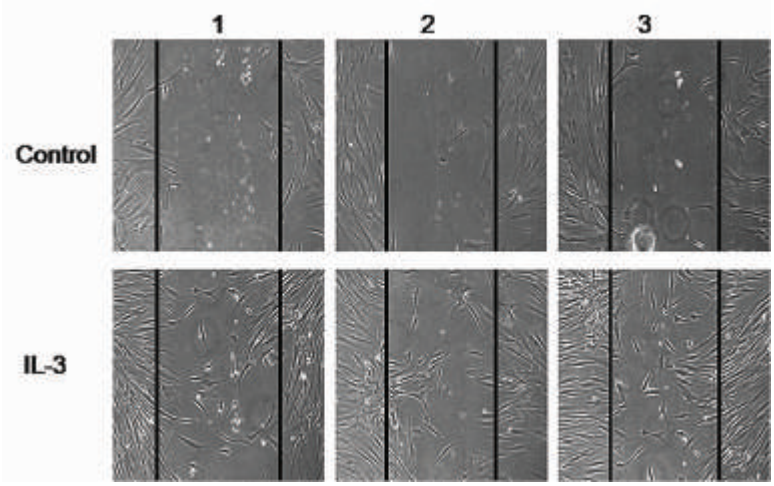

Fig. 2: Effect of IL-3 on wound healing ability of human MSCs. Human MSCs (10^5 cells) were plated in 35 mm culture plates. After 80-90% confluency wounds were created using 200 µl pipette tip. Culture plates were washed to remove cell debris and cells were further incubated for 20 hours in the presence or absence of IL-3. The wound healing was observed under microscope. Magnification, 20X. 1, 2 and 3 are triplicate cultures.

on expression of surface markers of MSCs was evaluated. Purified human MSCs were incubated with or without IL-3 for 7 days and percent total expression of CD29, CD44, CD73, CD90, CD105 and CD34, CD45 was analyzed by flow cytometry. We observed that IL-3 did not alter the expression of MSC surface markers. The number of $CD45^+$ and $CD34^+$ cells was negligible (< 5%) and IL-3 treatment did not increase the number of these hematopoietic cells. These results suggest that IL-3 enhances human osteoblast differentiation and bone formation by direct action on MSCs.

Osteoblasts and adipocytes share a common progenitor and decrease in bone mass of age-related osteoporotic patient is accompanied by an increase in marrow adipose tissue. Increased adipocyte differentiation may lead to increased fat deposition in the body. Therefore, the role of IL-3 on differentiation of human MSCs into adipocytes was also studied. MSCs were incubated for 28 days with repeated cycles of adipogenic induction and maintenance media in the presence or absence of different concentrations of IL-3. Quantitative analysis of lipid droplets was performed by extracting the Oil Red O dye. It was observed that lipid droplet accumulation was not altered during adipocytes differentiation in the presence of IL-3. These results indicate that IL-3 enhances osteoblast differentiation without affecting adipocytes formation.

Future Work

1. To investigate the mechanism(s) of IL-3 induced migration of MSCs.
2. To investigate the role of IL-3 on bone remodeling in ovarietctomised induced bone loss in animal models of human osteoporosis.

Research Report

Chromatin Architecture & Gene Regulation

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Global gene regulation by tumor suppressor SMAR1

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Background

The nuclear matrix forms a scaffold upon which the chromatin is organized into periodic loop domains called Matrix Attachment Regions (MAR) by binding to various MAR binding proteins (MARBPs). Aberrant expression of MARBPs modulates the chromatin organization and disrupt transcriptional network that leads to oncogenesis. Dysregulation of nuclear matrix associated MARBPs have been reported in different types of cancers. Some of these proteins have tumor specific expression and are therefore considered as promising diagnostic or prognostic markers in few cancers. SMAR1 (Scaffold/ Matrix attachment region binding protein 1), is one such nuclear matrix associated candidate tumor suppressor whose expression is drastically reduced in higher grades of breast cancer. *SMAR1* gene is located on human chromosome 16q24.3 locus, the loss of heterozygosity (LOH) of which has been reported in several types of cancers.

SMAR1 was identified as novel DNA binding protein from murine thymocyte expression library. The human homolog of SMAR1 named BANP was isolated as a BTG3 associated nuclear protein using yeast two hybrid approach. The BTG3 (B-cell translocation gene) is the member of anti-proliferative gene family which includes BTG1 and BTG2. The human SMAR1 is present on chromosome 16q24.3, while mouse SMAR1 maps to distal portion of chromosome 8 at a distance of 111.8 cM. Both human and mouse *SMAR1* contains 14 exons, but in mouse there is 23kb insertion between exon 1 and 2. *SMAR1* gene encodes a 2.1 kb long mRNA that corresponds to 60 kDa full length protein (548 amino acids). Protein sequence analysis revealed that SMAR1 shares homology with other MARBPs such as Cux/CDP, Bright and SATB1 in the MAR binding regions. The MAR binding domain of SMAR1 resides within 352-394 amino acids. In addition to this, 398-456 aa region of SMAR1 is homologous to the tetramer domain of Bright. The small domain of SMAR1 within 160-350 aa contains nuclear localization signal (NLS). Additionally, SMAR1 also contains the BEN domain residing between 218-291 amino acid residues. The BEN domain is predicted to mediate protein-DNA and protein-protein interactions during chromatin organization. The 160-350 aa region which contains BEN domain interacts

with MAR-binding protein Cux/CDP, and the HDAC/SIN3complexes. The C-terminal region of the BEN domain overlaps with the MAR-binding region in SMAR1.

Since SMAR1 has been mapped to the 16q24.3 loci, its potential role in breast and other cancers was investigated. The expression analysis of SMAR1 mRNA and protein in an array of breast cancer derived lines revealed that SMAR1 level was significantly low in MCF-7, MDA-MB-231, MDA-MB 468, HBL-100, ZR75.1 and ZR75.3. Further studies on human breast tumor clinical specimens of different histological grades confirmed that SMAR1 expression is down-regulated in infiltrating ductal carcinomas (IDC) of grades I, II and III.

Studies from our lab have well established the role of SMAR1 in inflicting cell cycle arrest and its role in DNA damage induced apoptosis was elucidated recently by Sinha et al. (2010). This study demonstrated that depending on the extent of DNA damage, SMAR1 modulates p53 activity to dictate the cell fate i.e. towards arrest or apoptosis.

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

Alternative splicing of CD44 by SMAR1 and its Implication in cancer

Cancer is a complex process where signals follow distinct paths from the extracellular matrix to the nucleus and back again. 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 found that nuclear matrix protein SMAR1 interacts with splicing co-activator SRm160 which is known to regulate Ras dependent CD44 alternative splicing and also enhances constitutive splicing. Inclusion of variable exons in CD44 mRNA is dependent on MAP kinase signaling pathway. CD44 has 10 constant exons and 10 variable exons residing between constant exon 5 and 6. Higher levels of CD44 variants confer strong metastatic potential to tumors. Our study revealed that knock down of SMAR1 enhances the inclusion of CD44 variable exons. We found SMAR1 interacts with STAR family protein Sam68 endogenously and MAP kinase mediated activation induces phosphorylation of SMAR1 on the threonine residue of the consensus TP site by ERK and mediates the translocation of protein from the nucleus to cytoplasm. Inhibition of ERK with U0126 and nuclear export inhibitor Leptomycin B retains the protein in the nucleus. Using RNA immunoprecipitation and Chromatin IP, we found that SMAR1 is accumulated into the coding region along the variable exons of CD44 endogenously, which inversely correlates with the RNAPII Ser2 phosho-CTD upon ERK activation. These results conclude that the low levels of SMAR1 in the higher grade tumors, confers the metastatic potential by favourable inclusion of the variable exons.

DNA damage repair by SMAR1 through Ku70 deacetylation

DNA is the blueprint by which the cell passes its hereditary information to its progeny. The integrity of the genome is fundamental to the propagation of life, illustrated by the complexity of the DNA replication and repair mechanisms. Besides the basal levels of DNA lesions generated from normal cell metabolism, damage is further induced by genotoxic stress. DNA damage plays a major role in mutagenesis, carcinogenesis and ageing. Failure to repair damaged DNA gives rise to mutations and chromosomal abnormalities. Hence any damage in this information can be detrimental to the cell leading to many consequences as infection, inflammation, altered protein expression, cancer, cell division arrest and ultimately apoptosis.

The DNA damage response pathway is a signal transduction pathway consisting of sensors, transducers and effectors. Two related and conserved proteins, ATM and ATR, are central components of the DNA damage response (DDR). Scaffold/matrix attachment region binding protein (SMAR1) is one such tumor suppressor stress responsive protein which is known to globally modify the topology of chromatin (Chattopadhyay *et al*., 2000) and architecture of chromatin depends upon DNA replication and efficient DNA repair because anchoring of the damaged DNA to the nuclear matrix is prerequisite for efficient repair.

Panoptic studies on SMAR1 has revealed that SMAR1 is a stress responsive protein as Doxorubicin, Camptothecin and UV mediated damage induces the expression levels of SMAR1. This has rationalized our intrigue to understand the regulation of DNA double strand breaks by SMAR1. In response to γ-irradiation SMAR1 is rapidly induced, is

phosphorylated at serine 370 by ATM which causes its nuclear translocation and forms irradiation induced foci (IRIF) which colocalizes with γH2AX and. Upon nuclear translocation, SMAR1 facilitates in the DSB repair but still the molecular mechanism is unknown and which has to be studied in detail. SMAR1 directly interacts with Ku70 protein and favors DNA repair by enhancing deacetylation of Ku70 via recruiting histone deacetylase HDAC6. The Ku protein plays a key role in multiple nuclear processes, e.g., DNA repair, chromosome maintenance, transcription regulation, and V(D)J recombination. Ku70 is a critical component of non-homologous end joining (NHEJ) repair process and gets recruited to chromatin in response to DNA damage. Since SMAR1 affects the acetylation status of Ku70, we investigated if the recruitment of Ku70 to chromatin fraction is dependent upon SMAR1 or not. To our surprise, Ku70 recruitment was drastically impaired in the absence of SMAR1 (Fig 1). Functionally SMAR1 facilitates DSB repair by assisting in anchorage of DNA repair factors like Ku70 at the site of repair and causing cell-cycle arrest hence providing ample time for repair. SMAR1 favors the BAX localization in the cytoplasm as evident by more BAX in cytoplasmic fraction upon SMAR1 overexpression and therefore less apoptosis is found in SMAR1 overexpressed cells. Upon SMAR1 knockdown the BAX showed preferential localization in mitochondrial fraction which might be the reason that enhanced apoptosis is found in SMAR1 knocked down cells. These observations provide new insights into how the nuclear matrix binding protein SMAR1 critically modulates DNA repair activity and contributes for the maintenance of genomic integrity. DNA repair mechanism is a promising target of many novel cancer therapies as inefficient repair in the cell is the prime reason for mutations in the genome and development of cancer and involves mutation in the DNA repair genes and tumor suppressor genes. Drugs that target DNA damage checkpoint proteins are already in clinical trials as anticancer drugs and studies on different cellular factors that can regulate DNA damage checkpoints in cancer stem cells and radioresistance will provide insights into novel cellular targets which can lead to improvement in treatment modalities.

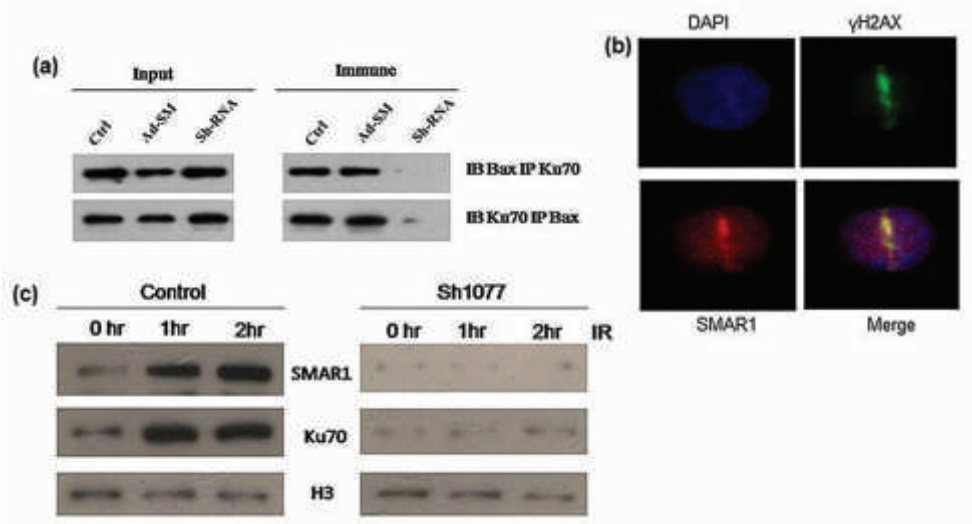

Fig. 1: (a) Immunoprecipitation analysis for Bax and Ku70 interaction upon SMAR1 over expression and knockdown by Ad-SMAR1 and ShRNA 1077 respectively (b) Colocalization of SMAR1 with gamma-H2AX upon laser-microirradiation (c) Recruitment of SMAR1 and Ku70 to chromatin in control and SMAR1 knockdown i.e. Sh1077 treated cells in response to IR.

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

We are working on a matrix attachment region binding protein (MARBP) SMAR1 that globally regulates gene transcription through recruitment of HDAC1-Sin3 complex at various promoters. Previous results from our lab suggested a critical role of SMAR1 in the differentiation of T helper cells to Th1 and Th2 subtypes by the regulation of T-bet promoter. Further extension of SMAR1 mediated regulation of T cell lineage elucidated on yet another function of SMAR1 in regulating Th17 differentiation. The expression level of SMAR1 is downregulated in naïve T cells polarized *in-vitro* towards Th17. Induced expression of SMAR1 inhibits Th17 polarization probably by binding to the MAR regions on the IL17 locus. Research on SMAR1 further assumes it to be a global regulator of gene transcription having multifarious functions in the regulation of other cytokine genes that drives specific T cell lineages. Th17 cells are the most important candidate for the immune response against inflammatory conditions. Hence, regulation of Th17 by a cell intrinsic factor can be a potent regulator of inflammatory responses. Understanding the regulation of the inflammatory responses by SMAR1 will be assessed using over-expressed and T cell specific conditional knock-down mice. In this regard, chemically induced colitis and rheumatoid arthritis models are under study to better understand the function of nuclear matrix proteins in T cell differentiation and thus in immunity through T cell polarization. Mice experimental models of chemically induced colitis showed a protective role of SMAR1 through the disease progression. Conserved MAR sequences were observed in IFNγ and IL17 locus which are the signature cytokines for Th1 and Th17 cell lineages respectively suggesting the regulation of these genes by SMAR1. Many reports described the chromatin modulation associated with the polarization of naïve T cells to different lineages. Inter and intra chromosomal interactions and the histone modifications culminate in the activation of genes specific for one particular lineage and the repression of others. The molecular mechanisms associated with the chromatin changes are still to be understood clearly.

Future work

1. Global gene regulation will be checked using proteomics approach where overexpression and downregulation of SMAR1 will be done in cancer cells.
2. We shall be using both asthma and colitis as disease models to find out role of SMAR1 in fine tune regulation of Th1, Th2 and Th17 differentiation.
3. We find that one of major target of SMAR1 is to repress transcription from miR 371-373 cluster. We are further looking into the anti metastatic properties of SMAR1 through regulation of miRNA 373. Also, in future we shall look into the role of miR 373 in nuronal differentiation from human ES cells where SMAR/ BANP plays a pivotal role.

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Mechanisms of global gene regulation by SATB1

Background

T-lymphocyte development is a complex and tightly controlled process that requires coordinated action of multiple signaling pathways, which alter transcription status of the cell at different stages of development. This process is executed by an elaborate orchestration of transcriptional regulation within chromatin domains. Chromatin remodelers, modifiers and organizers together control gene expression in a 3-dimensional matrix of chromatin within the nucleus and this is important in coordinating the developmental program of the cell. The T lineage-enriched chromatin organizer SATB1 was identified by its property to bind specialized genomic segments referred to as matrix attachment regions that are important for higher order loop domain structure of chromatin. SATB1 and orthologous proteins can act as global regulators of cell function in specific cell lineages.

Aims and Objectives

1. Study how SATB1 mediates Wnt signaling in T cells.
2. To delineate the role of SATB1 in T helper cell differentiation.

Work Achieved

SATB1 expression level seems to dictate T-cell fate

T cell development and differentiation is coordinated by multitude of signaling processes and transcription factors that impart distinct functional properties on progenitors. We have focused on understanding the role of 'Special AT-rich binding protein 1' (SATB1) in T cell development and differentiation. SATB1 is a T-lineage-enriched chromatin organizer and global regulator. In mice lacking SATB1 thymocyte development is stalled at double positive (DP) stage suggesting a critical role for SATB1 in thymocyte development. Recent reports from our laboratory and other groups have demonstrated that SATB1 is known to play an important role in regulation of Interleukin 4 (IL-4) gene locus in peripheral CD4+ T cells and thereby regulate T-helper 2 (TH2) differentiation. Here we show that SATB1 is differentially expressed in various subsets of thymocytes. This finding implicates that

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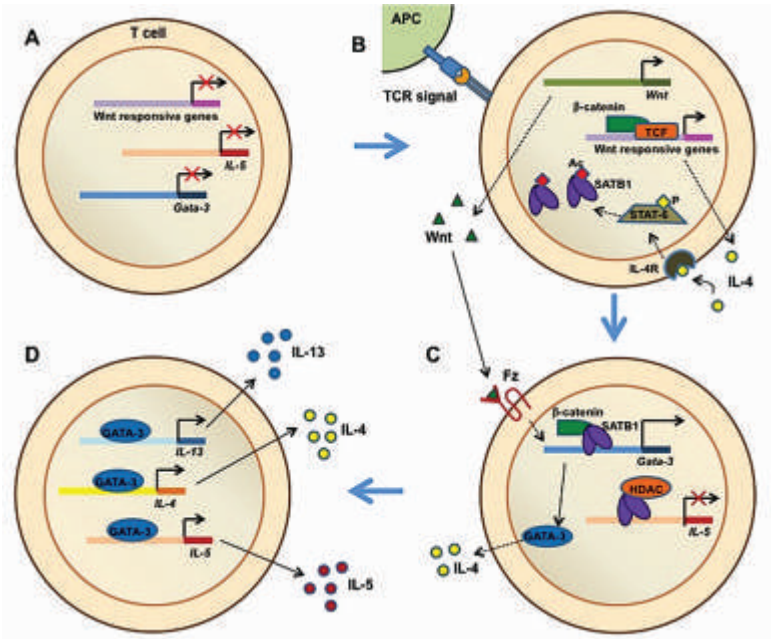

Fig. 1: Role of SATB1 during T_H2 differentiation. **A. The gene expression status of $CD4^+$ T cells prior to TCR encounter.** In precursor $CD4^+$ T helper cells (T_HP) Wnt responsive genes and T_H1/ T_H2 specific genes are repressed. **B. T_H0 polarization:** Upon TCR-mediated activation of T_HP cells, Wnt genes are expressed along with Wnt responsive genes culminating into production of Wnt and moderate level of IL-4 which then polarizes activated $CD4^+$ T helper cells (T_H0) cells and acetylates SATB1. **C. Early T_H2 differentiation.** After receiving Wnt signal b-catenin is stabilized, SATB1 is deacetylated and recruits chromatin modifiers to its targets. In T_H2 polarized cell SATB1 recruits b-catenin:p300 complex and drives expression of *Gata-3* while repressing late cytokine *Il-5* via recruitment of HDAC1. **D. T_H2 commitment.** During late stage of differentiation, GATA-3 coordinately regulates the T_H2 cytokine cluster leading to abundant production of the cytokines IL-4, IL-13, IL-5.

SATB1 might play an important role during the lineage commitment of various subsets of T cells in the thymus. The CD4+ single positive (SP) thymocytes exhibit two very distinct subsets based on SATB1 expression. We refer to these as $SATB1^{Hi}$ and $SATB1^{Lo}$ populations of CD4+ T cells. Mature thymocytes migrate to the peripheral lymphoid organs, where they carry out effector immune responses. Interestingly, we find that the peripheral $CD4^+$ T cells, unlike $CD4^+$ thymocytes have a single population corresponding to the $SATB1^{Lo}$ fraction of $CD4^+$ SP thymocytes. One important class of T lymphocytes involved in balancing the immune responses is the T regulatory (Treg) cells. The interleukin (IL)-2 cytokine is important in maintaining the balance between Treg and conventional T (Tconv) cells. However, the manner in which transcription factors interact to control expression of IL-2 in Treg cells is not well understood. We show that in natural $CD4^+FoxP3^+$T-regs SATB1 is downregulated. During TCR activation of T cells we demonstrate that SATB1 interacts with NFAT1 and positively regulates IL-2 expression. We therefore speculate that overexpression of SATB1 in natural T-regs would upregulate IL-2 in an NFAT-dependent manner leading to abrogation of the suppressor function of Tregs. In the present study we have dissected the role of SATB1 during thymocyte development and contributed towards a greater understanding of its regulation in the peripheral $CD4^+$ T cells. These studies support the notion that SATB1 functions as key orchestrator of T cell development and differentiation.

Future Work

1. To study the role of SATB1 in organization of chromatin domains segregating active and inactive regions of the genome.
2. To study the effect of repositioning of SATB1 targets during T cell development and differentiation.

Research Report

Infection & Immunity

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

Background

Inflammation is complex set of reactions involves a set of cytokines, chemokines, adhesion molecules and various cell types. Inflammation can happen in any organs or tissues in response to traumatic reaction, infection, early cancer, post-ischemic, toxic or autoimmune injury. The functional relationship between inflammation, innate immunity and autoimmunity are well recognized. However, the cellular and molecular mechanism that regulates inflammation and autoimmune diseases are still not well defined.

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. There are number of targets remain to be elucidated and discovered. It is important to note that signaling experiments are often performed on isolated cells, chemokine-engineered cells, only carried out with one chemokine ligand, mostly using one concentration and time points. Furthermore most of

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the experiments are performed in the absence of other important factors such as intigrins, growth factors, and pro-inflammatory cytokines- thus requiring careful data analysis and interpretation in order to draw a conclusion compared to the cells in natural three-dimensional microenvironment. How do intrinsic signaling from these chemokines, cytokines and cell adhesion molecules help in the controlling the inflammation and autoimmune diseases need to be investigated?

Aims and Objectives

1. How does inflammatory chemokine such as CCR6 activation along with other co-stimulatory or inflammatory signals affect Th1, Th2, Th17 and Treg development and function both *in vitro* and *in vivo*?
2. What are epigenetic modifications induced by CCR6 activation at different regulatory regions of genes associated with different subsets of T cell differentiation, and their function under inflammatory and tolerogenic conditions?

Work achieved

We have established an experimental animal model for studying autoimmune colitis. C57BL/6 mice were given 2% dextran sodium sulphate (DSS) in drinking water, and these mice developed colitis within 5-8 days as seen by body weight loss, loose stools, bloody diarrhea, and rectal prolepses. Further, immunohistochemical analysis of colon showed the infiltration of mononuclear cells in the DSS treated mice colon. Flow cytometry

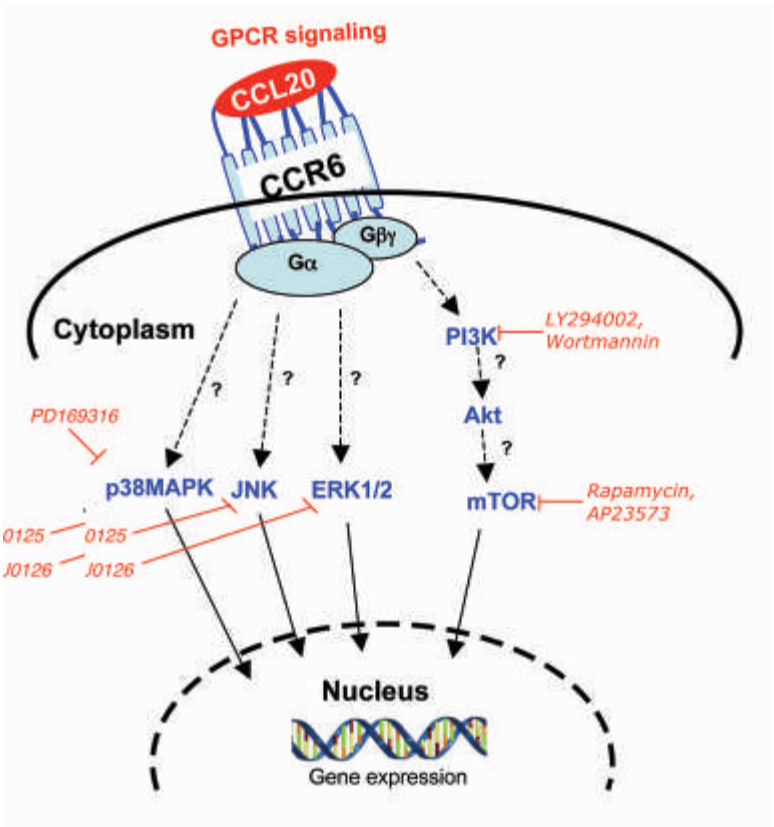

Fig. 1: The proposed model of CCR6 signaling

analysis of CD4 T cells showed increased CCR6 and decrease $Foxp3^+$ expression in spleen, mesenteric lymph node and Peyer's patches. Th17 cells are known to secrete the IL-17A and IL-17F. IL-17A has inflammatory function whereas IL-17F inhibits the proliferation of effector cells and help in the maintenance of mucosal tolerance. To investigate the effect of CCL20 in the differentiation of CD4 T cells, naïve CD4 T cells ($CD4^+CD25^-$ T cells) were cultured under various T cell subsets (Th1, Th2, Th17 and Treg) polarization conditions in presence or absence of CCL20. Results showed that CCL20 did not affect the secretion of IL-4 and polarization into Th2 subset. However, addition of CCL20 in the Treg condition decreased the foxp3 expression, and increased the expression of RORγτ. Intracellular cytokine staining showed that CCL20 enhanced IL-17A expression but did not affect the IL-17F expression in CD4 T cells. These results showed that interaction of CCR6-CCL20 signaling promotes the effector CD4 T cell differentiation (Th1 and Th17) and prevents the tolerogenic $Foxp3^+$ Treg generation in autoimmune colitis. Understanding of chemokine downstream signaling will help us to explore potential therapeutic targets to control autoimmune diseases.

Our lab is actively investigating the molecular mechanisms CCR6 downstream signaling and its function in CD4 T cells differentiation and function.

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Studies on the role of IL-3 in development of Th17 cells

Background

IL-3, a cytokine secreted by T helper (Th) cells, functions as a link between the immune and the hematopoietic system. IL-3 has an anti-inflammatory activity and prevents bone and cartilage damage in mice. Recently, we have demonstrated that IL-3 attenuates collagen-induced arthritis (CIA) by modulating the development of $Foxp3^+$ regulatory T (Treg) cells under both *in vitro* and *in vivo* conditions. The development of Treg cells is reciprocally linked with development of Th17 cells. Conditions that typically favor the development of Treg cells and promote tolerance are challenged by inflammatory signals towards supporting the generation of Th17 cells. In animal models, the enhancement of Th17 cell differentiation is at the expense of Treg cells, and these combined changes trigger autoimmunity. Th17 cells play an important role in the pathogenesis of a number of immune-mediated diseases, including psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease etc. Since IL-3 promotes development of Treg cells, we next investigated the role of IL-3 in development of Th17 cells.

Aims and Objectives

To investigate the role of IL-3 in development of Th17 cells.

Work Achieved

IL-3 inhibits the in vitro differentiation of Th17 cells

To evaluate the role of IL-3 in development of Th17 cells we first checked the expression of IL-3Rα on in vitro generated Th17 cells. Splenic naïve $CD4^+CD25^-CD62L^{hi}CD44^{lo}$ T cells were stimulated with plate-bound anti-CD3ε and anti-CD28 mAbs in serum-free X-VIVO 15 medium in the presence of rhTGF-β1, rmIL-6, rmIL-23, along with anti-IL-4 and anti-IFN-γ neutralizing antibodies. After 4 days, the expression of IL-3Rα was checked at mRNA level by RT-PCR. *In vitro* generated Th17 cells showed strong expression for IL-3Rα. Splenic naïve $CD4^+CD25^-CD62L^{hi}CD44^{lo}$ T cells were induced as described above for Th17 cell differentiation in the absence or presence of different concentrations of IL-3. After 4 days, Th17 cells were quantified by intracellular staining for IL-17A and IFN-γ

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expression by FACS. We observed that IL-3 inhibited the differentiation of Th17 cells with a significant reduction in the percentage of $CD4^{+}IL\text{-}17^{+}IFN\gamma^{-}$ T cells. Anti-IL-3 mAb completely abrogated the inhibitory effect of IL-3 on Th17 differentiation. We also found that IL-3 inhibited Th17 cells specific transcription factor Roryt which was completely abolished by anti-IL-3 mAb. These results clearly suggest that IL-3 inhibits the differentiation of Th17 cells.

IL-3 inhibits the secretion of Th17 cells effector cytokines

To determine the effect of IL-3 on Th17 cells effector cytokines we quantified the levels of IL-17A, IL-21 and TNFα in the culture supernatants of differentiated Th17 cells. Splenic naïve $CD4^{+}CD25^{-}CD62L^{hi}CD44^{lo}$ T cells stimulated as described above for Th17 cell differentiation were incubated in the absence or presence of different concentrations of IL-3. On day 5 culture supernatants were collected and analyzed for levels of IL-17A, TNFα and IL-21 by cytometric bead array. Interestingly, it was observed that IL-3 significantly inhibited the secretion of these cytokines. These results further strengthen that IL-3 inhibits the differentiation of Th17 cells and the secretion of effector cytokines of Th17 cell lineage. Thus IL-3 plays an important role in modulation of Treg and Th17 cell development (Fig. 1).

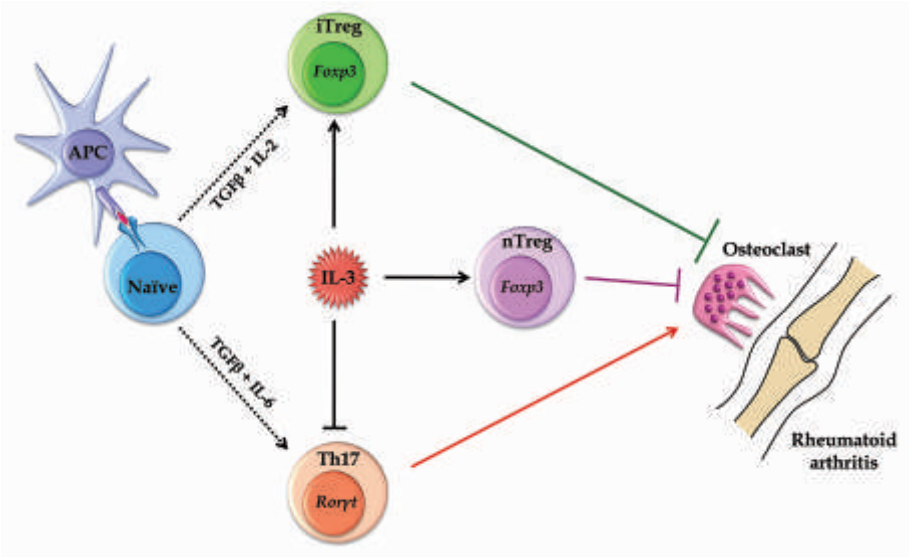

Fig 1: IL-3 modulates Treg-Th17 cell balance

Future Work

To investigate the role of IL-3 in development of human T reg and Th17 cells.

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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

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. We have shown earlier that Nef not only interacts with the heat shock protein 40 (Hsp40) but it also induces the expression of Hsp40 in HIV-1 infected cells. This interaction seems to be necessary for Nef mediated up regulation of viral gene expression. 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 now shown that Hsp40 and Hsp70 reciprocally regulate HIV-1 gene expression

and replication. We have also shown that HSF-1 positively regulates HIV-1 gene expression and replication by two distinct mechanisms. Firstly, along with Nef it activates HSP40 promoter and the increased HSP40 then promotes viral gene expression and replication as reported earlier. 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 also 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 the CEM-GFP cells with HIV-1 NL4.3 virus. RNA from these cells along with uninfected CEM-GFP cells was used to study the modulation of different members of all the HSP family by using PCR Array, which analyzed the expression of 84 heat shock protein genes. Our results indicate that in case of HSP40 family, 17 genes out of 37 are modulated during HIV-1 infection. In HSP70 family, out of 10 members, 6 members have shown significant modulation. While expression of most of the HSP60 members remains unchanged during infection, two members of HSP90 family seem to be modulated. In case of HSP100 family only one member is significantly up regulated upon HIV-1 infection. Real time PCR analysis for various heat shock protein genes thus suggest that the expression of selected members of various HSP family is differentially modulated during HIV-1 infection. Validation of these results is currently in progress.

In order to find out the molecular mechanism involved in reciprocal regulation of viral gene expression by Hsp40 and Hsp70, we have now looked at their role at the level of positive transcription elongation factor b (P-TEFb), which comprises of CDK9 and cyclin T1. As shown in Fig-1a, CDK9 interacts with both Hsp40 and Hsp70, indicating that Hsp70 is part of the Nef–Hsp40–CDK9 complex reported earlier. P-TEFb promotes general elongation of transcription at many promoters and can phosphorylate the C-terminal domain of the largest subunit of RNA polymerase II. HIV-1 Tat stimulates transcription elongation by recruitment of the P-TEFb, to the transactivation response (TAR) RNA. CDK9 is known to be activated by phosphorylation at a number of serine and threonine residues at the C-terminal end, but evidences suggest that conserved threonine 186 phosphorylation in the T-loop of CDK9 plays a major role in its activation. Although reports exist both for Thr186 auto-phosphorylation and phosphorylation by an unknown nuclear kinase, it is evident that phosphorylation of Thr186 is necessary for the high-affinity binding ofHIV-1 Tat- P-TEFb complex to TAR RNA. We have previously shown that the Hsp40–Nef complex binds to CDK9. As our present results show that Hsp70 inhibits viral gene expression and replication, therefore, it would be pertinent to see whether Hsp70 has any effect on CDK9 phosphorylation. The result in Fig. 1b shows that overexpression of Hsp70 inhibits the phosphorylation of serine residues of CDK9. We then wanted to see whether Hsp40 and Hsp70 have any effect on the phosphorylation of

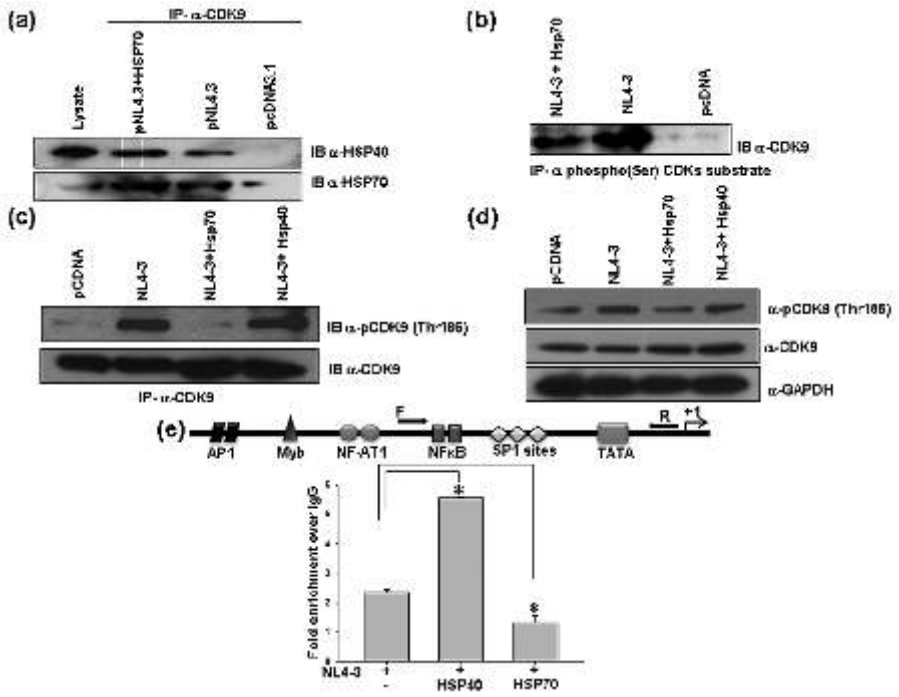

Fig. 1: Hsp70 overexpression leads to a decrease in CDK9 phosphorylation and RNA Pol II recruitment at the LTR promoter, whereas Hsp40 overexpression leads to an increase in CDK9 phosphorylation and recruitment of Pol II at the promoter. (a) Hsp70 is also a part of the Hsp40–CDK9 complex. Transfected cell lysates were immunoprecipitated with CDK9 antibody followed by immunoblotting with Hsp40 and Hsp70 antibodies. (b) Hsp70 overexpression decreases the CDK9 phosphorylation in 293T cells transfected with NL4-3 molecular clone. (c) Hsp70 overexpression inhibits CDK9 Thr186 phosphorylation, whereas Hsp40 overexpression leads to increased phosphorylation. (d) Hsp70 decreases CDK9 Thr186 phosphorylation, whereas Hsp40 increases CDK9 Thr186 phosphorylation in 293T cells transfected with NL4-3 molecular clone. (e) Hsp40 induces RNA Pol II recruitment on HIV-1 LTR, whereas Hsp70 reduces Pol II recruitment in HIV-1 NL4-3-transfected 293T cells as analyzed by chromatin immunoprecipitation. Result is presented as fold enrichment of Pol II as compared to only NL4-3-transfected cells. Data represented are mean±S.D. of three independent experiments. Student's t-test was used to asses statistical significance of the samples; *, $p \leq 0.05$.

Thr186 in the presence of the virus. Results obtained by immunoprecipation with anti-CDK9 followed by immunoblotting with anti-phosphoCDK9 of transfected 293T cell lysates clearly indicate that Hsp70 overexpression leads to inhibition of CDK9 Thr186 phosphorylation, whereas Hsp40 overexpression leads to modest increase in phosphorylation (Fig-1c). Similar results were also obtained by direct immunoblotting with anti-phosphoCDK9 of pNL4.3 transfected 293T cell lysates (Fig. 1d), without any change in the levels of CDK9 expression. These results indicate that Hsp40 seems to induce viral gene expression by increasing CDK9 phosphorylation at Thr186, whereas Hsp70 inhibits viral gene expression by reducing CDK9 phosphorylation. Finally, we also wanted to study the recruitment of RNA Pol II at the HIV-1 LTR in the presence of Hsp70 or Hsp40. Chromatin immunoprecipitation results clearly indicate that Hsp40 overexpression leads to increased recruitment of Pol II at the LTR, whereas Hsp70 overexpression results in reduced recruitment (Fig.1e). Thus, our results suggest that these two proteins not only work at the level of CDK9 activation but also seem to be involved in the recruitment of Pol II at the LTR promoter. Thus, Hsp40 and Hsp70 proteins, which are known to be closely associated with each other in their chaperone function, seem to act contrary to each other for HIV-1 gene expression.

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 (pTEFb) complex to the LTR promoter. The pTEFb complex then hyperphosphorylates 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 NFB 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 NFκB 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 now 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. Tat was also 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 NFκB enhancer sites in the promoter. At the end, one can speculate that Tat might be repressing the *c-Rel* expression to reduce its possible inhibition of Tat induced T-cell apoptosis during HIV-1 infection. Finally, down regulation of an NFκB family transcription factor C-Rel by Tat could also be a viral strategy to induce persistent infection in T cells.

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 comprises of five enzyme complexes, subunits of which have been implicated in various functions in addition to their primary role in energy generating process. Using differential gene expression analysis, we have now shown that Cytochrome Oxidase-II (COX-II), a subunit of Complex-IV is induced in HIV infected apoptotic T-cells. Further analysis indicates increase in expression of majority of complex-IV subunits with concomitant increase in Complex-IV activity in HIV infected T cells. Silencing of COX-II expression leads to reduced apoptosis in infected T-cells, indicating its importance in apoptosis. Furthermore, 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 now initiated studies on the role of autophagy in HIV-1 infection.

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

The current therapeutic strategy involving the use of reverse transcriptase and protease inhibitors in combination (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. One of the strategies has been to identify anti-HIV compounds in natural resources. Our studies with NIPER have resulted in identification of a number of novel derivatives of quinoline 2, 4-diol, β-carboline and dimeric phloroglucinol molecules showing potent anti-HIV activity. Several of these molecules have shown high safety or therapeutic index in cell based assays, which are now being further studied for potential development of a microbicide formulation. In addition to these, efforts have been made to develop combination of two or more molecules which target multiple stages of viral life cycle. We have reported synthesis and anti-viral activity of several novel caffeoyl anilide compounds, which shows significant inhibition of virus by both inhibiting entry through CCR5 co-receptor and by inhibiting Integrase function. We are also working with IICB Kolkata for analyzing the potential of Accasiaside B as a potential anti-HIV microbicide. Accasiside 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. Finally, we have recently initiated structure based development of new Integrase inhibitors in collaboration with Birla Institute of Technology, Mesra.

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 like Hsp27, Hsp60, Hsp90 and Hsp105 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 by yeast two hybrid screening for their functional relevance in HIV lifecycle. Furthermore, we are studying the recruitment of Tat protein on the chromatin during HIV infection that might lead to the elucidation of the 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 mitochondrial oxidative phosphorylation system and autophagy. Finally, studies to identify novel 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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Glycosomal metabolism in *Leishmania major*

Background

The parasitic protozoa *Leishmania* belongs to the order Kinetoplastida and are the causative agents of a disabling and incurable diseases known as leishmaniasis. During its digenetic life cycle, *Leishmania* alternates between the alimentary tract of the sand fly vector as an extra cellular promastigote and the acidic phagolysosomes of mammalian macrophages as an intracellular amastigote. Apart from the changing metabolic environment with variable glucose contents, the striking feature related to the glucose metabolism in kinetoplastids including *Leishmania,* is that the first 7 glycolytic enzymes are sequestered in peroxisome-like organelles called glycosomes. Glycosomes do not have nucleic acids or protein synthesizing machinery but contain 10% of the total cellular proteins. It has been implied that the genes encoding glycosomal proteins (GPs) are located in the nucleus and these gene products are synthesized in the cytoplasm on free polysomes. The complete genome sequence information of most important *Leishmania* species is available and therefore systematic impartial analysis of the protein content of the parasite by experimental proteome analysis is important. Studying proteomics of glycosomes is of interest as it contains a focused set of proteins that fulfills discrete but varied cellular functions. Comparative analysis of glycosomes from two stages of *Trypanosoma brucei* has been reported and has shown identification of novel glycosomal constituents.

Aims and Objectives

To identify total glycosomal protein contents from the promastigote form of *Leishmania major* and to identify various peroxisomal targeting signals present on these proteins.

Work achieved

A linear gradient of sucrose ranging from 70-20% was used for the isolation of pure glycosome fraction from cell free extracts of *L. major.* Western blot analysis of the sucrose density gradient fractions with anti-LmHK antibodies revealed that HK was present in the 50-60% fraction of sucrose which corresponded to the buoyant density of glycosomes (1.15-1.2 g/cm^3). The protein mixture from the pooled fractions was subjected to In-gel

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tryptic digestion after which the insolution tryptic digests were fractionated via strong cation exchange and offgel fractionation techniques. The fractionated peptides obtained were analyzed using high resolution Fourier transform mass spectrometry. This study resulted in identification of total 2730 proteins from glycosomal fraction as compared to 160 proteins reported from earlier study performed with *T. brucei*.

Out of these 180 proteins showed a tri-peptide PTS-1 signal that is localized at the C-terminal end of the protein while 140 proteins showed presence of N-terminal nine amino acid PTS-2 sequence. PTS-1 sequence for *T. brucei* PGK does not function in mammalian cells and the spectrum of variants in PTS-1 signal that is functional in *T. brucei* diverge distinctly from that of mammalian cells. Similar observation of degeneracy of the signal was seen for various PTS-1 signals found in *L. major* glycosomal proteins. The PTS-2 signals found in *L. major* also showed much variation in PTS-2 motifs found in plants and human. The degeneracy was more profound at position 9 in the signal in which signal is defined as (R/K)(L/V/I/Q)XXXXX(H/Q)(L/A/F) in humans, while in *Leishmania* it is [RKH][VLIWF]XXXXX[HKQR] [VFAYLI] (Figure-1).

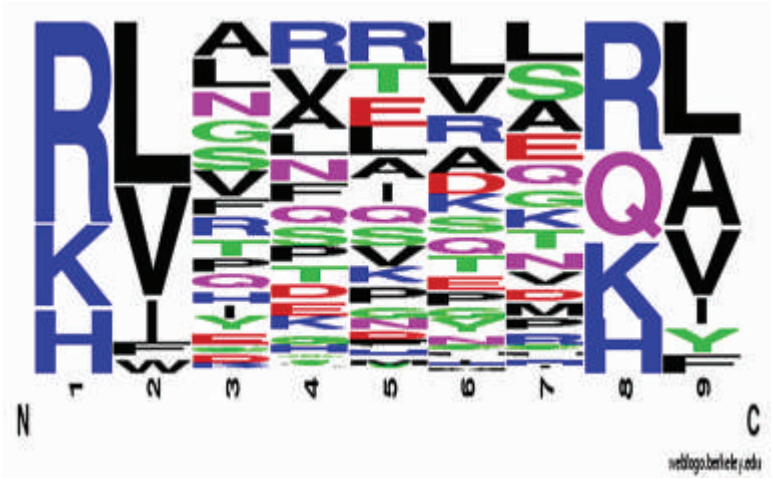

Fig. 1: Amino acid compositions of PTS2 motifs in proteins detected in glycosomes of *L. major*.

All glycolytic enzymes, previously reported to be glycosomal, were identified by mass spectrophotometry in *Leishmania* glycosomal preparation. Most proteins having defined function and with unknown function detected by glycosomes of *T. brucei* were detected in the present study. The proteins detected in the *Leishmania* glycosomes were found to be involved in glycolysis and pyruvate metabolism, pentose phosphate pathway, purine and pyrimidine metabolism, beta oxidations of lipids, glycerol-ether lipid biosynthetic enzymes, and protein-targeting membrane-bound peroxins.

One of the important features of glycosomal proteome is presence of proteins responsible for superoxide metabolism and heat shock proteins. Both these class of proteins are shown to be stress responsive proteins in plants. Superoxide dismutases (SOD) are metallo-enzymes that degrade toxic reactive oxygen intermediates, generated by several oxidative enzymes and during the auto-oxidation of various biomolecules, to

oxygen and H_2O_2. Here, an iron-dependent SOD is detected in the glycosomes of promastigote *L. major*. The peroxides formed during the SOD reaction can be metabolized by the trypanothione metabolism. In *Leishmania,* the thiol metabolism couple glutathione/glutathione reductase is replaced by trypanothione and the NADPH-dependent enzyme trypanothione reductase. Reducing equivalents are transferred via trypanothione and tryparedoxin to a trypanothione/tryparedoxin-dependent peroxidase, which is concomitantly reduced and converts hydroperoxides to molecular oxygen. Both tryparedoxin and peroxidase were detected in glycosomes. Trypanothione reductase, the first enzyme of the trypanothione metabolism and ascorbate-oxidoreductase, a key-enzyme of the ascorbate-glutathione, were also detected in the glycosomes of promastigote. In addition to this heat shock proteins, stress inducible protein STI, ATP-dependent Clp proteases and DNAJ-like proteins were found in glycosomes.

In addition to this, many structural proteins and proteins of mitochondrial, nuclear and cytoplasm were also detected. Presences of similar proteins in glycosomal proteome are reported in the earlier study. To ascertain that all the proteins are indeed targeted to glycosomes, fusion proteins with GFP and RFP will be expressed in *Leishmania* to study its localization.

Future Work

The future work includes proteomic mapping of glycosomes from *Leishmania* donovani and metabolic profiling of glycosomes of *Leishmania.* Preparation of glycosomes from true amastigote form of *Leishmania* will be attempted to find the difference in proteins expressed in two different stages.

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Cytokine profiles & antibody levels in *Plasmodium falciparum* and *Plasmodium vivax* malaria patients

Background

Studies have been carried out to evaluate levels of antibodies to total parasite antigens as well as specific parasite peptide antigens in different endemic areas of the world. However, studies regarding the efficacy of patients' sera in inhibiting parasite growth & cross reactive antibodies between *P. falciparum* & *P. vivax* antigens are scanty, particularly with reference to endemic regions of India.

Aims and Objectives

1. Estimation of levels of different antibodies (IgM, IgG, IgA & IgE) to *Plasodium falciparum* 3D7, native field parasite isolate antigens & virulent antigens.
2. Determination of the efficacy of antibodies in sera from malaria patients in inhibiting *in vitro* merozoite invasion & intraerythrocytic parasite growth.
3. To study cross reactive antibodies in sera from patients infected with *P. falciparum* & *P. vivax*.

Worked achieved

We have estimated the total level of IgG antibodies in the sera of patients infected with *P. falciparum*, using soluble antigens from laboratory-cultured erythrocytic stages of 3D7 *P. falciparum* and antigen prepared from *P. falciparum* from the same endemic region for comparison. (The field isolates were cultured for six days and were used for antigen preparation). It has been reported that field parasites, when cultured, undergo antigenic variations. The levels of IgG antibodies in sera samples are more or less similar with 3D7 antigens and with field parasite antigens in most patients. However, some patients show difference in the levels of IgG antibodies to these two antigens. The sera samples of patients infected with *P. falciparum* show low levels of IgM antibodies as compared to the IgA_1+A_2 antibodies. Comparative levels of IgG, IgM and IgA antibodies in sera samples of patients infected with *P. vivax* were studied by ELISA using antigen prepared from *P. vivax* field parasites. In all the sera samples tested, the antibody levels of IgM were high, followed by levels of IgG, while the levels of IgA were the lowest. Further, these sera

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samples were also tested for cross reactive IgG, IgM and IgA antibodies to the *P. falciparum* 3D7 antigen. All the sera samples showed high levels of IgM, followed by IgA_1+A_2, and levels of IgG cross-reactive antibodies were the lowest. The *P. berghei* antigen (prepared from Balb/C and C57BL/ 6 mice) showed cross reaction with sera samples of patients infected with *P. falciparum* and *P. vivax*. The antigens seem to react with IgG antibodies in these sera samples. This shows that *P. berghei* shares same common antigenic epitopes with *P. falciparum* and *P. vivax* malarial parasites. The cytokines IL-12(p40) and IL-10 levels were estimated using ELISA kits in sera samples of patients infected with *P. falciparum* and *P. vivax*. The ratio of IL-12(p40)/IL-10 seems to determine the severity of the disease. This ratio is >10 in 7 out of 10 *P. falciparum* cases, whereas the ratio is <10 in 7 out of 10 *P. vivax* patient samples. This might indicate that the infection with the *P. falciparum* parasite is more severe than with the *P. vivax* parasite. The preliminary experiments resulted in some interesting findings. Further testing of larger samples is in progress. There is a need to study more parameters to understand the antigenic variation in the field isolates and the cross reactive antibodies to other related parasites. Detailed studies need to be carried out on cytokine profiles in correlation with parasitemia and severity of the disease. This would help understand the role of Th1 & Th2 responses in determining the nature of the immunopathophysiology of the disease, which would be crucial for the development of new strategies to arrest the disease.

Future Work

1. Studies on growth inhibition of the parasite by sera of patients suffering from malaria.
2. Studies on cross reactive antibodies in sera samples from patients infected with *P. falciparum* & *P. vivax*.

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CD40 signaling in the regulation of immune response

Background

Antigen-presenting cell expressed CD40 interacts with the T cell expressed CD40-L and plays a significant role in immune response to *Leishmania major* infection. The CD40-CD40-L interaction results in signaling that is controlled reciprocally between two pathways. We have examined the involvement of kinases in the signaling and how a *Leishmania* protein may interfere with CD40 signaling.

Aims and objectives

The aim of the project is to analyze the role of CD40 in the regulation of immune response. Specifically, we had three aims:

1. To decipher the modular arrangement of kinases.
2. To find out a *Leishmania*-derived factor that modulates CD40 signaling.
3. To find out a peptide agonist of CD40.

Work Achieved

Bimodular arrangement of kinases

Depending on the strength of signal dose, CD40 receptor (CD40) controls ERK-1/2 and p38MAPK activation. At low signal dose, ERK-1/2 is maximally phosphorylated but p38MAPK is minimally phosphorylated; as the signal dose increases, ERK-1/2 phosphorylation is reduced whereas p38MAPK phosphorylation is reciprocally enhanced. The mechanism of reciprocal activation of these two MAPKs remains un-elucidated. Here, our computational model, coupled to experimental perturbations, shows that the observed reciprocity is a system-level behavior of an assembly of kinases arranged in two modules. Experimental perturbations with kinase inhibitors suggest that a minimum of two trans-modular negative feedback loops are required to reproduce the experimentally observed reciprocity. The bi-modular architecture of the signaling pathways endows the system with an inherent plasticity which is further expressed in the skewing of the CD40-induced productions of IL-10 and IL-12, the respective anti-inflammatory and pro-inflammatory cytokines- as effector functions. Targeting the

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plasticity of CD40 signaling significantly reduces *Leishmania* major infection in a susceptible mouse strain. Thus, for the first time, using CD40 signaling as a model, we show how a bi-modular assembly of kinases imposes reciprocity to a receptor signaling (Figure 1). The findings unravel that the signaling plasticity is inherent to a reciprocal system and that the principle can be used for designing a therapy.

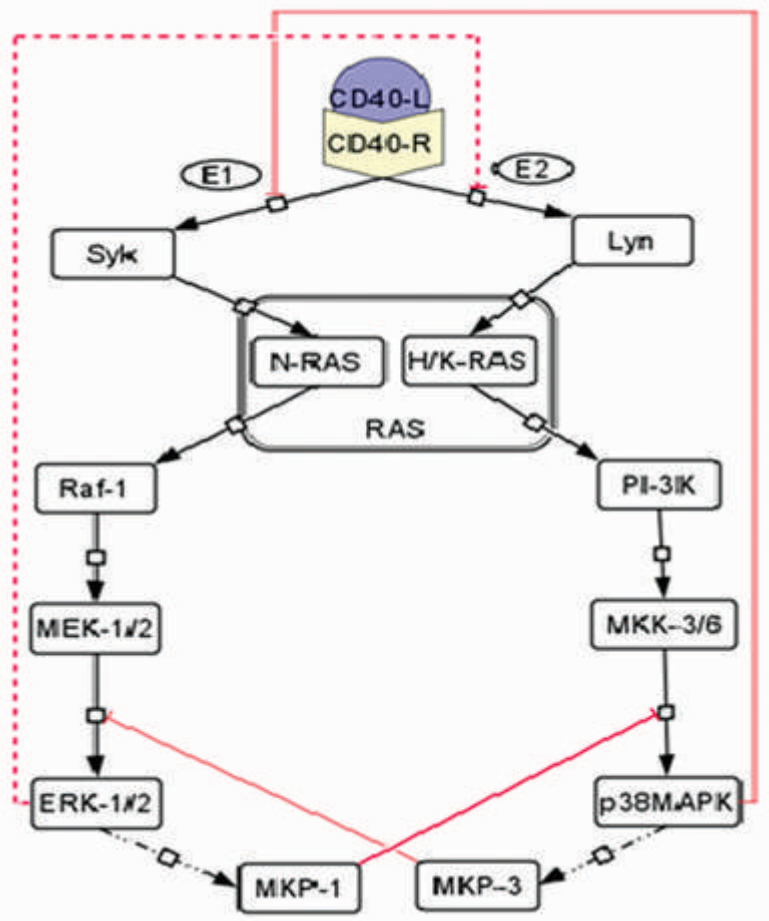

Fig. 1: Bimodular arrangement of kinases in the CD40 signaling pathway in macrophages.

LmjMAPK4 influences CD40 signaling

As *Leishmania* resides in macrophages, wherein the CD40 signals reciprocally, we argued that leishmanial MAP kinases might intercept CD40 signaling. Therefore, we searched for the leishmanial MP kinases that show greatest homologies with p38MAPK or ERK. It was observed that LmjMAPK4 had 42% homology with ERK1. So, we cloned and expressed the gene and purified the protein. It was observed that LmjMAPK4 interacts with MEK-1 and skews the CD40 signaling towards ERK-1/2. Because CD40 signaling through ERK-1/2 exacerbates *L. major* infection, we argued that an inhibitor of LmjMAPK4 might reduce CD40-induced ERK-1/2 activation and ameliorate the disease. Therefore, we identified the compound by virtual screening and examined its anti-leishmanial activities. It was observed that the compound killed amastigotes in vitro and in vivo and restored CD40 signaling with reduced ERK-1/2 activation. Therefore, LmjMAPK4 intercepts CD40 signaling and works for the survival of the parasite.

Peptide agonists of CD40

The antigen-presenting cell expressed CD40 is implied in the regulation of counteractive immune responses such as induction of pro-inflammatory and anti-inflammatory cytokines, IL-12 and IL-10, respectively. The mechanism of this duality in CD40 function remains unknown. Here, we addressed if such duality depends on ligand binding. We identify two dodecameric peptides- peptide-7 and peptide-19 - from the phage peptide library. Peptide-7 induces IL-10 and increases *Leishmania donovani* infection in macrophages whereas peptide-19 induces IL-12 and reduces *L. donovani* infection. CD40-peptide interaction analyses by surface plasmon resonance and atomic force microscopy suggest that the functional differences are not associated with the studied interaction parameters. The molecular dynamic simulation of the CD40-peptides interaction suggests that these two peptides bind to two different places on CD40. Thus, we suggest for the first time that differential binding of the ligands imparts functional duality to CD40.

Future work

1. To extend the members in CD40 signaling kinase modules.
2. To find out the mechanism of CD40 signaling modulation by the *Leishmania*-derived factor.
3. To find out more peptides that bind CD40.

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Role of viral complement control proteins in immune evasion

Background

There exists a constant struggle between viruses and their hosts. Viruses continuously pursue hosts for their survival and propagation. The hosts on the other hand resists these assails owing to the ability of their immune system to fight infections, components of which work in concert. The complement system is one of the essential components of this host immune network. It possesses the ability to neutralize viruses directly as a result of opsonization, aggregation and lysis. And, in addition, support their control by augmenting virus-specific antibody as well as cell-mediated immune responses. Given that viral pathogens survive successfully in their host, it is likely that they subvert the complement system before gaining entry into the host cells. Consistent with this premise, viruses have developed multiple strategies to counteract and bypass the host complement system. One of the central mechanisms utilized by the large DNA viruses like herpes and pox viruses is the molecular mimicry of human complement regulators: they encode homologs of the human regulator-of-complement-activation (RCA) proteins. Our laboratory focuses on characterization of these viral homologs with respect to their function and role in viral pathogenesis.

Aims and Objectives

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

Work Achieved

Smallpox, an abysmal disease caused by variola virus, claimed millions of human lives over the centuries until its successful eradication in 1977 as a result of extensive mass vaccinations using vaccinia virus as the inoculating agent. Despite this success, there are looming concerns about its re-emergence owing to the possible usage of variola virus as a bioterrorism agent, as a large percentage of the current human population is susceptible to smallpox. Thus, there is a need to further define the factors that influence variola virus virulence, pathogenesis and control.

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Both variola and vaccinia viruses are known to encode homologs of human complement regulators. The homolog of variola is known as SPICE, while the homolog of vaccinia is known as VCP. Earlier, we and others have shown that SPICE is much more potent against human complement compared to VCP. Intriguingly, this is consistent with the species tropism of variola. Unlike variola which infects only humans, vaccinia virus is known to infect a range of domestic animals and its outbreaks are frequently reported in dairy cattle in Brazil. We therefore asked, does VCP exhibit preference in inhibiting bovine complement? And if yes, what dictates the species specificity in SPICE and VCP?

VCP exhibits preference in inhibiting bovine alternative complement pathway

To answer whether VCP exhibits specificity towards bovine complement, we examined the inhibitory activity of VCP and SPICE against the alternative complement pathway of various domestic animals and primates. Our data revealed that VCP preferentially inhibited complement of non-primate species, while SPICE preferentially inhibited complement of primates. With respect to specificity of VCP and SPICE against bovine and human complement, VCP was about 34-fold better in inhibiting bovine complement compared to SPICE, while SPICE was about 23-fold better in inhibiting human complement compared to VCP. Earlier, we have shown that four residues in SPICE (Y98, Y103, K108 and K120) are responsible for its specificity towards human complement. We thus next sought to determine which residues in VCP are responsible for its specificity towards bovine complement.

Glutamates at positions 108, 120 and 144 are the key functional determinants of VCP for its specificity towards bovine complement

VCP possesses the ability to inactivate the alternative pathway by targeting the C3-convertase – by supporting the cleavage of C3b by serine protease factor I (termed cofactor activity) and by accelerating the dissociation of C3-convertase C3b, Bb (termed decay-accelerating activity). Because VCP differs from SPICE in only 11 residues (Fig. 1), we generated 11 single amino acid mutants by substituting each of the eleven variant VCP residues onto SPICE template and measured their cofactor and decay-accelerating activities using purified bovine complement components.

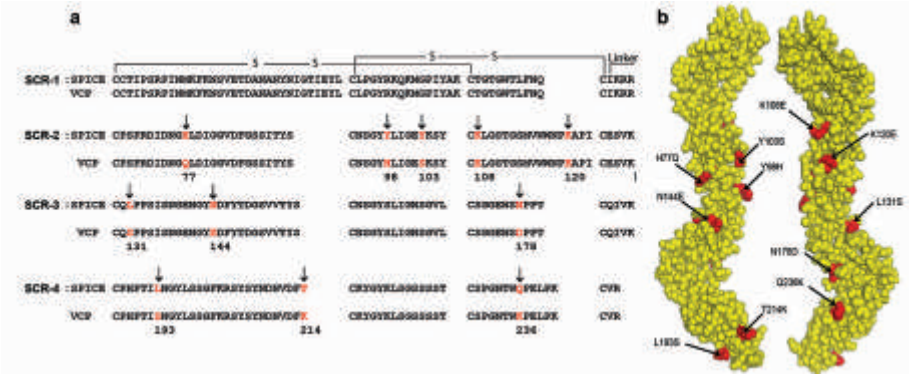

Fig. 1: Structure of SPICE and VCP. (a) Amino acid sequence alignment of SPICE and VCP depicting in red the 11 variant amino acids scattered on domains 2 to 4. The arrows indicate the amino acids in SPICE that were changed with the corresponding amino acid of VCP to generate the single- and multi-amino acid substitution mutants of SPICE. The numbers denote their corresponding position in the mature protein. (b) Structure of VCP showing positions of mutations in red.

Examination of cofactor activity of VCP compared to SPICE showed that VCP is 12-fold more efficient than SPICE in inactivating bovine complement protein C3b. Among the 11 mutants of SPICE, only Y103S, K108E, K120E and N144E exhibited notably higher (3- to 6-folds) cofactor activity compared to SPICE, suggesting that primarily negatively charged residues of VCP are responsible for shaping the higher cofactor activity of the molecule against bovine C3b. Next, we measured if the increased activity of VCP against bovine complement is also due, in part, to its enhanced decay-accelerating activity. Interestingly, VCP displayed 36-fold more potent decay activity than SPICE, and among the single amino-acid substitution mutants, K108E depicted a substantial (~16-fold) increase, indicating that the negatively charged Glu at 108 position of VCP is largely responsible for its increased decay-accelerating activity.

The above results obtained utilizing single amino acid substitution mutants suggested that four (S103, E108, E120 and E144) of the eleven variant residues of VCP are vital for imparting specificity towards bovine complement. We thus assessed how many of the four residues are needed to alter the specificity of SPICE towards bovine complement. To answer this, we generated double (K108E/K120E), triple (K108E/K120E/N144E) and tetra (Y103S/K108E/K120E/N144E) residue mutants of SPICE and measured their inhibitory activity towards bovine C3b and alternative pathway C3-convertase. Functionally, all the three multi-residue mutants of SPICE exhibited equally good ability to inactivate bovine C3b (Figs. 2a and 2b), but only triple and tetra mutants were more similar to VCP in their ability to decay the bovine alternative pathway C3-convertase (Fig. 2c). Binding analysis revealed that binding of triple and tetra mutants, but not the double mutant was comparable to VCP (Fig. 2d). Together these data indicate that primarily the glutamates at position 108, 120 and 144 direct VCP's specificity towards bovine complement.

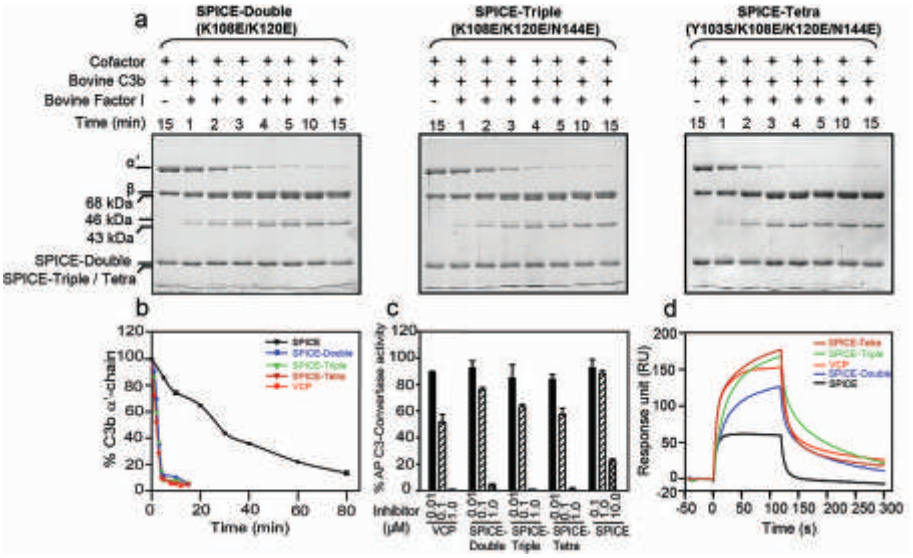

Fig. 2: SPICE double-, triple- and tetra mutants display functional activities comparable to VCP. **(a, b)** Factor I cofactor activity of multi-amino acid substitution mutants of SPICE was examined by measuring the cleavage of α'-chain of bovine C3b in the presence of factor I. Quantitation of the α'-chain by densitometric analysis is represented graphically in panel b. **(c)** Decay-acceleration of bovine C3 convertase by multi-amino acid substitution mutants of SPICE in comparison to VCP and SPICE. The decay was assessed after incubation of the convertase with the indicated proteins. **(d)** SPR-based binding analysis of multi-amino acid substitution mutants of SPICE to bovine C3b in comparison to VCP and SPICE.

Switch in host complement specificity of VCP and SPICE is primarily determined by the charge reversal

It is notable that Glu at positions 108 and 120 in VCP is replaced by Lys in SPICE (Fig. 1). In addition, VCP also contains Glu at 144, which is substituted by Asn in SPICE. Thus, it was tempting to speculate that the major determinant of switch in host specificity of VCP and SPICE is the opposite charge at these positions. To validate this premise, we measured the inhibitory activities of double (K108E/K120E), triple (K108E/K120E/N144E) and tetra (Y103S/K108E/K120E/N144E) mutants of SPICE as well as double (E108K/E120K) and tetra (S103Y/E108K/E120K/E144N) mutants of VCP towards bovine and human complement. As speculated, the triple-mutant of SPICE wherein Glu was substituted in SPICE at three positions, displayed an essentially similar inhibitory activity against bovine complement to that of VCP (Fig. 3a) and there was a loss in activity of this mutant towards human complement (Fig. 3b). On the other hand, the double-mutant of VCP wherein Lys was substituted in VCP at two positions exhibited appreciable gain in activity towards human complement and loss in activity towards bovine complement (Figs. 3c and 3d); admittedly complete gain in activity towards human complement was observed only in VCP-tetra mutant. In short, largely, the reversal in charge switched the complement specificities of VCP and SPICE. It is pertinent to point out here that overall bovine C3b is mostly electropositive, whereas human C3b is mostly electronegative.

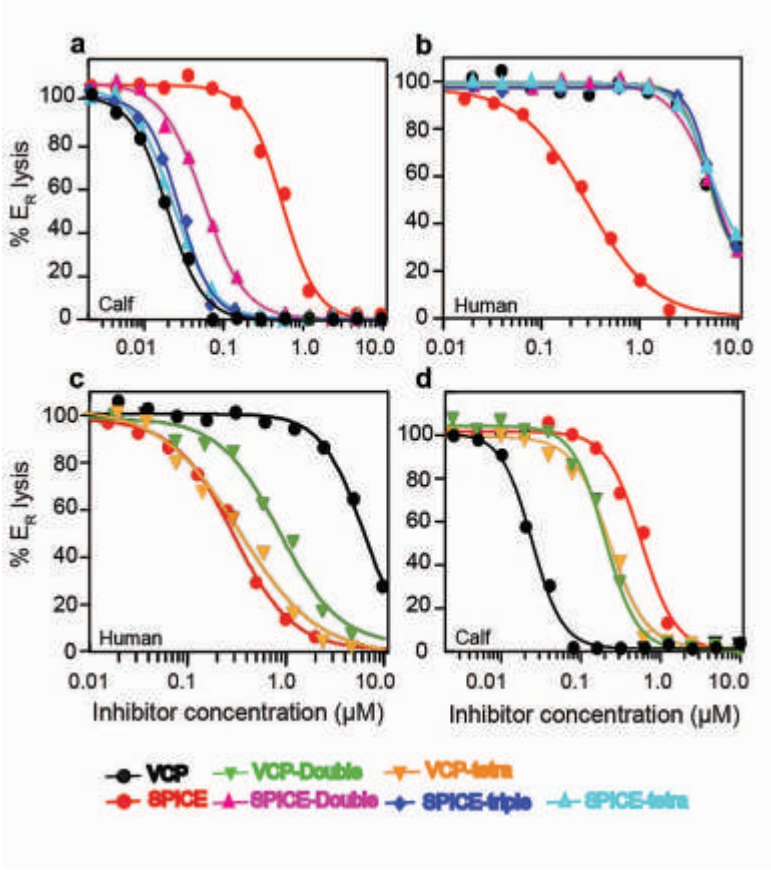

Fig. 3: Substitution of charged variant amino acids in VCP and SPICE skews their complement specificity. (a,d) Relative ability of multi-amino acid mutants of SPICE and VCP to inhibit bovine alternative complement pathway in comparison to VCP and SPICE. **(b,c)** Relative ability of multi-amino acid mutants of SPICE and VCP to inhibit human alternative complement pathway in comparison to VCP and SPICE. Key: SPICE-double, K108E/K120E; SPICE-triple, K108E/K120E/N144E; SPICE-tetra, Y103S/K108E/K120E/N144E; VCP-double, E108K/E120K; VCP-tetra, S103Y/E108K/E120K/E144N.

Determinants of species selective cofactor and C3-convertase decay-accelerating activities of VCP and SPICE

The cofactor activity is a result of interaction between three proteins – C3b, viral regulator and the protease factor I. Thus, we sought to determine if the enhanced cofactor activity of VCP and SPICE against bovine and human C3b, respectively, is influenced by their interaction with C3b or with factor I. We thus performed the C3b cofactor activity assays using four different combinations of bovine/human C3b and bovine/human factor I and assessed the functional activity of VCP, SPICE and their multi-amino acid mutants. Our results indicated that species specific cofactor activity of VCP and SPICE is primarily dictated by its interaction with human factor I.

The process of dissociation of the catalytic subunit Bb from the C3-convertase C3b,Bb is preceded by the binding of regulator to both the subunits of C3-convertase, C3b and Bb. We therefore asked whether the superior decay-acceleration activity of VCP against bovine C3-convertase is determined by its interaction with C3b or with Bb. Consequently, the C3-convertase C3b,Bb was formed by using different combinations of bovine and human complement proteins C3b and factor B and the decay activity of VCP, SPICE and their multi-residue mutants was assessed. Our data indicated that the specificity of VCP towards bovine C3-convertase is primarily due to its better interaction with C3b. Unlike VCP, SPICE displayed a very limited decay activity against human as well as bovine C3 convertase.

Future Work

1. Determining the influence of electrostatic potential on the species specificity of poxviral complement regulators.
2. Fine mapping of functional determinants in viral complement regulators.
3. Designing pathway specific complement inhibitors.

Research Report

Microbial Biology

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Microbial Diversity and Genomics

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Background

Microorganisms are omnipresent and can thrive under conditions where no other living forms can exist. Their metabolic, physiologic and genetic diversity is far greater than any other life form and thus they play a very important role in all ecosystems. There is very little information available about their diversity as compared to higher life forms. Recently developed massively parallel sequencing technologies have enabled us to get a better understanding of the uncultivable majority of microbes. Our laboratory uses these methodologies to understand the microbial community structure and function of selected unique ecological niches.

Aims and Objectives

To understand the structure-function relationship of microbes in unique ecosystems like insect gut, human gut and a hyper-saline, hyper-alkaline lake.

Work Achieved

Human gut microbiology

It is increasingly clear that the complex ecology of the human gut microbiome is of great medical importance. Recent observations from genetic analysis of the presence and abundance of bacterial species in the human gut, shows that individual gut microbiomes are not fixed, but instead show sustained change over many months within each individual. Furthermore, shift in the distribution of gut microbes have high correlation with a variety of human health issues including those that directly involve the GI tract such as inflammatory bowel disease, gastric ulcers. Moreover, the gut microbiome is also implicated in diseases not obviously linked to the GI tract, including obesity and diabetes and disorders of the central nervous system as well as autism. Recognizing gut microbial communities as complex dynamic systems raises many possibilities and leads to novel testable hypotheses. Most of the studies on human gut flora have been performed with European and American populations. The Indian population is different in physiology from the western population (YY paradox) and thus the gut flora in the Indian populations

is likely to differ from the extensively studied gut flora in western populations. Thus, the Indian population is an unexplored source for studying gut microflora.

The gut flora of Indian families, with three individuals belonging to three successive generations living under the same roof, was studied using culture-dependent and molecular methods. The results suggest that the gut flora does change within genetically related individuals of different age, living under the same roof. The *Firmicutes/ Bacteroidetes* ratio has been shown to be important when correlating gut flora with disease conditions. The *Firmicutes/ Bacteroidetes* ratio changes with age in Indian individuals are different from the ratio reported for the European population, suggesting that the composition of gut flora in Indian individuals is different from the western population. The isolation of potentially novel bacterial species from the gut of these individuals further emphasizes the need to explore the gut flora of Indian individuals/population.

In another study, the gut flora of diabetic and non-diabetic individuals was analyzed. In this study, the selected families had either the father, or the mother or both parents diabetic, and their children were at the risk of developing diabetes. Based on 16S rRNA gene clone libraries and real time PCR analysis, some extreme skewing in the gut flora towards the presence of *Firmicutes* was observed (Fig.1).

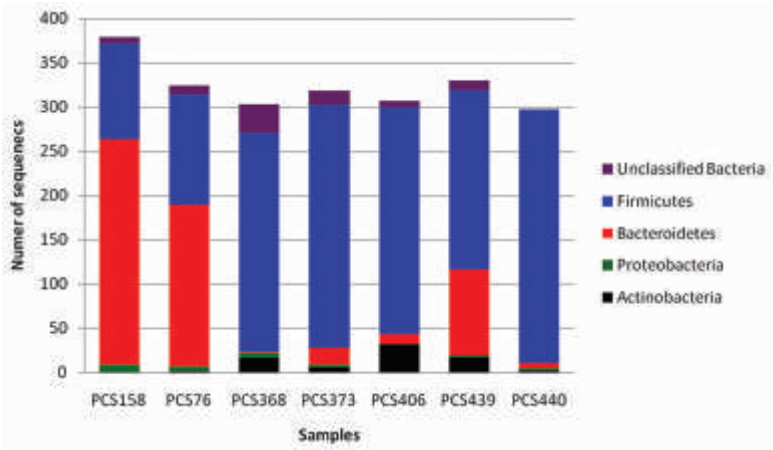


Fig. 1: Phylum-level distribution among different samples.

Probiotic potential of bacteria isolated from herbal preparations

The intestinal population of beneficial commensalic microorganisms, popularly called probiotics, helps in maintaining human health, and some of these bacteria have been found to significantly reduce the risk of gut-associated disease. The genomic characterization of probiotic bacteria is now under way and will help to deepen our understanding of their beneficial effects. In this regard, herbal biomedicine preprarations were used for the isolation and characterization of potential probiotic microorganisms. *Lactobacillus plantarum* isolated from these sources exhibited probiotic attributes like tolerance to low pH, bile salts and simulated gastric juice. It was also found to be adherent in both *in vitro* and *in vivo* models (Fig. 2).

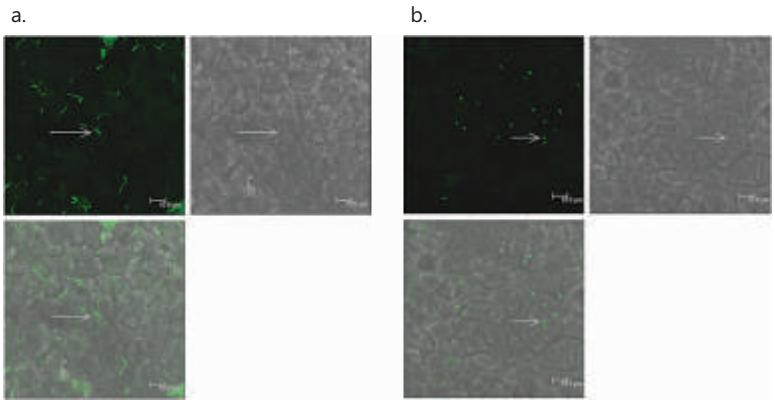


Fig. 2: Adherence pattern of *Lactobacillus rhamnosus* GG 'a' and *Lactobacillus plantarum* 'b' in intestinal HT-29 cell line.

Characterization of prokaryotic communities associated with Lonar crater basalts

Lonar lake is a unique saline and alkaline ecosystem formed by a meteor impact in the Deccan basalts in India around 52000 years ago. Until now, a great amount of data had been gathered about the cultured and uncultured bacterial diversity of the Lonar lake, whose biogeochemical properties have also been well characterized.

Also, various biogeochemical cycles like the carbon, nitrogen and sulphur cycles have reported to be well active in the hypersaline Lonar soda lake. The functional genes prevailing in the lake environment and playing an active role in these biogeochemical cycles are the key to study the functionality of these cycles and also to detect the diversity of the various classes of microorganisms harbouring these genes. These microorganisms include cyanobacteria, anaerobic sulphate reducers, methylotrophs & methanogens, photosynthetic bacteria and purple sulphur & purple non-sulphur bacteria.

Diversity of endolithic (rock-dwelling) prokaryotes

In the present study, *Bacteria* and *Archaea* inhabiting basalts were retrieved from four discrete sampling sites on the Lonar crater walls and the lake bed. These were assessed by us using cultivation-independent molecular methods. Cluster analysis of DGGE fingerprints (Fig. 3) and UniFrac analysis of clone library sequences suggested substantial

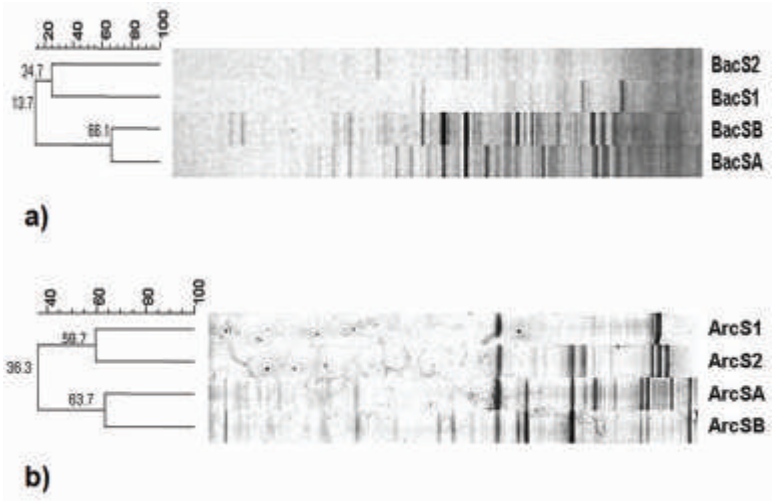
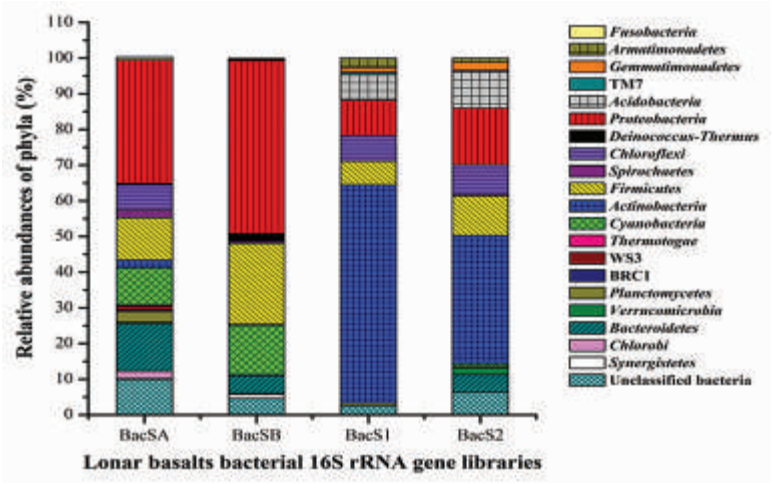


Fig. 3: Curve-based cluster analysis (Pearson correlation and UPGMA) of DGGE community fingerprints obtained from Lonar basalt **a)** bacterial and **b)** archaeal communities. Values at nodes represent percentage similarities. Surface basalts from two sites on the crater-wall (S1 and S2) and two sites on the lake-bed (SA and SB) were sampled.

variations in bacterial and archaeal diversity at the inter-site level. A combined total of ~1000 bacterial and ~700 archaeal 16S rRNA gene sequences were retrieved from the Lonar crater basalts, with the majority of these sequences being phylogenetically affiliated to *Actinobacteria, Proteobacteria, Firmicutes, Cyanobacteria, Thaumarchaeota and Euryarchaeota* (Fig. 4). Many of the detected phylotypes were closely related to those detected in soils or sediments and not to those previously found in terrestrial or marine basalts. A substantial number of our clone library sequences did not affiliate with extant *Bacteria* and *Archaea* and may represent novel lineages. Diversity indices and richness estimates suggested a high diversity of endolithic organisms in the Lonar crater basalts. The presence of prokaryotic lineages associated with C, N and S cycling indicates the potential ecological relevance of basaltic endolithic communities.

a.



b.

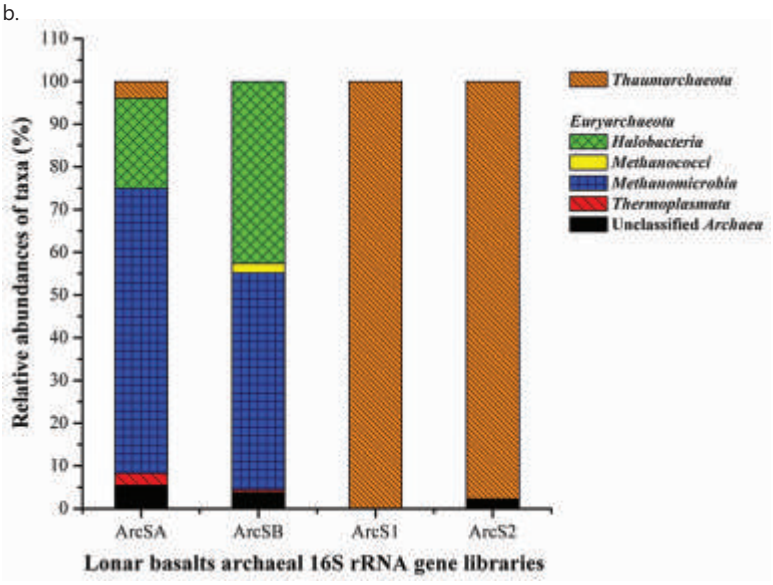


Fig. 4: Taxonomic classification of: a) Bacterial 16S rRNA gene clone sequences retrieved from Lonar lake bed surface basalts (SA and SB) and Lonar crater-wall surface basalts (S1 and S2); b) Archaeal 16S rRNA gene clone sequences retrieved from Lonar lake bed surface basalts (SA and SB) and Lonar crater-wall surface basalts (S1 and S2). All clone library sequences were identified using the RDP Classifier tool.

Heavy metal and antibiotic resistance

Heavy metal and antibiotic resistance of microbes of the Lonar lake have been studied by the metagenomic as well as culture-based approaches. Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life, including microbes, by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (by efflux, complexation, or reduction of metal ions) or to use them as terminal electron acceptors in anaerobic respiration. Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium, and nickel have been identified and described in detail.

Mercury-resistant bacteria were isolated from Lonar lake sediment sample by culture-dependent methods. Out of all the isolates obtained, isolate C2 which was identified as *Lysinibacillus* by 16S RNA analysis, showed resistance to antibiotics as well as mercury. While the highest MIC (minimum inhibitory concentration) for mercury-resistant bacteria reported to date has been no more than 10 μM, the Lonar Lake isolate C2 has shown resistance to a very high concentration, i.e. 325 μM of mercury. This isolate is also resistant to different classes of antibiotics like I, II & III generations of cephalosporins (cefixime, ceftazidime, ceftizoxime, cefaperazone & cefazolin), ß-Lactam antibiotics (penicillin & oxacillin), as well as the amnioglycoside, streptomycin. These results indicate close proximity between antibiotic resistance genes and heavy metal resistance genes on the chromosomal and/or plasmid DNA. Successful amplification of the cadmium resistance gene (CAD gene) from caesium chloride gradient-purified plasmid DNA showed that this isolate had a plasmid-borne gene for resistance to this heavy metal. Several other isolates showed the presence of antibiotic resistance genes like TEM and CTX-M.

Integrons are genetic elements that incorporate exogenous sources of mobile DNA called gene cassettes into the recipient genome via site-specific recombination. Class 1 integrons were discovered in association with antibiotic resistance genes and are widely studied for this association. However, the role of integrons in the natural environment is poorly studied. In this study we explored the prevalence of class 1 integron gene cassettes in the unique environment of the Lonar lake, in order to understand the role of integrons in the natural environment. Class1 integron gene cassettes were amplified using PCR primers previously reported. The PCR products were sequenced using the Ion-Torrent Next Generation Sequencing platform. A total of 55,104 reads were obtained out of which 41,499 reads were used for annotation and further analysis using MG-RAST (http://metagenomics.anl.gov). The results revealed that the integron gene cassette encoded for genes involved in basic metabolism of the microorganism. It includes pathways such as glycolysis, TCA cycle, fatty acid metabolism and amino acid synthesis. The association of genes involved in important metabolic pathways with integrons emphasizes the importance of integrons in adaptive strategies.

High-throughput sequencing

Recently developed high through put sequencing technologies were used for microbial genome sequencing as well as microbial community analysis using tagged amplicon sequencing. A new species of the genus *Janibacter, Janibacter hoyeli* was earlier reported from the stratosphere. The isolate was shown to have a high degree of resistance to ultra violet and gamma radiation. A 74X coverage of the genome with 122 contigs was successfully obtained. Many genes responsible for UV resistance were successfully mapped on the genome.

The microbial diversity of a hot water spring in Soldhar, Himachal Pradesh, was studied with high throughput DNA sequencing. For this, the V6 region of the 16S rRNA gene was amplified and sequenced. A total of 3,97,000 reads with a read length of 120 bases were obtained. After quality trimming and removal of chimeras, the remaining 57,029 reads formed 34,120 unique OTUs. These OTUs could be assigned to diverse genera like *Nitrincola* (23%), *Rheinheimera* (20%), *Pseudoacidovorax* (8%), *Hylemonella* (7%), *Thermodesulfatator & Tolumonas* (4%), *Methylobacterium*, *Novospingobium*, *Delftia* and *Micrococcinae* (2% each). This indicated the existence of a large, hitherto uncharacterized diversity in this ecosystem.

Future Work

1. To understand the functional microbial diversity in the Lonar lake and other extreme ecosystems.
2. Studies on larger human populations to understand the effects of genetic and environmental factors that determine the human microbiome.

Research Report

Signal Transduction

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Osteopontin-activated macrophages play a crucial role in MAPK- dependent cyclooxygenase-2 expression that regulates melanoma progression and angiogenesis

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Background

Cancer is a multistage genetic and epigenetic disease in which a group of normal cells transform into highly malignant cells which are further modulated by extrinsic signals from the various cellular components of the tumor microenvironment. A solid tumor is a heterogeneous population of cells that comprises of cancer cells and non-malignant stromal cells, mainly fibroblasts, endothelial and inflammatory cells such as lymphocytes, macrophages, neutrophils and mast cells. During tumor progression there is a fine tuned interaction between cancer and stromal cells that create a unique microenvironment permissive for tumor growth, metastasis and angiogenesis by secreting a wide array of growth factors, chemokines and proteases. Recent reports reveal that targeting stromal cells can reverse tumor progression. The study of stroma-tumor interaction may resolve many unanswered questions in tumor biology which might be helpful for designing novel agent(s) for anticancer therapy.

Accumulating evidences suggest that inflammatory cells and their products are clearly not innocent bystanders in cancer, but rather play an active role in promoting tumor initiation and progression, and also decide the outcome of cancer therapy. Among all the inflammatory cell types in the stroma, the most prominent subpopulation is that of tumor-associated macrophages. Upon activation, macrophages can release a vast array of cytokines, proteolytic enzymes, angiogenic factors and inflammatory mediators which profoundly affect endothelial, epithelial and mesenchymal cells in the tumor microenvironment. Moreover, through inappropriate secretion of cytokines and growth factors, dysfunctional macrophages may actually support tumor growth and invasion. Multiple studies have focused on elucidating the role of infiltrating macrophages in angiogenesis and tumor growth. However, so far there has been no characterization of the pattern of soluble mediators released by macrophages and the signaling events driven by these soluble mediators that initiate the phenotypic switch in macrophages or of their potential impact on the remodeling of the tumor microenvironment.

In this report, using both *in vitro* and *in vivo* models, we demonstrate the involvement of osteopontin (OPN) signaling in regulating macrophage phenotype, which further

dictates melanoma growth and angiogenesis. Our findings suggest that OPN signals via its α9 integrin cell surface receptor activates p38 and ERK signaling pathways, which ultimately leads to COX-2 expression, angiogenesis and tumor cell motility. Thus, our results emphasize the potential role of macrophage in the modulation of the tumor microenvironment via secretion of OPN, PGE_2, and MMP-9, suggesting that OPN signaling blockade may provide a means of targeting melanoma growth and angiogenesis.

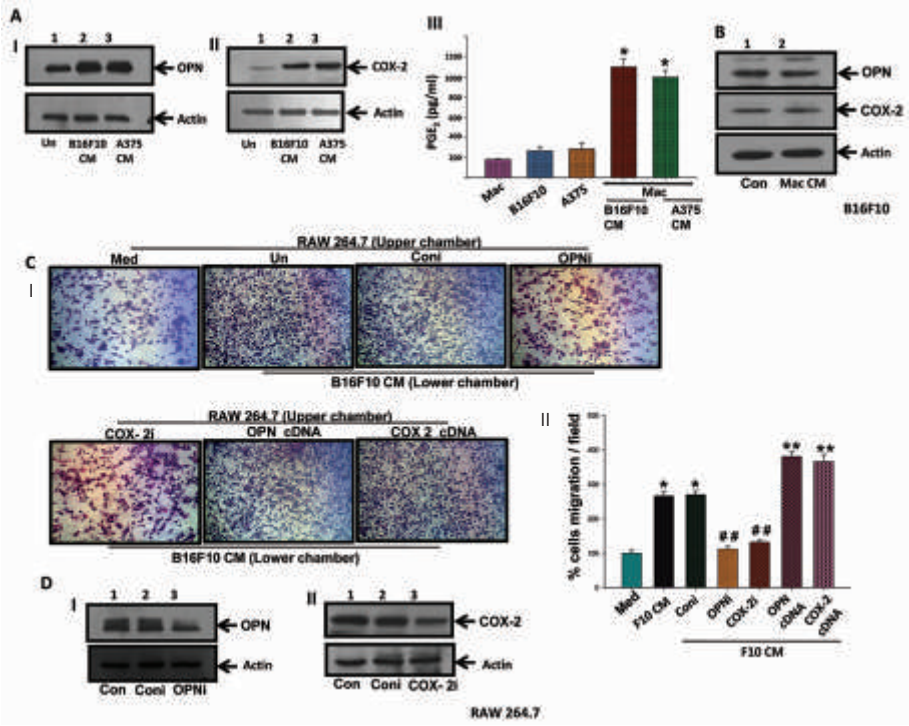

Fig. 1: Co-culture of macrophages with melanoma cells activates macrophage migration and OPN, COX-2 are highly elevated in macrophages upon co- culture with melanoma cells.
A: RAW264.7 cells were supplemented with conditioned media (CM) of B16F10 or A375 for 24 h and OPN expression was analyzed by Western blot from cell lysates. Actin was used as loading control (panels I). RAW264.7 cells were supplemented with CM of B16F10 and A375 for 24 h and COX-2 expression was analyzed by Western blot. Actin was used as loading control (panels II). Conditioned media were collected and the level of PGE_2 was estimated by enzyme immunoassay. Bars correspond to mean ± SEM (panels III). *P<0.01 as compared with the untreated macrophage cells. **B:** B16F10 cells were supplemented with CM of RAW264.7 for 24 h. The expression of OPN and COX-2 were analyzed by Western blot. Actin was used as loading control. **C:** Macrophages (1×10^6) alone or transfected with Coni, OPNi, COX-2i, OPN cDNA, COX-2 cDNA were used in the upper chamber, whereas CM of melanoma were used in the lower chamber (panel I). The macrophages that migrated toward the reverse side of the upper chamber were stained with Giemsa, photographed, counted in 4 hpf and represented in the form of a bar graph. Bars correspond to mean ± SEM (panel II). *P<0.001 as compared with the media control (Med), whereas ##P<0.001 as compared with cells co-cultured with CM of B16F10. **P<0.005 as compared with cells co-cultured with CM of B16F10. **D:** Levels of OPN and COX-2 in cells transfected with their specific siRNA were analyzed by Western blot. Actin was used as control. Control siRNA (Coni) was used as negative control (panels I and II).

Aims and Objectives

1. To investigate how OPN-activated macrophages infiltrate into the tumor and how that controls tumor growth and angiogenesis in a melanoma model.
2. To examine whether OPN regulates phosphorylation of ERK and p38 and how that controls COX-2 dependent MMPs (-2 & -9) activation in macrophages and the mechanism underlying this process.
3. To study whether OPN-activated macrophages are of proangiogenic phenotype and to check whether OPN-induced COX-2 signaling plays any role in tumor angiogenesis.
4. To analyze the expression of OPN, COX-2 and MMPs (-2&-9) in infiltrating macrophages of clinical specimens of melanoma and their correlation with tumor angiogenesis in different grades of human clinical specimens.

Work Achieved

In this study, we report that genetic ablation of OPN expressed in macrophages of stromal origin suppresses melanoma growth in mice and that macrophages are the crucial

components in stroma responsible for melanoma growth. Tumor-associated macrophages (TAMs) abundantly produce OPN and its expression is associated with tumor growth and angiogenesis. In the tumor microenvironment, OPN activates macrophages and influences angiogenesis by enhancing COX-2 dependent PGE_2 production in an autocrine manner. Furthermore, we identify α9β1 integrin as a functional receptor for OPN that mediates its effect and activates intracellular ERK and p38 signaling, which ultimately leads to COX-2 expression in macrophages. The major role played by OPN and PGE_2 in angiogenesis are further amplified by upregulation of MMP-9. OPN-activated macrophages promote the migration of cancer cells and enhance their adherence to extracellular matrix via PGE_2. These findings provide evidence that TAMs serve as a source of key components such as OPN and COX-2 derived PGE_2 and MMP-9 that modulate the tumor microenvironment and trigger angiogenesis and melanoma progression. These data provide compelling evidence that OPN and COX-2 expressing macrophages are obligatory players in melanoma progression. In summary, OPN signaling is involved in the migration of macrophages into tumor, and thus blockade of OPN and its regulated signaling network provides a unique strategy to eradicate melanoma by manipulating TAMs.

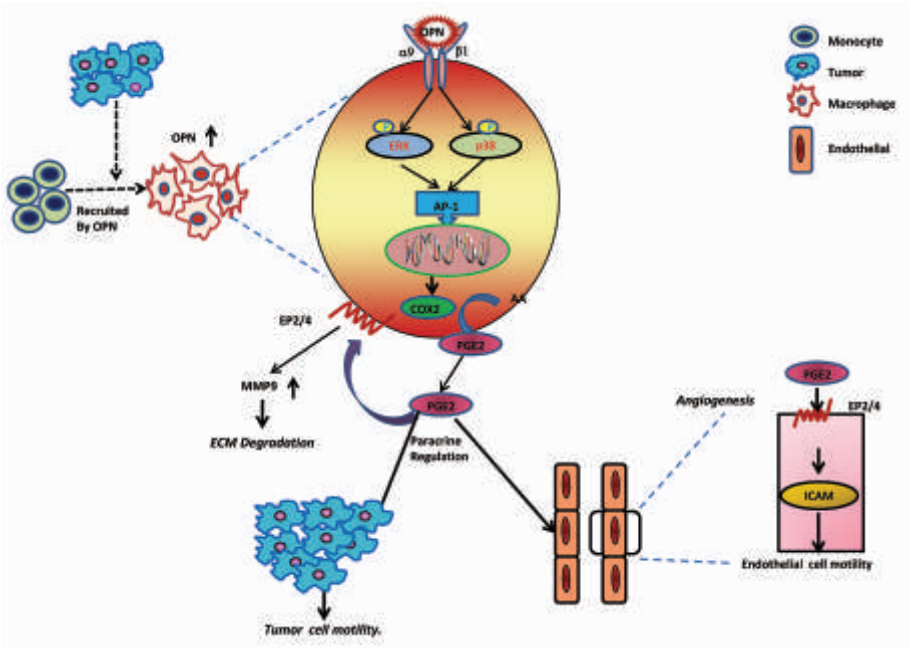

Fig. 2: Schematic representation of OPN-activated macrophages that play a crucial role in MAPK-dependent cyclooxygenase-2 expression leading to melanoma progression and angiogenesis

Future Work

Epithelial to mesenchymal transition plays a crucial role in the control of tumor growth and metastasis. The role of OPN and other associated genes in the regulation of epithelial to mesenchymal transition leading to cancer progression and angiogenesis are in progress. The role of hypoxia in the regulation of OPN expression and OPN-dependent HIF1alpha-mediated VEGF expression leading to breast tumor growth and angiogenesis will be examined further.

Musti Krishnasastry

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Cell Membrane Repair Pathway Involves Sensing of Dynamics of Caveolae and Caspase-1

Background

The nature of cellular signalling events, particularly the membrane repair pathways activated by toxins, caused by the binding and assembly of the pore forming toxins, has just begun to unfold. In this event, it is the activation of the sterol regulatory element-binding (SREBP) pathway through K^+ efflux and caspase-1 which promote biosynthesis of lipids, cholesterol and fatty acids. The caspase-1 plays an important role in promoting cell survival by activating lipid metabolic pathways. However, the sensors that sense the initial changes soon after the binding of membrane damaging molecules are still unclear.

Recently, we have shown the arrest of dynamics of caveolae at the cell surface by the monomeric form of staphylococcal toxin, H35N, which remains as a membrane bound monomer. It must be noted that the caveolae of mammalian cells undergo a continuous cycle of 'kiss and run' dynamics with the plasma membrane that become immobile upon the binding of the monomer. We have also shown that in the absence of an adequate membrane repair response, the target cells induce apoptosis via intrinsic mitochondrial pathway. The cells treated with H35N were capable of activating the membrane repair mechanism involving caspase-1 dependent activation of sterol regulatory element binding protein-1. However, there were several questions that still remain unanswered.

Aims and Objectives

1. To determine whether SREBP-1 translocation induced by H35N was dependent on casapse-1 or casapase-3.
2. Furthermore, to determine whether blockade of the dynamics of caveolae by silencing of KIAA0999 and MAP3K2 (both were known to regulate the dynamics of caveolae) leads to the activation of membrane repair pathway or whether the two events i.e. blockade of dynamics of caveolae and activation of membrane repair pathway are independent of each other.

Research Findings

The aim of our work is to understand in detail whether the activation of membrane repair

Participants

Saumya Srivastava, *SRF*
Santosh Kumar, *JRF*
Ekansh Mittal, *JRF*
Anil Kumar, *JRF*
Shika Nag, *JRF*
Anil Lotke, *Technician*

Collaborators

Dr. Prakash Deshpande, *NCCS, Pune*
Dr. Bhaskar Saha, *NCCS, Pune*

pathway is the outcome of the arrest of dynamics of caveolae, induced by H35N, or whether the two events are independent of each other.

H35N induced SREBP-1 activation is mediated by caspase-1 but not by caspase-3

Translocation of SREBP-1 to the nucleus is one of the first steps for initiation of transcription of lipogenic genes. We had ascertained that the activation of SREBP-1 is by caspase-1 by inhibiting the activation of the latter. Under physiological conditions, SREBP resides in the ER. Upon treatment with H35N, SREBP-1 was processed into its matured form which migrated to the nucleus, whereas in the cells treated with H35N, post silencing of caspase-1 there was no translocation of SREBP-1 to the nucleus. Hence, the inhibition of caspase-1 expression by siRNA has blocked the H35N induced SREBP-1 translocation to the nucleus.

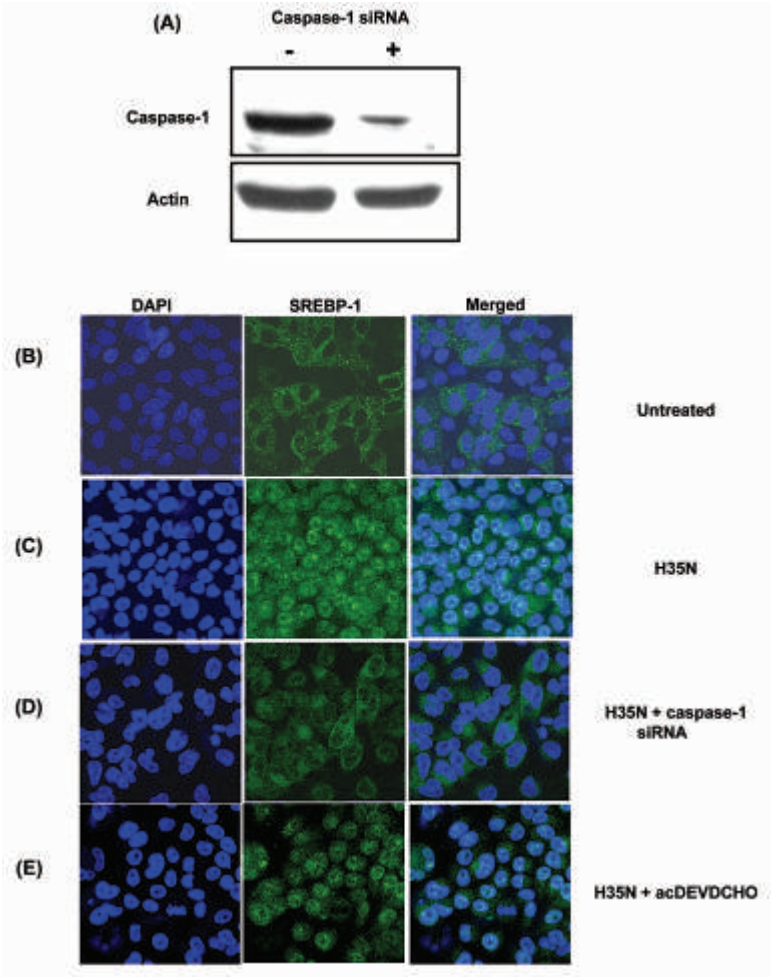


Fig. 1: H35N induced SREBP-1 is mediated by caspase-1 and not caspase-3: HeLa cells were left untransfected or transfected by caspase-1 siRNA and subjected to immunoblotting after harvesting. (A) Hela cells were left untreated (B) Treated with H35N for 7 hr (C) HeLa cells were transiently transfected with casapse-1 siRNA followed by treatment with H35N for 7 hr (D) Pretreated with acDEVDCHO for 2 hr followed by H35N treatment for 7 hr (E) Cells were fixed, permeabilised and stained with SREBP-1 antibody followed by anti mouse FITC secondary antibody. The nuclei were counterstained with DAPI.

H35N triggers decrease in cytosolic potassium

We have examined whether the K^+ efflux occurs in H35N treated cells which induced membrane repair pathway. For this, we examined the intracellular K^+ level in H35N treated HeLa cells loaded with the cell-permeant acetoxymethyl ester derivative of the

fluorescent potassium sensitive dye PBFI-AM. We have observed that the H35N treated HeLa cells showed a decrease in the UV fluorescence by about 32 % indicating the fall in intracellular potassium level in comparison to the untreated cells. This process of incomplete assembly could be responsible for arresting the caveoale dynamics at the cell surface and may result in improper sealing of the membrane areas between the caveolae and the non raft fractions. This improper sealing may allow the passage of monovalent K^+ ions (ionic radius of 0.138 A^0) but not amenable to larger marker molecules such as propidiumiodide (ionic radius of iodide ion is 0.3 A^0).

H35N induces activation of SREBP-2

The major isoforms of SREBP are SREBP-1a, SREBP-1 c and SREBP-2. While the former two are encoded by a single gene (involved in fatty acid metabolism), the SREBP-2, is encoded by a different gene that controls the cholesterol and lipid biogenesis. Since blockade of caveolae dynamics can imbalance the membrane cholesterol homeostasis, we have examined whether H35N treatment also triggers the SREBP-2 processing.

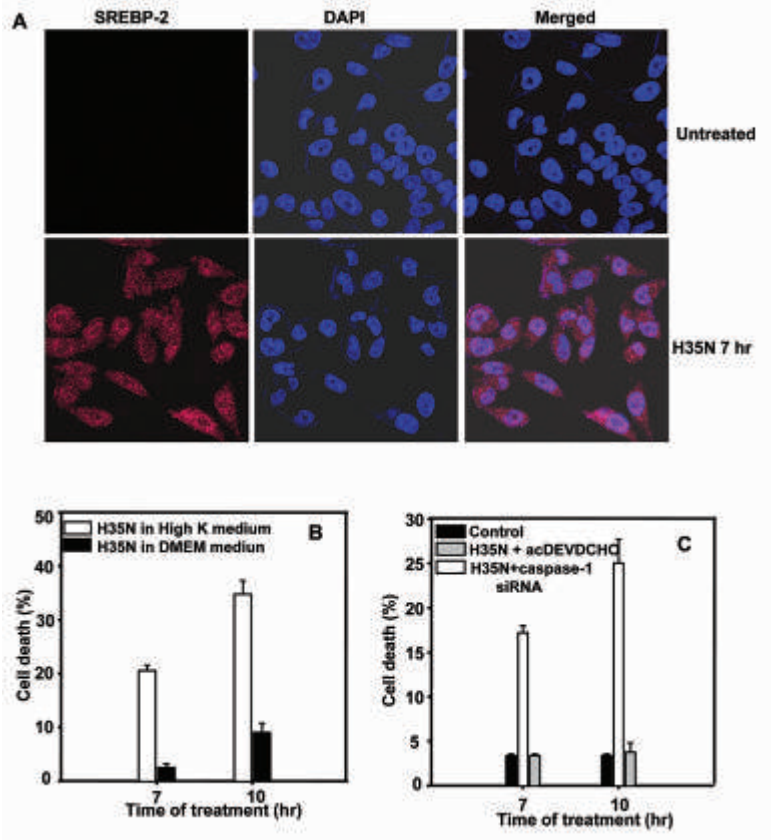


Fig. 2: Effect of H35N on SREBP-2 translocation and prevention of K^+ efflux and caspase-1 activation promotes H35N induced cell death: HeLa cells were left untreated or treated with H35N for 7 hr. Cells were fixed, permeabilised and incubated with SREBP-2 antibody followed by staining with Cy-3 (Red). The nuclei were counterstained with DAPI **(A)** HeLa cells were treated with H35N for the following time points in normal (DMEM) culture media or in modified Hank's media in which the sodium and potassium concentrations were inverted (high K^+ media) and the cell death was analysed by trypan blue staining. **(B)** HeLa cells were left untreated or transiently transfected with caspase-1 siRNA followed by H35N treatment for the mentioned time points and the cell death was analysed by trypan blue staining **(C)**.

Inhibition of KIAA0999 and MAP3K2 induces SREBP-1 activation

Under physiological conditions caveolae are known to undergo a continuous cycle of 'kiss and run dynamics' with the plasma membrane. In our earlier study, we have shown that binding of H35N to the host cell arrests the dynamics of caveolae and activated the membrane repair pathway. However, it was unclear whether the arrest of dynamics of

caveolae dynamics by the kinases that control the process leads to the activation of membrane repair pathway or the two events are independent of each other. It has been shown in the literature that the two serine/threonine KIAA0999 and MAP3K2 regulate the dynamics of caveolae at cell surface as examined by total internal reflection fluorescence microscopy. Silencing of these kinases results in loss of dynamics or the visible kiss and run dynamics leading to accumulation of caveolar structure at the cell surface. To explore the role of these kinases in SREBP activation, we used siRNAS specific for these two kinases and the status of SREBP-1 was examined by confocal microscopy after silencing of the kinases. The arrest of dynamics of caveolae dynamics at the cell surface, post silencing the KIAA0999 and MAP3K2, was confirmed by transfecting the same coverslips with caveolin-1 GFP and using TIRF microscopy. The silencing of KIAA0999 leads to accumulation of caveolar structures at the cell surface as compared to control. Similarly, silencing of MAP3K2 also leads to arrest of caveolae at the cell surface. The coverslips were then stained for SREBP-1 antibody and the localisation of the same was examined by confocal microscopy. We have observed that when the caveolae dynamics are blocked by silencing the expression of MAP3K2 or KIAA0999 ser/thr kinases, there was significant migration of SREBP-1 to the nucleus (indicative of the activation of membrane repair pathway). This shows that arrest of caveolae dynamics at the cell surface is one of the events that lead to the activation of membrane repair pathway.

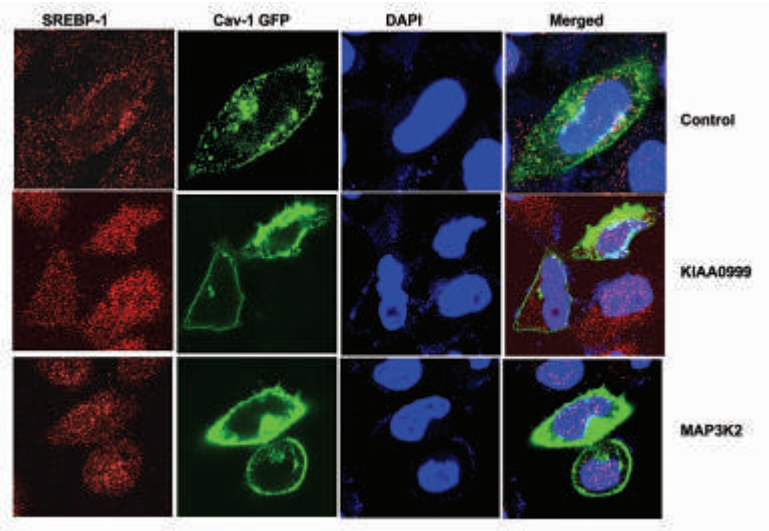


Fig. 3: Effect of silencing of MAP3K2 and KIAA0999 ser/ thr kinases on SREBP-1 activation: HeLa cells were transiently transfected with cav-1 GFP only or co-transfected with MAP3K2 or KIAA0999 siRNA and Cav-1-GFP. Cells were fixed, permeabilised and incubated with SREBP-1 antibody followed by staining with anti mouse secondary antibody and (red) and visualized by confocal microscopy.

Recent findings in the literature demonstrate the changes in the cellular signalling triggered by the assembly of pore forming toxins. Some of these pathways are involved in warning the immune system such as the activation of NF-κB whereas others are known to promote the host cell survival. Osmotic stress induced by the toxin was known to activate p38 MAPK. p38 MAPK activation in turn activates the release of chemokines such as IL-8 and also known to play an important role in activating the survival response. The work done using the present model system employing α-HL identified the range of cellular changes that take place during the assembly of α-HL on the mammalian cell surface that starts with the arrest of dynamics of caveolae at the cell surface within 2 hr, K^+ efflux by 6 hr,

followed by caspase-1 activation and finally the translocation of SREBP to the nucleus by 7 hr. In absence of adequate membrane repair response, the balance between the survival (activation of repair mechanism) and death shifts towards cell death and the host cell undergoes apoptosis via intrinsic mitochondrial pathway. Although there are many missing important links between the dynamics of caveolae and the activation of caspase-1, it is important to understand the kinases/proteins involved in sensing the dynamics of caveolae and translating the same to activate the membrane repair mechanism.

Future Work

To the best of our knowledge, this is the first observation linking the dynamics of caveolae, KIAA099 and MAP3K2 kinases and membrane repair pathway. It is presently not known whether the activation of the membrane repair pathway post silencing of KIAA0999 or MAP3K2 involves K^+ efflux or not. It would be interesting to examine the effector downstream signals generated by the blockade of dynamics of caveolae.

Support Units

Ramanamurthy Bopanna

Rahul Bankar

Experimental Animal Facility

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
- NOD/LtJ
- SWISS#
- BALB/c*
- NMRI $^{nu/nu}$
- NZB
- AKR#

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

RATS:

- WISTAR

RABBITS:

- NEWZEALAND WHITE

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

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 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 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: blood and tissue sampling, immunizations, surgical procedures, injection of tumor cells in SCID/nude mice etc., assistance in the writing of Animal Study Protocols, education and assistance regarding interpretations of animal use regulations and the procurement of animals.

The breeding of laboratory animals has been planned to meet the needs of scientists / research scholars for various animal experiments. A total of 9,689 laboratory animals were supplied on demand for the ongoing research projects during the period.

#: Outbred
*BALB/c with cataract mutation

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:

1. The 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 total number of samples analyzed from April 2011 to March 2012 was approximately 498, including 174 external samples.

2. The 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 from April 2011 to March 2012 was approximately 136, including 16 external samples.

3. The 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 a 4800 MALDI-TOF/TOF system for proteome analysis.

4. The Eksigent Express Micro LC-Ultra System is an advanced micro-LC technology with pneumatic pumps, integrated autosampler, ultra-sensitive, full-spectral UV detector and temperature-controlled column oven. 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, with only a small fraction of solvent used.

5. The Eksigent EKSpot MALDI Spotter couples the Nano MDLC to the 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. Each of the targets can hold up to 1,000 spots and it can generate up to 8,000 spots on an overnight run.

Participants

Dr. Vijay Sathe, *Analyst*

Snigdha Dhali, *Technician*

4800 MALDI-TOF/TOF

4000 Q-Trap LC-MS/MS

6. The Shimadzu Prominence UFLC is a higher speed and uncompromised separation liquid chromatography instrument. It provides ten times higher speed and three times better separation when compared with a 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.

7. The 2-D DIGE proteomics set-up includes Ettan IPGphor isoelectric focusing unit, Hoefer mini electrophoresis system, SE600 RUBY unit, Ettan DALT unit, DIGE Typhoon FLA 9000 scanner, DeCyder 2-D DIGE analysis software, and an 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

The bioinformatics facility at NCCS provides access to high-performance compute 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 1300 Cluster
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

Sunfire X 4150 (Intel Xeon 2.33 GHz Quad Core Dual Processor) Standard Libraries and compilers.

Specialized Workstations: HP workstations of Intel Core 2 Duo @3.00GHz with 8 GB of DDR2 memory, 320 GB of SATA storage and 19" LCD wide Display with Linux operating system. HP Z800 workstations were also recently procured and installed.

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 and normal LCD display and loaded with Windows XP. Apart from these, HP Elite CMT 8200 PC with Windows 7 were recently procured and installed.

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

UPS: 10KVA (APC make) to run clusters

Software infrastructure: The Bioinformatics Facility at NCCS has procured several softwares for scientific research with commercial and/or academic licenses. These are:

Technical Staff:
Mrs. Virashree Jamdar, *Technician*

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 Visualisation: Rasmol, MolMol, WinCoot, Swiss PDB viewer, MolScript, VMD

Ab intio 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

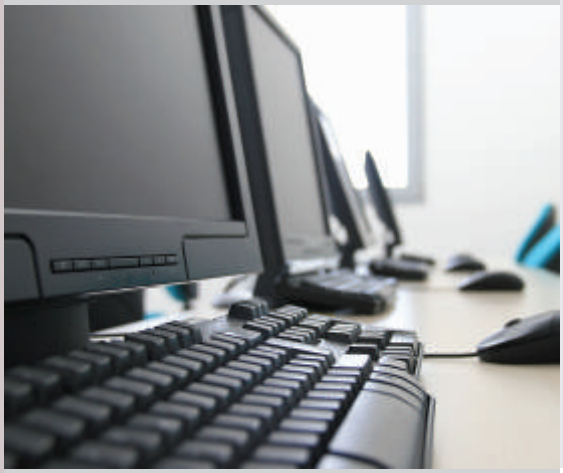
The NCCS library has a collection of publications in frontier areas of biotechnology that are relevant to NCCS research activities. The library holds approximately eleven thousand five hundred bound journals, two thousand six hundred and fifty books and one hundred and seventy two Ph.D. theses of NCCS research scholars. The library subscribes to sixty five scientific journals and twenty three other periodicals in print form, and eight online scientific journals.

The library collection is expanded in consultation with NCCS scientists since the library's priority is to support NCCS research activities. Our print collection is growing by approximately 1000 volumes per year. The library is equipped with Linux-based SLIM21 with RFID Interfaced library software for library housekeeping operations and Web-OPAC for online searching of library documents. The barcode technology has also been installed for circulation (issue & return) of library documents. Information useful for the faculty and students is maintained on the library webpage, which includes free Online Medical database links, a list of NCCS research publications, the collection of Ph.D. theses, NCCS in the news, a list of NCCS Alumni and links related to scientific grants, funds and fellowships. Library-associated forms are also available online in Hindi. A digital archive of Ph.D. theses of NCCS research scholars is also maintained, which is updated every year. Additional 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. The NCCS library is a member of 'DeLCON', the DBT online journal consortium, which provides access to 883 journals from various publishers. A list of these journals is maintained on the library webpage.

Team

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



Computer Section

- The NCCS new building was connected with the NCCS LAN through a fiber optic cable with the installation and configuration of CISCO and 3COM switches and accessories.
- A new Cyberoam Unified Threat Management device was installed and configured for the NCCS network for better protection against viruses, threats and spam. All the internet links were terminated on it for load balancing.
- The Symantec protection suite SPSS 3.0 was installed and configured with 300 user licenses (at present, and likely to be increased to 500) and configured for gateway level e-mail antivirus, antispam, desktop and laptop antivirus, antispam and network access control.
- A New NCCS Intranet website was launched. This website gives information about the various facilities, the faculty in-charge of these facilities, and seminars. It also has different forms uploaded, which can be filled in.
- Several operating systems and common application softwares were installed/updated at NCCS. MS Office 2010, Adobe Suite X, Sigma Plot Suite 12.0 and Reference Manager 12.0 were installed on user computers and Win. Vista was upgraded to Win7. In addition, Paypack salary software was installed and configured for the NCCS salary process which takes care of TDS and EPF.

Team

Mr. Rajesh Solanki

Mr. Shivaji Jadhav

Ms. Rajashri Patwardhan

Ms. Kirti Jadhav

NCCS Facilities

a) DNA Sequencer

A total of 15, 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	Total Samples Acquired
FACS Calibur	2410	2487	104	5001
FACS Canto II (Old)	6536	22	-	6558
FACS Canto II (New) *	911	-	-	911

*Made functional from Nov.2011

STERILE SORTING

EQUIPMENT	SORTING	ACQUISITION**	TOTAL
FACS Aria II SORP	411	1422	1833
FACS Aria III SORP	297	935	1232

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

BD Pathway 855

Around ten 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 non-NCCS users

We have analyzed the following samples on FACS Calibur for non-NCCS users from institutes like IRSHA and IISER:

Surface/ Intracellular staining-12 and DNA cell cycle analysis- 94.

Procurement of equipments in the facility

- FACS ARIA III SORP was received under the buy back scheme of FACS Vantage, and it was installed and made functional from April 2011.
- New Canto II machine was supplied as FOC by BD under this buy back scheme. Installation of the machine was completed and it was made functional from November 2011.
- Training for operators on BD Pathway 855 was organized by BD in April 2011 and it was made functional from June 2011.
- The FACS Aria SORP was upgraded to FACS ARIA III Standard and made functional from December 2011.

Training

Under the BD-NCCS COE Programme, training for NCCS students on Calibur was started from January 2012 by BD. The training will be conducted every month throughout the year.

c) Confocal Microscopy

Technical Officers: Ashwini N. Atre & Nitin S. Sonawane

The imaging facility has two scanning confocal laser microscopes, namely Zeiss LSM510 META and Leica SP5 II system. Both these systems are inverted microscopes and have a wide range of lasers such as Blue Diode laser (405nm), Argon laser(458/477/488/514 nm), He-Ne [543nm, 594nm, and 633nm] 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. The Leica SP5 II microscope will be upgraded with hybrid detectors. In addition to the above two microscopes, we have added another FLUOVIEW FV10i microscope from M/s Olympus, which has a compact design and does not require a dedicated darkroom. All three instruments are used exhaustively by in-house users as well as by users from neighboring organizations.

d) IVIS Imaging System Facility

In-charge: Dr. Gopal C. Kundu

Technical Staff: Mr. Mahadeo Gorain, Technician.

The IVIS imaging system facility is a common facility of NCCS. This instrument provides bioluminescent and fluorescent imaging of cells or whole small animals under in-vitro and 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 for 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 is included a custom lens with a 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°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.

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

IVIS Imaging System

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.

Microbial Culture Collection

The Microbial Culture Collection (MCC) was established by the Department of Biotechnology (DBT), Government of India in April 2009 at the National Centre for Cell Science (NCCS) in Pune, with a broad charter to preserve, characterize and authenticate microbial resources. MCC is now an affiliate member of the World Federation for Culture Collections and registered with the World Data Centre for Microorganisms. In April 2011, MCC was recognized by the World Intellectual Property Organization, Geneva, Switzerland as an International Depositary Authority (IDA) for the deposit of patent microorganisms under the Budapest Treaty.

MCC holds one of the largest culture collections in the world and offers numerous services to its customers. MCC was originally created to serve the preservation and supply requirements of the DBT's microbial prospecting project under which more than 1,50,000 bacterial cultures were collected from diverse ecological niches in India, such as soils from the Western Ghats, the North East of India, mangroves, the marine environment, industrial effluent polluted sites and insect guts. At present, MCC has successfully preserved all these cultures. A major effort is now underway to identify and characterize these safe deposit cultures using rRNA gene sequencing and fatty acid methyl ester (FAME) analyses. About 8,000 cultures have already been identified using 16S rRNA sequencing. In addition, MCC will soon begin to supply all these cultures to industrial partners selected for various bioactivity screening programs. Recently, MCC has begun to accept other deposits, and it currently holds 43 cultures (26 bacterial and 17 fungi) under general deposit and five cultures as an IDA. MCC also offers rRNA gene sequence based microbial identification services to academic and industrial clients. In the last year, approximately 2000 samples of bacteria and fungi have been identified using these services. Very soon, MCC expects to offer additional identification services, such as phenotypic characterization, FAME analysis, G+C mol%, and DNA-DNA hybridization. MCC is also working towards obtaining ISO certification for its service activities so as to achieve the highest quality standards.

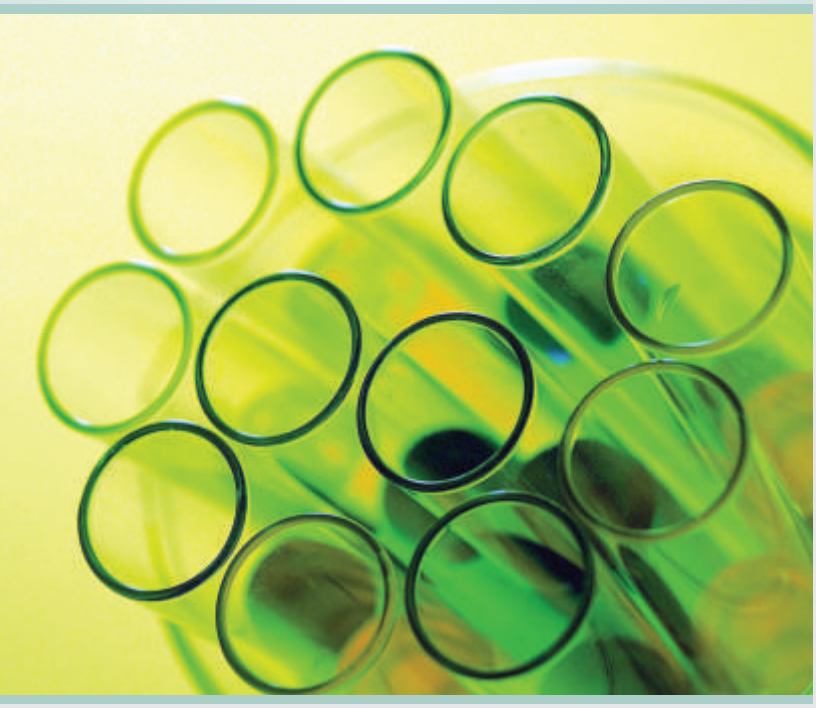
Along with providing high-quality services to its valued customers, MCC faculty and staff also conduct active research in microbial ecology and systematics. Currently, its 48 members include 12 scientists with diverse expertise. They are working in specific areas to improve the quality of services at MCC. In addition, MCC is actively involved in training new manpower to carry out research in microbial ecology and systematics by conducting workshops and symposia at both the local and national level. Further, MCC is continually recruiting new team members to keep up with rising customer demands.

MCC has undergone a major transition since its establishment. For almost three years, it was housed in a 6000 sq. ft. start-up facility located 15 km north of NCCS, Pune. In March 2012, it moved to an interim facility on the main NCCS campus. In early 2013, MCC is expected to move locally to a much larger (40,000 sq. ft.) long-term facility, where additional services will be offered. In the near future, MCC will transform into an autonomous institute under DBT, tentatively named as the National Centre for Microbial Resource, and will continue to provide the highest-standard services along with focused research on microbial ecology and systematics.

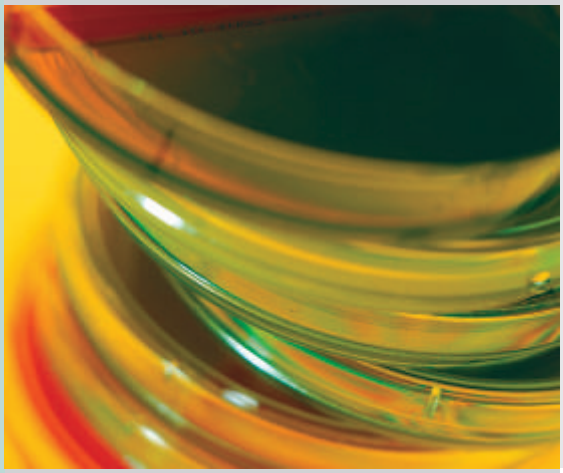
By following internationally accepted guidelines and validated protocols, MCC is strongly committed to providing the highest quality services for microbial preservation and identification, and for supplying authentic cultures. MCC intends to become a premier microbial resource centre in India with a strong focus on in-house research and periodic training of personnel. Details about the services offered, fee structure and other information are available on the MCC website at http://www.nccs.res.in/mcc.

Services Offered by MCC

- Deposit Services
  General Deposit for Public Access; Safe Deposits; IDA Deposits
- Supply of Cultures
- Identification Services
  16S/18S rRNA gene sequencing
  Phenotypic characterization
  Phylogenetic Tree
  FAME analysis (available soon)
  BIOLOG system (available soon)
  API® NE, API® 50 CH, API® ZYM, Vitek® *(available soon)*
  G+C mol% (T_m and HPLC) *(available soon)*
  DNA – DNA Hybridization *(available soon)*
- Educational Services
  Training and Workshops



Other Information



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Book Chapters / Invited Reviews

- Singh S and Shinde S. Stochastic simulation for biochemical reaction networks in infectious disease. In: Medicinal Chemistry and Drug Design, Ekinc D (Ed), pp 329 – 356, InTech Publishers, 2012.
- Suresh A, Naik R and Bapat SA. Role of cancer stem cells in oral cancer. In: Oral Cancer - A Comprehensive Review, Saranath D & Kuriakose MA (Eds) (in press).
- Bapat SA. Epigenetic regulation of cancer stem cells. In: Epigenetics: Development and Disease - Subcellular Biochemistry, Kundu TK (Ed). Springer Publishers (in press).
- Dhamija N, Rawat P and Mitra D. Epigenetic Regulation of HIV-1 Persistence and Evolving Strategies for Virus Eradication. In: Epigenetics: Development and Disease - Subcellular Biochemistry, Vol. 61, Kundu TK (Ed). Springer Publishers (in press).
- Dr. Nibedita Lenka: Editor (with Dr. Deepa Bhartiya) for Pluripotent Stem Cells, Book 2, ISBN: 980-953-307-463-9, InTech Publishers.

Patents Filed / Sealed

Dr. Vaijayanti Kale

1. Creation of artificial bone-marrow environment and uses thereof.

   Granted and sealed in India (Apr. 2011), Australia (Jan. 2012), Singapore (Aug. 2011) and New Zealand (Aug. 2010)

Dr. Manas Santra

1. SRPX for treatment of cancer.

   (US Patent Application # 61/534655, Filed: September 2011)

Dr. Sandhya Sitasawad

1. Anti-tumor activity of AECHL-1, a novel triterpenoid isolated from *Ailanthus excelsa in vitro* and *in vivo*.

   (PCT Application # PCT/IN2008/000795 dated 02/12/2008; United States Patent Application Publication number: US 2010/0311987 A1)

Awards/ Honours/ Memberships/ Extramural Funding

Awards/ Honours/ Memberships

Rahul Bankar

- Member, Laboratory Animal Scientists Association (LASA)

Sharmila Bapat

- ICMR Award – Prem Nath Wahi Award 2007 announced in 2011 and received in November 2011
- Elected Executive Member - Indian Association of Cancer Research (IACR), 2012-2014
- Elected member of Guha Research Conference, 2011
- Member, Basic Biology Task Force, Department of Biotechnology, Govt. of India.
- Honorary Life member of the Indian Association of Head and Neck Oncology.

Manoj Kumar Bhat

- Fellow of the National Academy of Sciences, India, 2011

Sanjeev Galande

- Elected Fellow of the Indian National Academy of Sciences, New Delhi, 2012

Jomon Joseph

- Member - Indian Society of Cell Biology

Vaijayanti Kale

- Appointed as "Associate Editor" of the international journal "Stem Cells and Development", published in USA
- Appointed as "Associate Editor" of the journal "Annals of Neurobiology".
- Appointed as "Senior Editor" for the international journal "InSciences
- Member of International Society of Experimental Hematologists
- Member of International Society of Stem Cell Research (ISSCR)
- Life member of Indian Association of Cell Biology
- Life member of Indian Association of Biotechnology
- Life member of Indian Society of Microbiologists

Gopal Kundu

- Platinum Jubilee Award, 99th India Science Congress, KIIT University, January, 2012
- First Ila Roy Memorial Award, Indian Science News Association, Kolkata, March, 2012

Girdhari Lal

- Life member, Biological Chemists (SBC), India (since 2011).
- 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

- Editor, Pluripotent Stem Cells / Book 2", ISBN: 980-953-307-463-9 (InTech Publisher) (Reviewing In Progress).
- ? Member (Expert), Stem Cells Research Monitoring Subcommittee, NIRRH, Mumbai (2012).
- ? Member, Editorial Board, International Scholarly Research Network (ISRN) Cell Biology.
- ? Member, Editorial Team, Journal of Clinical Medicine and Research.
- ? Member Editorial Advisory Board, Annals of Neurosciences.
- ? Life Member, Indian Academy of Neuroscience.
- ? Life Member, Stem Cell Research Forum of India (SCRFI)

Lalita Limaye

- ? Member of the International Society of experimental Haematology
- ? Life member of the Indian society of cell biology
- ? Life member of the Biotechnology society of India
- ? Life member of the Indian women scientists association
- ? Life member of the Indian association of Microbiologists of India

G C Mishra

- ? 'Raj Kisto Dutt Memorial Award' for the year 2011-2012, at the 99th Indian Science Congress, KIIT University, Bhubaneshwar, January, 2012.

Debashis Mitra

- ? Prof. Y.T. Thathachari prestigious research award for Science, 2011
- ? DBT Tata Innovation Fellowship, 2012
- ? Member, Infectious Disease Biology Task Force, Department of Biotechnology, Govt. of India.

B. Ramanamurthy

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

Srikanth Rapole

- ? Life Member of Indian Society for Mass Spectrometry
- ? Life Member of Proteomics Society, India
- ? Member of American Society for Mass Spectrometry

Arvind Sahu

- ? 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)

Manas Kumar Santra

- Life member for the Society of Biological Chemists
- ? Life member for Indian Association for Cancer Research

Anjali Shiras

- ? Awarded the DBT-CREST Fellowship, DBT, India. Sabbatical with: Dr. Meenhard Herlyn, Director, The Wistar Institute Melanoma Research Center, Wistar Institute, Philadelphia, USA.
- ? Awarded Travel Award for Participation at the 10th Annual Meeting - International Society for Stem Cell Research (ISSCR) Yokohma Japan.
- ? Member of the Editorial Board for International Journal: Journal of Clinical Rehabitative Tissue Engineering Research
- ? Member of the Editorial Board of World Journal of Stem Cells
- ? Member of the International Society of Stem Cell Research (ISSCR), USA
- ? Member of Indian Association of Cancer Research (IACR), India
- ? Member of the Neuro-oncology Society of India (NOSI), INDIA

Shailza Singh

- ? DST Young Scientist Award
- ? International Travel Award by CSIR and DBT
- ? 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

Mohan Wani

- ? Fellow, National Academy of Sciences (FNASc), Allahabad, India, Elected 2011.
- ? Chancellor nominee as an Executive Council (Governing Body) member, Maharashtra Animal and Fisheries Sciences University (MAFSU), Nagpur (20012-2014).
- ? BD Biosciences Research Grant Program, 2011.
- ? Member, Editorial board of World Journal of Immunology.
- ? Member, Peer Review Committee of Maharashtra Animal and Fisheries Sciences University (MAFSU), Nagpur.

Extramural Funding

Sharmila Bapat

NATIONAL:

1. Epigenetic mechanisms in ovarian cancer progression. 2008 - 2011 (DBT)

INTERNATIONAL:

1. Prognostic evaluation of the e-box binding transcription factors in ovarian, breast, prostate and head and neck cancers. 2010 - 2013 (Indo - Finnish Grant)
2. Role of carf and mortalin in ovarian cancer and cancer stem cells. 2010 - 2013 (Indo-Japan Grant)

3. Molecular pathways regulating ovarian cancer cell plasticity and stem cell properties. 2012 - 2015 (Indo-Australia Biotechnology Fund Round 6)

Jomon Joseph

1. Regulation of RNA metabolism by Dishevelled, a critical player of Wnt signalling. 2011-2014 (DBT)

Vaijayanti Kale

1. Effect of hematopoiesis-regulatory factors/signaling molecules expressed in the stromal cells on the fate of the HSCs interacting with them. 2 years (DBT)
2. A study to determine the safety and efficacy of extra-cellular matrix (ECM) embedded bone marrow-derived endothelial progenitor cells (EPCs) in treatment of impaired wound healing. 3 years (BRNS)
3. Identification of signaling mechanisms involved in the regulation of hematopoietic stem cells (HSCs). 3 years (DBT)
4. Creation of an *in vitro* model of the BM niche and studies on its impact on the He matopoietic Stem Cells. 3 years (DBT)
5. Detection of Minimal Residual Disease in acute Leukemia by Molecular Methods. 3 years (DBT)

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 *in vitro* and *in vivo* models. 2010-2013 (DBT)

Girdhari Lal

1. Ramalingaswami Fellowship. 2011-2016 (DBT)
2. Autoimmunity grant (PR4610). 2011-2015 (DBT)
3. Innovative Young Biotechnologist Award grant. 2011-2014 (DBT)

Lalita Limaye

1. *Ex vivo* and *In vivo* studies on megakaryocytes and platelets generated in cultures from hematopoietic stem cells with special reference to role of nutraceuticals as supplement in the culture media. 2010-2012 (DBT)
2. Functional characterization of the in vitro generated dendritic cells from cord blood derived hematopoietic stem cells. 2012- 2012 (DBT)

Shekhar Mande

1. Construction of regulatory networks in *Mycobacterium tuberculosis* analysis of gene expression data and transcription regulation predictions. 2010 - Nov 2012 (DST)
2. *Mycobacterium tuberculosis* bioinformatics and structural strategies towards treatment. Nov 2010 - Nov 2012 (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. Characterization of anti-HIV activity of Acaciaside-B and pre-clinical studies towards its development as a potential microbicide-spermicide formulation. 2009-2012 (DBT).
   Co-Principal Investigators: Debashis Mitra, NCCS and Syed N. Kabir, IICB, Kolkata.
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.

Arvind Sahu

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

Manas Kumar Santra

1. Molecular Mechanism of transcriptional gene silencing (Ramalingaswami Fellowship). 2010-2015 (DBT)
2. 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

1. 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)
2. Exploring the potential of TF antigen binding property of lectin from *Sclerotium rolfsii* for Tumour suppressive activity. 2010-2013 (DBT)

Anjali Shiras

1. Unravelling 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 - 2011 (DBT)
2. Deciphering cancer stem cells – endothelial niche interactions in glioblastoma. 2012 - 2014 (DBT)
3. Identification of biomarkers for diagnosis and prognostication by Next Gen sequencing of oligodendroglial tumour exome. 2012 - 2014 (DBT)

Yogesh Shouche

NATIONAL:

1. Characterization of Hox complex and regulatory elements from *Anopheles stephensi* and *Aedes aegypti*. 2008-2011 (DBT)
2. Establishment of Microbial Culture Collection and Biological Research Centre. 2009-2013 (DBT)
3. Screening of bio-molecules from bacterial diversity isolated from various niches of Western Ghats. 2012-2014 (DBT)
4. Small intestinal and whole gut met genome in patients with celiac disease, their first degree relatives and controls.2011-2013 (DBT)
5. The origins and process of microbiota development in different geographic areas: creating new nutritional tools for microbiota modulation. 2011-2014 (DBT)

INTERNATIONAL:

1. Methanotrophic communities in a meteor impact crater lake in India. 2008-2011 (UKIREI-UK India Research and Educational Initiative)
2. Microbial diversity & development of antibiotic resistance associated with industrial waste water treatment. 2009-2011 (Swedish Research Council)
3. Mid gut bacteria in *Aedes aegypti* and vector competence. 2 years (Forgarty International Research Collaboration Award)

Shailza Singh

1. RGYI Award. 2012 (DBT)
2. Drug Target Identification in *L. major* and *S. mansoni* through Biochemical Network Modeling. 2012-2015 (DBT)
3. Systems Biology of *L. major*: Therapeutic Implications. 2012-15 (DBT)

Mohan Wani

1. Studies on understanding the role of IL-3 in regulation of human osteoclasts and osteoblasts differentiation. 2010-2013 (DBT)
2. BD Biosciences Research Grant Program, 2011

Seminars

Seminar talks by NCCS Scientists

Sharmila Bapat

- ? Modeling Cancer Stem Cell Biology in Culture Systems, August 2011, Abasaheb Garware College
- ? Cancer Stem Cells - Insights from Ovarian Cancer, May 2011 Graduate students symposium at ACTREC:
- ? Modern Research Trends and Applications in Life-Sciences - Inaugural Talk delivered as Chief Guest at the National Symposium on Biotechnology applications : Science, Technology & Society, Elphinston College, 7 January 2012
- ? Stem Cells in Ovarian Cancer - at International Gynecological Cancer Society meeting, 3 April 2011
- ? Gene Expression Analyses towards Molecular Classification of Ovarian Cancer, Plenary talk during meeting organized by Sigma-Aldrich, 19 July 2011 - Frontiers in Gene Silencing and Targeted Gene Editing in Eukaryotes, Pune
- ? Cancer Stem Cells - Elphinston College, National Symposium on Biotechnology applications 7 January 2012, on Modern Research Trends and Applications in Life-Sciences
- ? EMT-MET systems networks in ovarian cancer: defining metastable states or distinct molecular classes? International Symposium on Cancer Biology National Institute of Immunology 14-16 November 2011
- ? Ovarian cancer stem cells - A developmental perspective in Cross talk in Hematological Research between Indo-EU Investigators: Focus on Cancer, Stem Cells, Genomics and Signaling National Centre for Biological Sciences, 6-8 November 2011
- ? Ovarian cancer stem cells and transformation-associated pathways - 23th European Congress of Pathology, Helsinki, Finland, 27 August - 1 September 2011
- ? Ovarian cancer stem cells. 1st CME cum Workshop on Stem Cell therapy and research, Kolkatta, 26 December, 2011
- ? A Systems View towards Molecular Classification of Serous Ovarian Adenocarcinoma. 2nd International conference on Perspectives of Cell Signaling and Molecular Medicine, Kolkatta, 8-11 January 2012
- ? EMT- MET in ovarian cancer: metastable states or distinct molecular classes? AACR International Conference - New Horizons in Cancer Research: Biology to Prevention to Therapy, Gurgaon, 13-16 December, 2011
- ? Ovarian cancer stem cells and transformation-associated pathways. 2nd International Conference on Stem Cells and Cancer (ICSCC-2011), Pune, 15-18 October, 2011
- Tumor heterogeneity and ovarian cancer progression. Indo-US Cytometry Workshop, DY. Patil University, Pune, 19 - 20 October, 2011

Manoj Kumar Bhat

- ? Adipocyte differentiation *in vitro* and *in vivo* impact of obesity on cancer - Workshop on Pathologic Adipose Tissue, Jaipur, 22- 23 March 2012.
- ? Involvement of kinases in cancer cell killing - 80th Annual meeting of Society of Biological Chemists (I) meeting, Lucknow, 12-15, November 2011.

Sanjeev Galande

- ? Unfolding the chromatin 'loopscape': integrating higher-order chromatin architecture with gene regulation - at the University of Hamamatsu, Japan, May 13, 2011.
- ? From Sequence to Consequenc - at the Department of Biochemistry, University of Cambridge, UK, October 15, 2010, and at Kyoto Institute of Technology, May 17, 2011.
- ? Role of chromatin organizer SATB1 in the Wnt signaling pathway - Japanese Society of Developmental Biology Annual Meeting, Okinawa, Japan, May 20, 2011.
- ? How to write an effective grant proposal - BIRAP workshop, Pune, July 14, 2011.
- ? From Sequence to Consequence - CSIR foundation lecture series, Indian Institute of Chemical Biology, Calcutta, September 2011.
- ? Epigenetics of regeneration in Hydra - Workshop on emerging model systems to study stem cell biology, Instem, Bangalore, October 1, 2011.
- ? Epigenetic regulation by chromatin organizer SATB1 - International meeting on 'Epigenomics and Gene Regulatory Networks Controlling Cellular Responses', Mauno Koivisto Center, Biocity Turku, Finland, 3-4 October 2011.
- ? Chromatin organizer SATB1 as a molecular target for anticancer therapy - Chemical Biology Symposium, IISER, Pune, 14th October 2011.
- ? Genome sequence of freshwater cnidarian Hydra vulgaris: Insights into dynamic Epigenetic regulation - Institute of Genomics and Integrative Biology, New Delhi, December 2011.
- ? From Genome to Epigenome – 4 Lectures delivered at several schools and colleges in Pune city as well as outside Pune during the period April 2011-Mar 2012.

Jomon Joseph

- ? Seeing life through microscopic eyes. Navodya Vidyalaya Samiti, Pune Region, Regional Science Congress, Pune, India, November 16, 2011.
- ? Wnt signalling and mRNA regulation. Workshop on Developmental Mechanisms in Model Organisms, Jaipur, India, February 24-25, 2012.
- ? Wnt signaling and mRNA regulation. VI RNA group meeting, Indian Institute of Science, Bangalore, India, March 30-31, 2012.
- ? Ran GTPase: Running beyond nucleo-cytoplasmic transport. Jigyasa, Biochemical Sciences department, NCL, Pune, India, July 9, 2012

Vaijayanti Kale

- ? Invited to deliver a talk on "Stromal cell biology: Creation of in vitro niche (IVN) to modulate stem cell functions" in collaborative nucleator meeting with scientists from California Institute for Regenerative Medicine. The meeting was held at InStem/NCBS, Bangalore, on March 21, 22, 2011

- Invited to deliver a talk on "Stromal cell biology: Creation of in vitro niche (IVN) to modulate stem cell functions" by the Stem Cell Society, Singapore, on 8th June 2011.
- Invited to participate and deliver a talk on "Creation of "*in vitro* niches" - IVNs - to modulate stem cell functions" in discussion forum on "HSC fate in the niche" held on 6th January 2012 at CMC, Vellore.

Gopal Kundu

- 'Understanding the mechanism of tumor progression and angiogenesis in breast and other cancers: potential role of osteopontin as cancer diagnostics and therapeutics', Indian Institute of Chemical Technology (IICT), Hyderabad, 13 April 2011.
- 'Role of stroma and tumor derived osteopontin in regulation of tumor progression, angiogenesis and metastasis', Hokkaido University, Japan, 23 May 2011.
- 'Signal Transduction, Tumor Biology, Angiogenesis and Cancer Therapeutics', DST-INSPIRE Talk, University of Burdwan, Burdwan, West Bengal, 12 July 2011.
- 'Therapeutic potential of osteopontin in cancer', MLSC, Manipal University, Manipal, Karnataka, 14 July 2011.
- 'Role of stroma and tumor derived osteopontin in regulation of tumor progression, angiogenesis and metastasis', Jefferson Medical Center, Philadelphia, USA, 8 September 2011.
- 'Role of stroma and tumor derived osteopontin in regulation of tumor progression and angiogenesis', National Cancer Institute, NIH, USA, 9 September 2011.
- 'Development of novel diagnostic and prognostic biomarker in breast cancer', 2nd World Congress on Biomarkers & Clinical Research, Baltimore, MD, USA, 12 September 2011.
- 'Applications of Flow Cytometry in Cancer Biology and Angiogenesis', Indo-US Flow Cytometry Workshop, Manipal University, 14 October 2011.
- 'Osteopontin: a potentially important therapeutic target in cancer', 2nd Intl Conference on Stem Cells & Cancer (ICSCC), YASHADA, Pune, 15 October 2011.
- 'Therapeutic Significance of osteopontin in cancer', 80th Annual Meeting of Society of Biological Chemist (SBC), CIMAP, Lucknow, 13 November, 2011.
- 'Therapeutic and Diagnostic Significance of Osteopontin in Cancer', AFRI, Jodhpur, 5 December, 2011.
- 'Osteopontin: a potentially important therapeutic target and diagnostic marker in breast & other cancer', Society of Translational Cancer Research Symposium on Recent Development in Cancer Prevention, Udaipur, 17 December 2011.
- 'Osteopontin: a key therapeutic target in cancer', Platinum Jubilee Award Lecture, 99th Indian Science Congress, KIIT University, Bhubaneswar, 4 January 2012.
- 'Osteopontin regulated signaling plays crucial role in tumor progression and angiogenesis', International Cell Signaling & Molecular Medicine Symposium, Bose Institute , Kolkata, 9 January 2012.
- 'Transcriptional regulation of osteopontin in cancer and angiogenesis', 15th Transcription Assembly, Mahabaleswar, Maharashtra, 24 January 2012.
- Cancer Awareness in India. DST-INSPIRE Program, KIIT University, Bhubaneswar, 7 February 2012.

- ? 'Nanoparticle-mediated Drug Delivery in Cancer: Current Strategies and Future Prospects', DBT Nanotechnology Brain Storming Session, Kolkata, 11 February 2012.
- ? 'Role of stroma, tumor and cancer stem cell-derived osteopontin in regulation of inflammation, angiogenesis and cancer', National Conference on Biotechnology, MGM University, Aurangabad, Maharashtra, 16 February 2012.
- ? 'Nanoparticle-mediated Drug Delivery in Cancer: Osteopontin as Important Therapeutic Target', Nano-Bio-2012, Amrita Nano Science Center, Kochi, 22 February 2012.
- ? 'Osteopontin: its role in regulation of tumor growth and angiogenesis', 1st International Angiogenesis Symposium, Chennai, 1 March 2012
- ? 'Natural product derived targeted therapy in cancer', National Innovation Foundation, Rashtrapati Bhavan, New Delhi, 9 March 2012
- ? 'Application of Nanotechnology & Nanomedicine in Cancer', Krishna Institute of Medical Sciences University, Karad, Maharashtra, 17 March 2012.
- ? 'Cancer Stem Cells as Novel Therapeutic Target for the Prevention of Tumor Progression and Metastasis'. 1st Ila Roy Memorial Award Lecture, Kolkata, 28 March 2012

Girdhari Lal

- ? An epigenetic approaches to induce transplantation tolerance and control auto immunity: Invited talk at the Symposium on "Biotalk" at Hislop College, Nagpur, India, October 10-11, 2011.

Nibedita Lenka

- ? Invited talk: 'Investigating the Guiding Cues Underlying Early Neurogenesis from Embryonic Stem Cells *in vitro*', BIT Life Sciences, the 4th Annual World Congress of Regenerative Medicine & Stem Cell (RMSC-2011), Beijing, China, 2011.
- ? Invited talk: 'Cell Fate Modulation by Wnt during Embryonic Stem Cells Maintenance and Differentiation', XXXV All India Cell Biology Conference, NISER, Bhubaneswar, Odisha, 2011.
- ? Invited talk: 'Notch, the master regulator during cell fate specification and neurogenesis. National Seminar on Living Systems in Post Genomic Era', Fakir Mohan University, Balasore, Odisha, 2011.
- ? Invited talk: 'Temporal influence of cell fate modulators during early Neurogenesis from Embryonic Stem cells *in vitro*', Workshop on 'Developmental Mechanisms in Model Organisms' organized under the auspices of the Indian Society of Developmental Biologists, Jaipur, India, 2012.
- ? Invited plenary talk: 'Investigation of Cell Fate Specification and Underlying Modulators using Embryonic Stem Cells Model', Symposium on recent Advances in Stem Cells, Panjab University, Chandigarh, 2012.

Lalita Limaye

- ? Invited lecture: 'Hematopoietic stem cells', at the Modern College, Shivajinagar, Pune, 5th October 2011.

Shekhar Mande

- ? Invited talk at the National Chemical Laboratory, Pune, November 2011
- ? Invited talk at the Centre for Bioinformatics, University of Pune, Pune, December 2011
- ? Invited talk at the Department of Zoology, University of Pune, Pune, February 2012
- ? Invited talk at University of Delhi South Campus, March 2012
- ? Francis Crick Memorial talk, Aurora Degree College, Hyderabad, March 2012

Debashis Mitra

- ? 'Cyclin K inhibits HIV-1 gene expression and replication in Nef dependent Manner', 2nd Molecular virology meeting, Indian Institute of Science, Bangalore, April 29-30, 2011.
- ? Invited talk: 'Cellular heat shock proteins regulate HIV-1 gene expression and replication in Nef dependent manner', ACTREC, Navi Mumbai, 16th September 2011.
- ? Plenary Talk: 'Novel anti-HIV molecules from the nature and their potential as microbicide candidates', 12th International Congress of Ethnopharmacology, Kolkata, India, February 17-19, 2012.

Arvind Sahu

- ? Invited talk: 'Studies on complement evasion in poxviruses provide insight into their host tropism', the International Workshop on Platforms for Molecular cross-talk in Modern Biology, Regional Centre for Biotechnology (RCB), Gurgaon. 8 May 2012.

Manas Kumar Santra

- ? Invited talk: 'The F-Box Protein FBXO31 Directs Degradation of MDM2 to Facilitate p53-Directed Senescence Induction', North Eastern Hill University, 15 March, 2012.

Vasudevan Seshadri

- ? 'miR196b mediated translation regulation of mouse Insulin2', RNA2012, IISC, Bangalore, March 30, 2012.

Anjali Shiras

- ? Invited Talk: 'Therapeutic potential of Stem Cells', Training program for Medical Graduates, Moving Academy, March, 2012, Pune, India.
- ? Invited Talk: 'Facts and fallacies in Stem Cell Therapy', Indian Medical Association Meeting, April 2012, Pune, India.
- ? 'Identification of novel molecular targets for melanoma', University of Pennsylvania, October 2011, Philadelphia, USA.

Yogesh Shouche

- ? 'Space Microbiology', AMI, Chandigarh 2011.
- ? 'Understanding microbial community dynamics of Lonar Lake', Genomeet, New Delhi, December 2011.

Shailza Singh

- ? Invited Talk: 'Network Modeling and Validation for Infectious Disease', ACTREC, Kharghar, Mumbai, 24 June 2011.
- ? Invited Talk: 'Molecular Simulation to Biochemical Network Perturbation in Infectious Disease', Jamia Millia Islamia, New Delhi, in IISC, 15-17 November, 2011.
- ? Talk in SBC: 'Systems Biology for Exploring Novel Lipid Pathways in Infectious Diseases', CIMAP, Lucknow, November 12-5, 2011.
- ? 'Nanoliposomes in Drug Delivery Systems: A New Avenue for Pharmacological Intervention' the 4th Bangalore Nano, 8 December 2011.
- ? Invited Talk: 'Homology Modeling and its Applications', Bioinformatics workshop organized for Teachers and students at ACTREC, Navi Mumbai, Kharghar, 10 March 2012.

Mohan Wani

- ? 'Potential of human dental tissues-derived mesenchymal stem cells in regenerative medicine', Manipal Institute of Regenerative Medicine, Bangalore, July 6, 2011.
- ? 'Use of anesthetics and surgical techniques in laboratory animals', National Institute of Virology, September 7, 2011.

Seminar talks by Visiting Scientists

1. **Dr. Vishal S. Vaidya,** Brigham and Women's Hospital, Harvard Medical School, Boston, USA. 'Mechanisms of Kidney Exposure Biology', May 2011.

2. **Mr. Sachin Nikarage,** Program Coordinator, United States-India Educational Foundation (USIEF), Mumbai, India. 'Fulbright Fellowship Opportunities to the U.S.', July, 2011.

3. **Prof. Jürgen Wolfrum,** Professor (em.) for Physical Chemistry, Founding Director BioQuant, Heidelberg, Germany. 'Systems Biology - a new quantitative approach in life sciences and medicine', November 2011.

4. **Dr. Renu Wadhwa,** National Institute of Advanced Industrial Science and Technology (AIST), Japan. ''Molecular biology of p53 regulation by Mortalin and CARF in human cancer cells', November, 2011.

5. **Dr. Arunima Biswas,** Indian Institute of Chemical Biology, Jadabhpur, Kolkata, India. 'cAMP signaling in the survival and infectivity of protozoan parasite, *Leishmania donovani*'', November, 2011.

6. **Dr. Pawanbir Singh,** Senior Scientist, Stem Cell Technologies Inc. Canada. 'Culture systems for Mesenchymal Stem Cells' & Feeder-independent culture and *in vitro* differentiation of pluripotent stem cells', December 2011.

7. **Dr. Soumen Kanti Manna,** National Cancer Institute NIH, USA. 'Metabolomics : bridging the gap, between basic and translational chemical biology', January 2012.

8. **Dr. Radha Devi Chauhan,** The Rockefeller University, New York, USA. 'The Role of Nup93 Sub-complex in Nuclear Pore Complex Assembly and Function', January, 2012.

9. **Dr. Debabrata Biswas,** The Rockefeller University, New York, USA. 'MLL fusion protein and fusion partner protein complexes in transcriptional regulation as well as their novel exciting role in the field of DNA replication', January 2012.

10. **Dr. Deepa Subramanyam,** University of California, San Francisco, USA. 'Cell fate transitions in development and cancer: regulation by microRNAs and epigenetic mechanisms', January 2012.

11. **Prof. Lalitha Ramakrishnan,** Department of Microbiology, University of Washington, Seattle, USA. 'Host Genotype directed treatments for TB', January, 2012.

12. **Prof. Tom Blundell,** Department of Biochemistry, Cambridge University, UK. (Interactions & Discussions), February 2012.

13. **Prof. Jerome Galon,** Research Director, INSERM, Paris, France. 'Immune contexture: A novel paradigm for cancer', February 2012.

14. **Dr. Arianna Bertossi,** Max Plank Institute of Biochemistry, Germany. 'Role of the RNA-binding protein roquin in immune homeostasis and autoimmunity', February 2012.

15. **Dr. Purusharth Rajyaguru,** University of Arizona, USA. 'Regulation of mRNA translation and decay', February 2012.

16. **Dr. Rajakumar Eerappa,** Memorial Sloan-Kettering Cancer Centre, New York, USA. 'Mechanism of the histone code and cytosine methylated DNA read-out and interpretation by chromatin associated modules', February 2012.

17. **Dr. Ghanshyam Swarup,** the Centre for Cellular & Molecular Biology, Hyderabad. 'Functional Defects Caused by Glaucoma Associated Mutants of Optineurin', February 2012.

18. **Dr. Achut Malur,** Brody School of Medicine, East Carolina University, USA. 'Contributions of the accessory proteins to the human, parainfluenza virus type 3 pathogenesis', March 2012.

19. **Dr. Rajeeva Karandikar,** Director, Chennai Mathematical Institute, Chennai. 'Is there a science behind opinion polls?' March, 2012.

20. **Dr. Subhrajit Biswas,** Dept. of Medicine, Vanderbilt University Medical Center, Nashville, USA. 'Pro-apoptotic Bcl-2 Family Members in Hematopoietic Differentiation and Leukemogenesis', March 2012.

21. **Dr. Arvindya Dutta,** University of Virginia School of Medicine., Charlottesville. 'Strange new NA's in the call : tRFs. & microDNAs', April 2012.

22. **Prof. Madhav Gadgil,** 'What are endangered Indian birds?', May 2012.

23. **Dr. Mithilesh Mishra,** 'Studying cytokinesis *in vitro*', June 2012.

24. **Dr. Max Muehlig,** Life Technologies. 'GeneArt - Knockout genome Studies - TAL Endonuclease' (Technical Seminar), August 2011.

Conferences/ Workshops

Sharmila Bapat

- ? Ovarian cancer stem cells. 1st CME cum Workshop on Stem Cell therapy and research, Kolkatta, 26 December, 2011
- ? A Systems View towards Molecular Classification of Serous Ovarian Adenocarcinoma. 2nd international conference on Perspectives of Cell Signaling and Molecular Medicine, Kolkatta, 8-11 January 2012
- ? EMT- MET in ovarian cancer: metastable states or distinct molecular classes? AACR International Conference - New Horizons in Cancer Research: Biology to Prevention to Therapy, Gurgaon, 13-16 December, 2011
- ? Ovarian cancer stem cells and transformation-associated pathways. 2nd International Conference on Stem Cells and Cancer (ICSCC-2011), Pune, 15-18 October, 2011
- ? Tumor heterogeneity and ovarian cancer progression. Indo-US Cytometry Workshop, DY. Patil University, Pune, 19 - 20 October, 2011

Manoj Kumar Bhat

- ? Impact of managing diet-induced obesity with antiobesity drug orlistat on progression of melanoma. Second AACR Special Conference on Metabolism and Cancer: Metabolism and Cancer, Baltimore, USA, 16 -19 October, 2011

Samit Chattopadhyay

- ? Regulation of T cell differentiation by nuclear matrix protein SMAR1: Its implication in immune response - Molecular Immunology Forum 2012, Matheran, 10-12 February, 2012
- ? p53 mediated global gene regulation in association with MAR binding protein SMAR1 - 15th Transcription Meeting, Mahabaleswar, 22-24 January, 2012
- ? Global gene regulation by SMAR1 - Guha Research Conference, Khimsar, Rajasthan, 1-5 December, 2011
- ? Regulation of T cell development and differentiation - International conference on stem cell and cancer, Pune, 15-18 October, 2011

Sanjeev Galande

- ? Biotechnology Industry Research Assistance Programme (BIRAP) Second Series of One-Day Regional Workshop on "How to write an effective grant proposal", Pune, July 14, 2011.
- ? Second Annual Fellows' Meeting- Wellcome trust-DBT India Alliance, Hyderabad, 29-30 September 2011.
- ? International meeting on 'Epigenomics and Gene Regulatory Networks Controlling Cellular Responses', Mauno Koivisto Center, Biocity Turku, Finland, 3-4 October 2011.
- ? Young Investigator Meeting (YIM) Boston, MIT, USA, 8-11 October 2011.
- ? Annual meeting of the Fellows of the Indian Academy of Sciences, Ahmedabad, November 2011.

- ? Guha Research Conference, Jodhpur, December 2012.
- ? Young Investigator Meeting, Lonavala, January 7-11, 2012.
- ? 15th Transcription Assembly, TIFR (organized at Mahabaleshwar), February, 2012.
- ? Molecular Immunology Forum meeting, Matheran, February 2012.
- ? Annual meeting of the Indian Society of Developmental Biologists, Jaipur, 24-25 February 2011.

Jomon Joseph

- ? Workshop on 'Developmental Mechanisms in Model Organisms', Feb 24-25, 2012, Jaipur, India.
- ? VI RNA group meeting, Mar 30-31, 2012, Indian Institute of Science, Bangalore, India.

Vaijayanti Kale

- ? Modulating stem cell functions *in vitro*', Invited talk in the Indo-Brazil Symposium on Biomedical Sciences held on 29th and 30th August, 2011 at the headquarters of the Brazilian Academy of Sciences, Rio de Janeiro, Brazil.
- ? M. Ranjita Devi and Vaijayanti P. Kale. "Role of FAK signalling in the regulation of HSC expansion" Presented in 8th Indo-Australia Biotechnology conference on "Stem Cell Biology" December 7-9, JNCASR, Bangalore.
- ? Characterization of hematopoietic stem/progenitor cells using *in vitro* and *in vivo* assays' Invited talk in National Seminar on "Interdisciplinary tools and techniques in life Sciences" organized by Ahmadnagar College on 1 - 3 March 2012.

Girdhari Lal

- ? 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 the American Transplantation Congress held at Boston, USA. June 2-6th. (Plenary 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 the American Transplantation Congress held at Boston, USA. June 2-6th. (Oral presentation).
- ? Lal G (2011) Role of B cell in the maintenance of transplantation tolerance. In 2nd International Conference on Stem cells and Cancer (ICSCC-2011), Pune, India. October 15-18th. (Invited speaker).
- ? Yin N, Lal G, XuJ, Ding Y and Bromberg JS (2011) "Pancreatic macrophage contribute to islet lymphangiogenesis and enhanced myeloid cell recruitment" in American Transplant Congress 2011, held at Philadelphia, USA. May 3rd. (Poster).
- ? Lal G, Yin N, Xu J, Ding Y and Bromberg J (2011) "Inflammatory signals induce epigenetic modifications that control natural regulatory CD4 T cells" in American Transplant Congress 2011, held at Philadelphia, USA. May 2nd. (Poster).
- ? Lal G, Nakayama Y, Burrell BE, Ding Y and Bromberg J (2011) "Non-humoral function of B cells is required for tolerance" in American Transplant Congress, held at Philadelphia, USA. May 1st. (Oral presentation).

Nibedita Lenka

- ? 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

Shekhar Mande

- ◆? National Conference on Genomics and Proteomics, University of Pondicherry, Puducherry, September 2011
- ◆? Conference on Bioinformatics and Computational Biology, CDAC, Pune, February 2012
- ◆? US-India Joint Workshop on Tuberculosis, ICGEB, New Delhi, February 2012
- ? Modern Trends in Biology, University of Delhi South Campus, March 2012

Debashis Mitra

- ◆? 2nd Molecular virology meeting, Indian Institute of Science, Bangalore, April 29-30, 2011.
- ◆? 12th International Congress of Ethnopharmacolgy, Kolkata, February 17-19, 2012.

Srikanth Rapole

- ◆? International conference on cancer biomarkers, 'Translational Biomarkers in Diagnostics and Therapeutics' organized by ISBHT at Mumbai, November 16-18, 2011.

Arvind Sahu

- ◆? Molecular Immunology Forum- 2012, Matheran, February10-12, 2012.
- ◆? Indo-US Workshop on Measuring Human Immune Response, Organized by THSTI, Oct 31-Nov 2, 2011.
- ◆? 'Studies on complement evasion in poxviruses provide insight into their host tropism', presented at the International Workshop on Platforms for Molecular cross-talk in Modern Biology, Organized by the Regional Centre for Biotechnology (RCB), Gurgaon, May 8, 2012.

Vasudevan Seshadri

- ◆? RNA 2012, Department of microbiology and Cell Biology, IISC, Bangalore, 30-31 march, 2012.

Anjali Shiras

- ◆? MicroRNA-145 functions as a tumor suppressor by targeting WNT signaling pathway in human glioma. International Symposium on Cancer Genomics and its Impact - 31st Annual Convention of Indian Association of Cancer Research, ACTREC, Mumbai, 26 - 29 Jan 2012
- ◆? Reprogramming of fibroblasts as well as cancer cells by non-coding RNA-Ginir. International Symposium on Stem Cell Biology, ACTREC Mumbai, January 30, 2012
- ◆? 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

Yogesh Shouche

- ◆? Annual meeting of the Association of Microbiologists of India, Chandigarh, December 2012.
- ◆? Genomeet, New Delhi, December 2011.

Sandhya Sitaswad

- 'A novel triterpenoid isolated from the root bark of *Ailanthus excelsa* Roxb (Tree of Heaven), AECHL-1 as a potential anti-cancer agent' Lavhale MS, Kumar S, Mishra SH, Sitasawad SL): oral paper presented at Recent Advances in Cancer Research: Bench to Bedside, School of Life Sciences, Central University of Gujarat, Gandhinagar, February 19-20, 2011.

Mohan Wani

- Stem Cell Conclave 2011, Mumbai, July 2-3, 2011.
- ? 'Interleukin-3 promotes osteoblast differentiation and bone formation of human mesenchymal stem cells' (poster): presented at the Annual Meeting of American Society for Bone and Mineral Research (ASBMR), San Diego, USA, September 16-20, 2011.
- ? Guha Research Conference (GRC) 2011, Khimsar Fort, Jodhpur, December 1-5, 2011.
- ? Molecular Immunology Forum 2012, Matheran, February 10-12, 2012

Conferences / Workshops attended by Students

- Aparajita Dasgupta presented a poster, 'To study the anti-angiogenic potential of AECHL-1 and elucidation of its mechanism' (Aparajita Dasgupta, Sandhya Sitasawad), at Recent Advances in Cancer Research: Bench to Bedside, February 19-20, 2011, School of Life Sciences, Central University of Gujarat, Gandhinagar.

- Arya Vindu presented a poster, 'Role of translation regulation in *Plasmodium falciparum* development and identification of stage specific transcript–protein interaction in *Plasmodium falciparum* life cycle', at the 35th All India Cell Biology (AICB) conference, 16-18 December 2011, National Institute of Science Education and Research (NISER), Orissa.

- Avtar Singh Meena presented a poster entitled 'Chemotherapeutic drugs induced caveolin-1 mediates cell death in breast cancer cells via activation of p53 and p38 pathways' (Meena AS, Upadhyay AK & Bhat MK), at the Fourth International Conference on Translational Cancer Research, Recent Developments in Cancer Prevention, 16 -19 December, 2011, Udaipur, India

- Avtar Singh Meena presented a poster entitled 'Molecular signature for inherent and acquired drug resistance in hepatocellular carcinoma cells' (Meena AS, Sharma A, Kumari R & Bhat MK) at the 70th Annual Meeting of the Japanese Cancer Association (JCA), 3 - 5 October, 2011, Nagoya, Japan.

- Kiran K Nakka made an oral presentation of a paper entitled 'Regulation of pre-mRNA Splicing by Nuclear Matrix Protein SMAR1' (Nakka KK & Chattopadhyay S) at RNA 2011, the Sixteenth Annual meeting of the RNA Society, 14 -18 June, 2011, Kyoto, Japan. He received a Travel Award from the Organizers to attend this prestigious meeting.

- Manasa Gayatri attended the 12th Indo-US Cytometry Workshop, 'Applications of laser flow cytometry in biomedical research', October 19-20, 2011, D. Y. Patil University, Pune.

- Manasa Gayatri attended the 5th Winter School in Immunology, January 9-15, 2012, Jodhpur, Rajasthan.

- Manasa Gayatri attended the 5th Congress of the Federation of Immunological Societies of Asia Oceania, 'Translational immunology in health and disease', March 14-17, 2012, New Delhi.

- Manasi Talwadekar presented a poster, '*In vitro* generation, characterizaton and cryopreservation of mesenchymal stem cells from various fractions of cord tissues' (Manasi Talwadekar, V. P. Kale and L. S. Limaye), at the XXXV All India Cell Biology Conference (AICBC) & Symposium on Membrane Dynamics and Disease, 16th-18th December 2011, National institute of Science Education and Research (NISER), Bhubaneswar, Odisha

- Mithila Sawant presented a poster, 'Investigation of cellular and molecular mechanisms underlying the anti-cancer activity of AECHL-1 on the breast cancer MCF-7 cells' (Mithila Sawant, Sandhya Sitasawad), at Recent Advances in Cancer Research: Bench to Bedside, February 19-20, 2011, School of Life Sciences, Central University of Gujarat, Gandhinagar.

- Naoshad Mohammad presented a poster entitled 'Methyl ß- cyclodextrin (MCD) enhances the cytotoxicity of tamoxifen in melanoma cells' (Mohammad N & Bhat MK), at the Fourth International Conference on Translational Cancer Research, Recent Developments in Cancer Prevention, 16 -19 December, 2011, Udaipur, India

- Navjot Kaur presented a poster entitled 'A novel non-coding RNA "Ginir" induces genomic instability and tumorigenicity by upregulation of BMI-1' at Cell Symposia: Epigenetics and the Inheritance of Acquired States, October 30 - November 1, 2011, Boston, MA, USA. She was awarded the DST Travel Award for participation.

- Neeru Dhamija presented an oral paper, 'Genome wide recruitment of Tat in HIV-1 infected T cells and its functional relevance', and received the Best Oral Presentation award, at the 2nd Molecular virology meeting, April 29-30, 2011, Indian Institute of Science, Bangalore.
- Pankhuri Vyas attended a Lecture series on 'Molecular mechanism of protein transport' organized by EMBO, Dec 2011, NCBS, Bangalore.

- Pabitra Kumar Sahoo made an oral presentation at the XXXV All India Cell Biology Conference at the National Institute of Science Education and Research (NISER), 16-18, December 2011, Bhubaneswar.

- Poonam Pandey presented an oral paper, 'Understanding the Mechanism of Differential Regulation of Insulin gene2 Splice Variants', at the RNA2012 meeting, 31 March, 2012, IISc, Bangalore.

- Pratima Rawat received the Best Poster Fellowship for the poster, 'Regulation of HIV-1 replication and gene expression by Heat Shock Factor 1', presented at the 2nd Molecular virology meeting, April 29-30, 2011, Indian Institute of Science, Bangalore.

- Rajkumar Singh presented a poster entitled 'RXR-G deficiency facilitates mechanistic benefit to transformed ovarian epithelial cells to escape apoptosis in disease progression' (Singh, R & Bapat SA), at the International Symposium on Cancer Biology, 14-16th November 2011, NII, New Delhi.

- Reecha Shah (research assistant on ICMR project) received the Shri Sitaram Joglekar Award for the Best Oral Presentation at the 31st Annual Convention of Indian Association for Cancer Research (IACR), Jan 2012, ACTREC, Navi Mumbai.

- Rutika Naik presented a poster entitled 'Resolution of tumor heterogeneity based on proliferative hierarchy, ploidy and tumor vasculature' (excellence award-winning poster), (Naik RR, Kusumbe AP & Bapat SA), at the International Symposium on Cancer Biology, 14-16th November 2011, NII, New Delhi.

- Shama Bansod presented a poster, 'Effect of oral administration of omega-3 fatty acids on hematopoiesis and megakaryopoiesis in mice' (Shama Bansod, Anitha K., V.P. Kale and L.S. Limaye), at the XXXV All India Cell Biology Conference (AICBC) & Symposium on Membrane Dynamics and Disease, 16th-18th December 2011, National institute of Science Education and Research (NISER), Bhubaneswar, Odisha

- Shardul Kulkarni, attended the workshop on Science & Communication organized by Well-come trust DBT Alliance, 28 - 30 March 2012, Hyderabad

- Sudeep Sabde presented a poster, 'Novel Caffeoyl anilide molecules as dual inhibitors of HIV-1', at the 2nd Molecular virology meeting, April 29-30, 2011, Indian Institute of Science, Bangalore.

- Surbhi Chouhan presented a poster entitled 'Hyperglycemia induced alterations on hepatocellular carcinoma and breast cancer cells' (Chouhan S, Pandey V, Chaube BK, Vijayakumar MV & Bhat M.K) at the Fourth International Conference on Translational Cancer Research, Recent Developments in Cancer Prevention, 16 -19 December, 2011, Udaipur, India

Conferences Organized by NCCS

- NCCS organized the 'Molecular Immunology Forum - 2012' at the Brightlands Resorts, Matheran (February 10-12, 2012).

  Organizing Committee: Mohan Wani, Arvind Sahu, Debashis Mitra

Students Awarded Ph.D.

Anjali Kusumbe

Title of the Thesis: Identification and characterization of the cellular types contributing to ovarian cancer progression.

Guide: Dr. Sharmila A. Bapat

Ratna Kumari

Title of the Thesis: Molecular mechanism of DNA damaging agent's action in solid tumor cell-lines: Role of oncogenes.

Guide: Dr. Manoj Bhat

Ranveer Jayani

Title of the Thesis: Role of SATB1 in establishing euchromatic and heterochromatic regions in nuclues.

Guide: Dr. Sanjeev Galande

Monika B. Sharma

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

Guide: Dr. Vaijayanti Kale

Amita Sneh

Title of the Thesis: Biophysical characterization of caveolin-1 and its interaction with other cellular molecules.

Guide: Dr. Musti Krishnasastry

Saravana Kumar Ramasamy

Title of the Thesis: Investigation of Early Neurogenic Proceedings and Neuronal Subtypes' Specification during *In Vitro* Neurogenesis Using Embryonic Stem Cell Model.

Guide: Dr. Nibedita Lenka

Sangeetha V.M.

Title of the Thesis: Studies on the biology of *ex vivo* expanded hematopoietic stem/progenitor cells: prevention of apoptosis to improve the efficiency of expansion, cryopreservation and engraftment.

Guide: Dr. L. S. Limaye

Namrata Shabrani

Title of the Thesis: Studies on the effect of nutraceuticals in promoting the differentiation of *ex vivo* generated megakaryocytes and platelets from hematopoietic stem cells.

Guide: Dr. L. S. Limaye

Amaresh C. P.

Title of the Thesis: Identification and characterization of the 5'UTR of mouse insulin mRNAs and the trans acting regulators.

Guide: Dr. Vasudevan Seshadri

Gowry Das

Title of the Thesis: Molecular mechanisms in survival and invasion of human gliomas – Role of NF-κB and PI3K/Akt pathways.

Guide: Dr. Padma Shastry

Radha Pujari

Title of the Thesis: Elucidation of signaling pathways in *Rhizoctonia bataticola* lectin (RBL)-induced mitogenesis.

Guide: Dr. Padma Shastry

Mruthuynjaya. S

Title of the Thesis: Differentiation of human bone marrow mesenchymal stem cells (MSC) towards neuronal lineages.

Guide: Dr. Padma Shastry

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

Kaustubh Gokhale

Title of the Thesis: Characterization of Hox Cluster from mosquito.

Guide: Dr. Yogesh Shouche

Vasundhara Kain

Title of the Thesis: Role of oxidative and nitrosative stress in defective intracellular Ca^{2+} signaling in diabetic cardiomyopathy.

Guide: Dr. Sandhya L. Sitasawad

Geetanjali Tomar

Title of the Thesis: Identification and characterization of mesenchymal stem cells derived from human gingiva.

Guide: Dr. Mohan Wani

Rupesh Kumar Srivastava

Title of the Thesis: Immunomodulatory role of interleukin-3 in Treg and Th17 differentiation.

Guide: Dr. Mohan Wani

Navita Gupta

Title of the Thesis: Role of interleukin-3 in the regulation of human osteoclast differentiation and activation.

Guide: Dr. Mohan Wani

Amruta P. Barhanpurkar

Title of the Thesis: Studies on role of interleukin-3 in regulation of osteoblast differentiation from mesenchymal stem cells.

Guide: Dr. Mohan Wani

Visit by the DBT Expert Committee

An Expert Committee constituted by the Department of Biotechnology visited NCCS on 11th & 12th December 2011, for an external review.

This committee, which was chaired by Prof. Inder Verma, comprised the following members:

Prof. Inder Verma
Laboratory of Genetics
Irwin and Joan Jacobs Chair in Exemplary Life Science
Salk Institute for Biological Studies
San Diego, California
USA

Dr. T. Balganesh
Head of Research
Astra Zeneca
Bangalore
India

Dr. Piet Borst
Group Leader
Division of Molecular Oncology
The Netherlands Cancer Institute
Amsterdam
The Netherlands

Dr. Vishva Dixit
Vice President
Physiological Chemistry
Genentech
San Francisco, California
USA

Prof. V. I. Mathan
Former Director and Head, Department of GI Sciences
Christian Medical College, Vellore,
Tamil Nadu
India

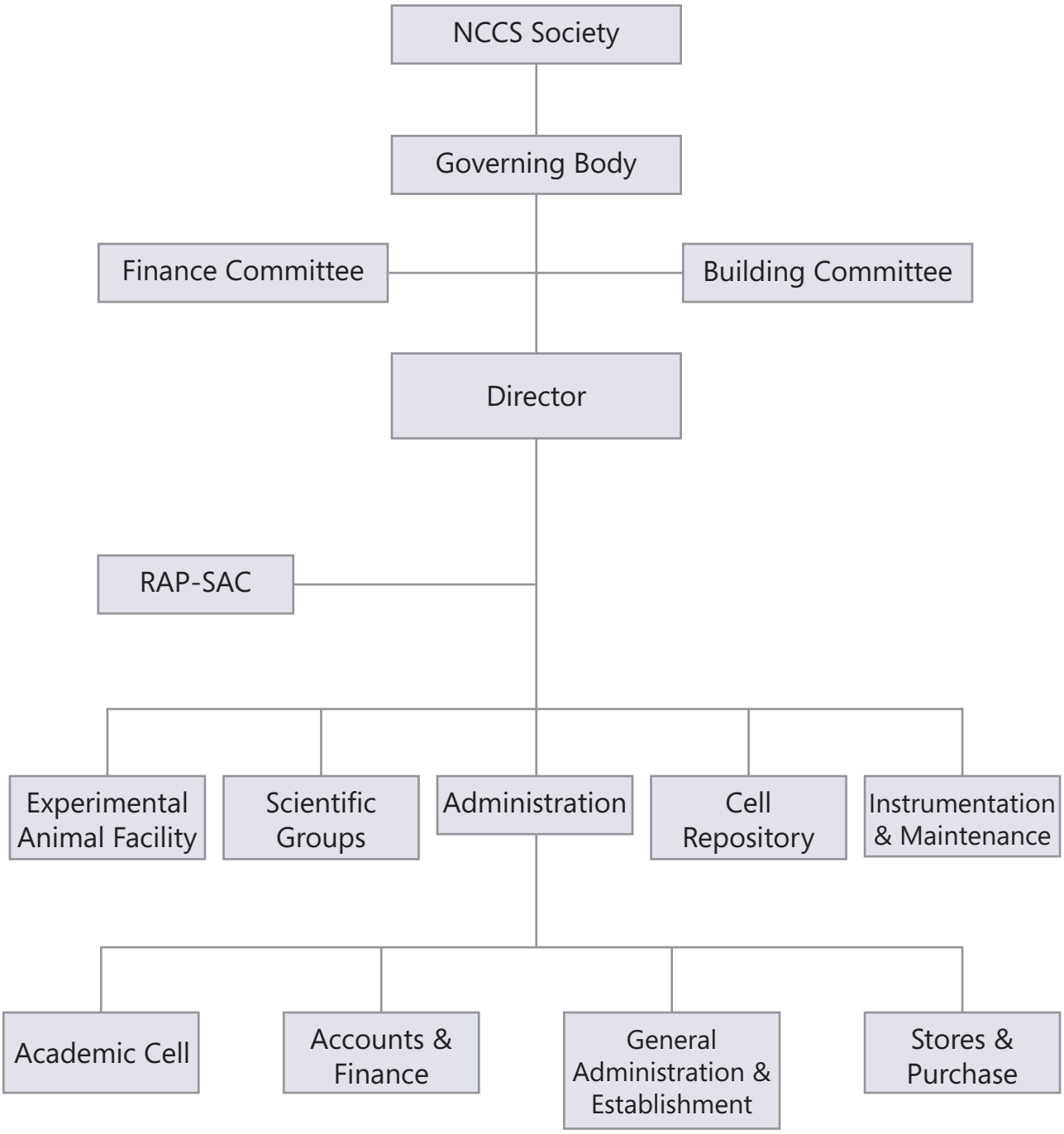
Dr. Carol Prives
Da Costa Professor
Biological Sciences
Columbia University
New York
USA

Dr. Shankar Subramaniam
Chair
Dept. of Bioengineering
University of California at San Diego
USA

Dr. Rafi Ahmed*
Director
Emory Vaccine Center
Atlanta, Georgia
USA
(* : he was unable to visit NCCS)

NCCS Organization

NCCS Organization



NCCS Committees

NCCS Society Members

1. **Shri Vilasrao Deshmukh** — President
   Honourable Minister of
   Science & Technology & Earth Sciences
   Anusandhan Bhawan,
   2, Rafi Ahmed Kidwai Marg,
   New Delhi – 110 001

2. **Prof. M. K. Bhan** — Member
   Secretary,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road, New Delhi 110 003

3. **Dr. Vishwa Mohan Katoch** — Member
   Secretary, Department of Health Research,
   & Director General,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911, New Delhi 110 029

4. **Dr. S. Ayyappan** — Member
   Director General,
   Indian Council of Agricultural Research
   And Secretary, Dept. of Agricultural Research
   & Education,
   Krishi Bhavan, New Delhi 110 114.

5. **Prof. W.N. Gade** — Member
   Vice Chancellor, University of Pune,
   Ganeshkhind, Pune 411 007.

6. **Prof. Umesh Varshney** — Member
   Dept. of Microbiology & Cell Biology,
   Indian Institute of Science, Bangalore 560 012

7. Ms. Anuradha Mitra — Member
Joint Secretary & Financial Adviser,
Department of Biotechnology,
Ministry of Science & Technology,
Block No.2, 7th floor, CGO Complex,
Lodi Road, New Delhi 110 003

8. Dr. Arvind Duggal — Member
Adviser,
Department of Biotechnology,
Ministry of Science & Technology,
Block No.2, 7th floor, CGO Complex,
Lodi Road, New Delhi 110 003

9. Prof. (Mrs.) S.S. Ghaskadbi — Member
Head
Department of Zoology,
University of Pune,
Ganeshkhind, Pune 411 007.

10. Dr. Kanuri Rao — Member
Sr. Scientist & Head,
Immunology Group,
International Centre for Genetic Engineering
and Biology,
Aruna Asaf Ali Marg, New Delhi 110 067

11. Dr. Padma Shastry — Member
Scientist 'G'
National Centre for Cell Science,
Ganeshkhind, Pune 411 007.

12. Dr. S. C. Mande — Member Secretary
Director
National Centre for Cell Science,
Ganeshkhind, Pune 411 007.

Governing Body Members

1. **Prof. M. K. Bhan** — Chairman
   Secretary
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor, CGO Complex,
   Lodi Road, New Delhi 110 003

2. **Dr. Vishwa Mohan Katoch** — Member
   Secretary, Department of Health Research,
   & Director General,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911, New Delhi 110 029

3. **Dr. S. Ayyappan** — Member
   Director General,
   Indian Council of Agricultural Research
   and Secretary, Dept. of Agricultural Research
   & Education,
   Krishi Bhavan, New Delhi 110 114.

4. **Prof. W. N. Gade** — Member
   Vice Chancellor, University of Pune,
   Ganeshkhind, Pune 411 007.

5. **Prof. Umesh Varshney** — Member
   Dept. of Microbiology & Cell Biology,
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6. **Ms. Anuradha Mitra** — Member
   Joint Secretary & Financial Adviser
   Department of Biotechnology,
   Ministry of Science & Technology,
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   Adviser
   Department of Biotechnology,
   Ministry of Science & Technology,
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8. **Dr. Kanury Rao** — Member
   Sr. Scientist & Head
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   and Biology,
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9. **Prof. (Mrs.) S.S. Ghaskadbi** — Member
   Head
   Department of Zoology,
   University of Pune,
   Ganeshkhind, Pune 411 007.

10. **Dr. Padma Shastry** — Member
    Scientist 'G'
    National Centre for Cell Science,
    Ganeshkhind, Pune 411 007.

11. **Dr. S. C. Mande** — Member
    Secretary
    Director
    National Centre for Cell Science,
    Ganeshkhind, Pune 411 007.

Finance Committee members

1. **Ms. Anuradha Mitra** — Chairperson
   Joint Secretary & Financial Adviser
   Department of Science & Technology,
   Technology Bhavan,
   New Mehraulli Road,
   New Delhi : 110 016

2. **Dr. Srikanth Tripathy** — Member
   Scientist 'F'
   Clinical Sciences Dept.,
   National Aids Research Institute,
   G-Block, P-73,
   Near Electronic Sadan,
   Bhosari, Pune 411 026

3. **Dr. Satish Kumar Gupta** — Member
   Chief – Reproductive Cell Biology Laboratory
   and Scientist 'H',
   National Institute of Immunology,
   Aruna Asaf Ali Road, New Delhi 110 067

4. **Prof (Mrs.) S.S. Ghaskadbi** — Member
   Head
   Department of Zoology,
   University of Pune,
   Ganeshkhind, Pune 411 007

5. **Dr. S. C. Mande** — Member Secretary
   Director
   National Centre for Cell Science,
   Ganeshkhind, Pune 411 007.

6. **Dr. Arvind Duggal** — Special Invitee
   Adviser
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodi Road,
   New Delhi - 110 003.

Building Committee members

1. **Dr. Dinakar Salunke** — Chairman
   Director,
   Regional Centre for Biotechnology,
   180, Phase – I, Udyog Vihar,
   Gurgaon – 122 016

2. **Dr. A. Duggal** — Member
   Nodal Officer & Adviser,
   Department of Biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi – 110 003

3. **Dr. J. Nagaraju** — Member
   Scientist,
   Centre for DNA Fingerprinting
   and Diagnostics,
   Bldg. 7, Gruhakalpa,
   5-4-399/B, Nampally,
   Hyderabad – 500 001

4. **Shri Sanjay Goel** — Member
   Director (Finance),
   Department of Biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi – 110 003

5. **Dr. V. H. Rao** — Member
   Consultant,
   National Institute of Animal Biotechnology,
   Visiting Scholars House,
   Lake View Guest House,
   University of Hyderabad Campus
   Prof. C.R. Rao Road, Gachibowli,
   Hyderabad – 46 (A.P.)

Scientific Advisory Committee Members

(Oct. 2011 – Sep. 2013)

1. **Dr. Kanury Rao** — Chairman
   Sr. Scientist & Head
   Immunology Group,
   International Centre for Genetic Engineering
   and Biology,
   Aruna Asaf Ali Marg, New Delhi 110 067

2. **Dr. R. A. Badve** — Member
   Director- Tata Memorial Hospital
   Dr. E Borges Road, Parel,
   Mumbai - 400 012 India

3. **Prof. Pinak Chakrabarti** — Member
   Department of Biophysics
   Bose Institute
   P1/12, CIT Scheme VIIM
   Kolkata - 700 054
   West Bengal, India

4. **Dr. Jyotsna Dhawan** — Member
   Dean, Institute of Stem Cell Biology
   & Regenerative Medicine,
   National Centre for Biological Sciences,
   Tata Institute of Fundamental Research, Bellary Road
   GKVK, Bengaluru,
   Karnataka 560065

5. **Dr. Arvind Duggal** — Member
   Adviser
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   7-8th floor, II Block
   NEW DELHI 110 003

6. **Prof. V. Nagaraja** — Member
   Professor,
   Microbiology & Cell Biology,
   Indian Institute of Science ,
   Bangalore 560012

6. **Dr. A. D. Sahastrabuddhe** — Member
   Director,
   College of Engineering,
   Shivaji Nagar,
   Pune – 411 005

7. **Chief Engineer** — Member
   Public Works Department, Pune Region,
   New Central Building,
   Pune – 411 001

8. **Dr. S. C. Mande** — Member
   Director,
   National Centre for Cell Science,
   Ganeshkhind,
   Pune

9. **Shri P. Y. Bhusnale** — Convener
   Tech. Officer 'C' (I&M)
   NCCS, Pune

10. **Shri B. G. Acharya** — Special Invitee
    Officer 'D' (Sr. Officer-Admin)
    NCCS, Pune

11. **Shri V. W. Shrirangbhattalwar** — Special Invitee
    Officer 'C' (Accts),
    NCCS, Pune

7. **Prof. Rajkumar T.** Member
Professor and Head,
Dept. of Molecular Oncology,
Cancer Institute (WIA), Adyar,
Chennai 600020

8. **Dr. Madan Rao** Member
Scientist,
Cellular Organisation and signaling,
National Centre For Biological Sciences (NCBS),
Tata Institute of Fundamental Research
GKVK, Bellary Road,
Bangalore 560065

9. **Dr. B. Ravindran** Member
Director,
Institute of Life Sciences,
Nalco Square, Chandrasekharpur
Bhubaneshwar751 023

10. **Prof. Ram Sasisekharan** Member
Professor of Health Sciences &
Technology and Bioengineering,
Department of Biological Engineering,
Massachusetts Institute of Technology (MIT),
Room 76-461, 600 Memorial Drive,
MIT, Cambridge , MA 02139-4307, USA

11. **Dr. Saumitra Das** Member
Associate Professor Department of
Microbiology and Cell Biology
Indian Institute of Science
Bangalore - 560 012, India

12. **Dr. S. D. Sharma** Member
Scientific Officer (E), RP&AD, BARC
CT&CRS Building, Anushaktinagar
Mumbai - 400 094

13. **Prof. L.S. Shashidhara** Member
Professor & Coordinator, Biology,
Indian Institute of Science Education &
Research (IISER),
Sai Trinity Building,
3rd Floor, Room no: 305
Sutarwadi, Pashan,
Pune - 411021

14. **Prof. Subrata Sinha** Member
Director,
National Brain Research Centre (NBRC),
NH-8, Manesar, Gurgaon,
Haryana - 122 050, India

15. **Dr. Kumarvel Somasundaram** Member
Associate Professor
Microbiology & Cell Biology,
Indian Institute of Science ,
Bangalore – 560 012

16. **Prof. Alok Srivastava** (MD, FRACP, FRCPA, FRCP) Member
Professor of Medicine
Head, Department of Haematology &
Centre for Stem Cell Research
Christian Medical College
Vellore - 632004

17. **Prof. Umesh Varshney** Member
Professor
Department of Microbiology and Cell Biology
Indian Institute of Science,
Bangalore 560 012

18. **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, and Stores & Purchase. The centre also has an Instrumentation & Maintenance unit. All these sections provide support 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.

IMPLEMENTATION OF THE 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 from the Administration Department have undergone training for Hindi typing conducted by the Hindi Teaching Scheme, while the others are under training. All the stenographers were given training in Hindi stenography and Hindi typing conducted by the Hindi Teaching Scheme. The Unicode Encoding System has been enabled on many of the computers so that work in Hindi can be carried out anywhere in the Institute.

The Centre also observes Hindi Week (Hindi Saptah) every year. In the year 2011-12, Hindi essay and letter writing competitions were held during the Hindi Week celebrations and the winners were awarded cash prizes and certificates. Shri. K. K. Gupta, Hindi Officer,

CWPRS, Pune was invited as the Chief Guest for the Hindi Day Function held on the 21st of September 2011, while Dr. G. C. Mishra, Eminent Scientist, NCCS was a special invitee.

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

VIGILANCE MATTERS

Dr. Bhaskar Saha, Scientist 'F', is the nominated Chief Vigilance Officer 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.

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

National Centre for Cell Science
An autonomous institution of the Department of Biotechnology, Govt. of India

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