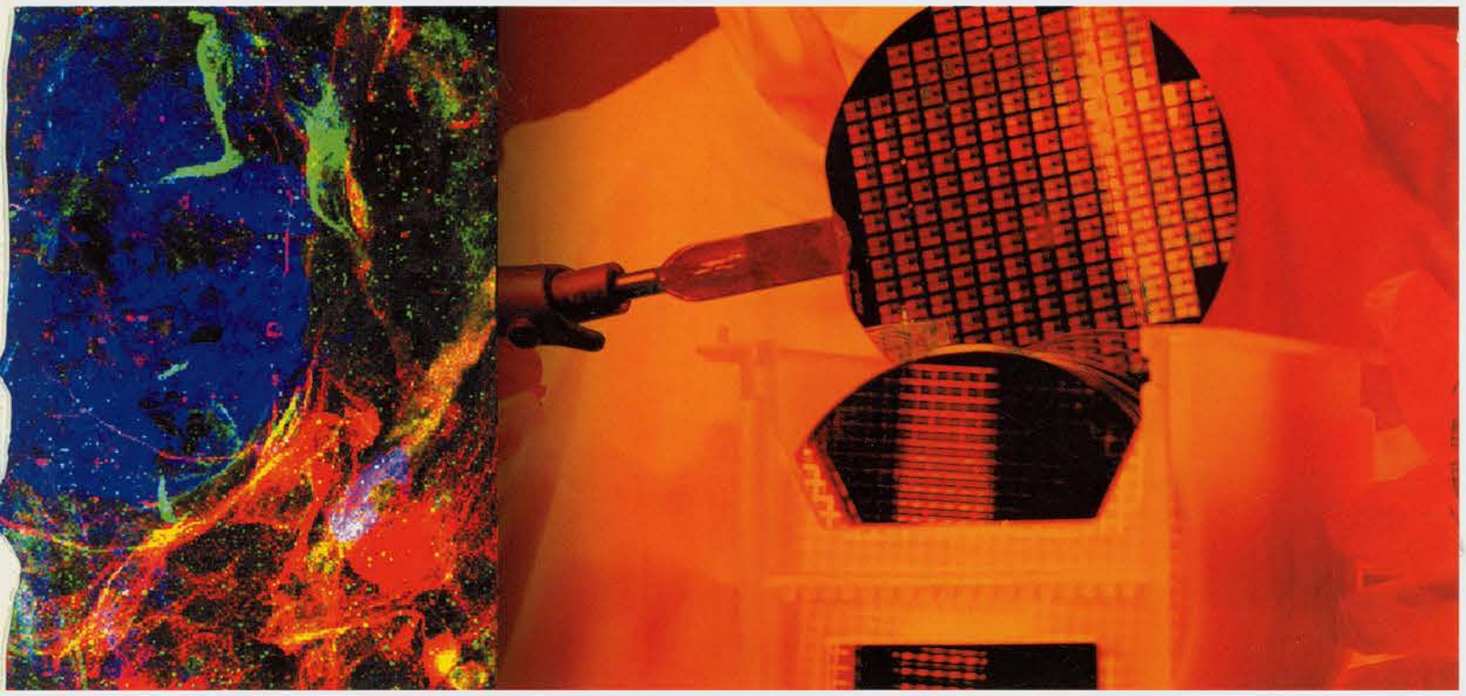
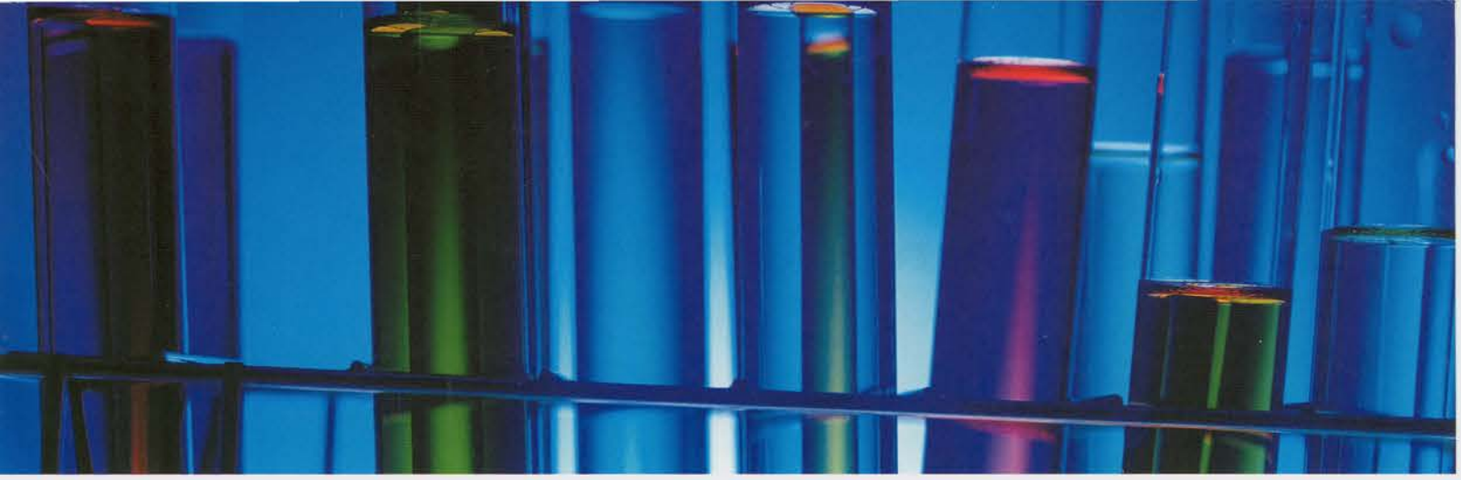
National Centre for Cell Science



Annual Report - 2007-2008



Contents

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The Mandate of NCCS

- To receive, identify, maintain, store, grow and supply:- Animal and human cell/cell cultures, cell lines of both existing (typed) and newly developed, hybrid cells including hybridomas; Tissues, organs, eggs (including fertilized) 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 National Reference Centre for tissue culture, tissue banking and cell products and 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 continious 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 Director's Desk

NCCS functions as a centre for biological research and human resource development, and is also the National Repository for cells. In 2007-2008, we have provided more than 1800 cell lines of different cell types to over 100 scientific institutions in the country. Our centre conducts cutting edge research activities in areas of stem cell biology, cancer biology, signal transduction, diabetes, insect molecular biology, infection and immunity, chromatin architecture and gene regulation. NCCS is committed to its contribution to technical manpower development, by way of reaching out to individuals at all levels including students, teachers and researchers in India. Basic training as well as custom-made programmes depending on specific requirements of smaller groups of researchers were, and will continue to be, conducted at NCCS and at the user's end. During the reporting year, we have conducted workshops and training programmes on basic techniques in tissue culture for 29 researchers from various institutes.

NCCS is a premiere research institute wherein different aspects of eukaryotic cell biology are studied at fundamental levels in order to generate scientific knowledge that have potential application in the bio-medical and industrial fields. This has been possible through the involvement of scientists from various fields, motivated research students, extensive infrastructural facilities, and trained technical and administrative staff. The institute's mission will continue to focus on important questions relevant to human health, specifically in the area of regenerative medicine, infectious diseases and lifestyle induced health adversities.

In the field of stem cell biology, NCCS primarily focuses on applications of stem cells in regenerative medicine through development of major

therapeutic strategies for diseases in which there is damage or loss of particular types of cells. The research area involves isolation, crypopreservation and expansion of stem cells, and optimizing the conditions for differentiation into specific cell types. Efforts are also in progress to understand the cellular and molecular mechanisms defining stem cell differentiation and maintenance of the stem cell niche in normal organs.

Cancer is a complex disease caused by misregulation of several signaling networks. In order to understand the biology of tumourigenesis and metastasis, NCCS is exploring pathways involving critical players such as Osteopontin, endothelial eNOS, p53, Cyclin D1, transcription factors such as Snail and Slug, and non-coding RNAs. Results from these studies are expected to provide tools for better therapeutic interventions for cancer treatment.

Recent increase in the incidence of diabetes amounting to 20 per cent of Indian population has prompted further interest in this area. Diabetes is a multifactorial metabolic disorder requiring divergent approaches for better management. NCCS focuses on the mechanisms involved in endocrine pancreas development and regulation of insulin biosynthesis. We have generated insulin producing islet-like clusters from pancreatic and non-pancreatic cells, with a potential to be used in cell replacement therapy. Role of oxidative stress on diabetes-induced cadiomyopathy is also being investigated.

The human body is continuously exposed to multitude of pathogens and the immune system functions to counteract them. The pathogens constantly evolve mechanisms to evade the immune system, leading to infections. Understanding the molecular mechanisms underlying the host parasite interactions is of paramount importance in developing strategies to combat infections. Scientists at NCCS study the viral evasion of human complement system, regulation of CD40 signaling in host cells by Leishmania, protein trafficking in Leishmania, Plasmodium replication in red blood cells, HIV biology and Host-pathogen interactions during Candida infections.

Epigenetic regulation of gene expression plays an important role in various cellular processes. We have shown that nuclear matrix associated proteins like SATB1 and SMAR1 regulate global gene expression by chromatin remodeling. Understanding the molecular mechanisms governing their functions in relation to development and tumourigenesis are being explored further.

Recent reports show that the microbial flora within an organism influences the metabolic processes of the host. Using modern methods such as whole genome sequencing and metagenomic analysis we are trying to unravel the complex microbial ecosystem in the midguts of humans and insects of clinical importance.

Novel biomolecule mining from hitherto untapped sources such as marine organisms and plants have been performed that would aid in treatments of AIDS, diabetes, malaria and osteroporosis. We are happy to report that a technology developed by NCCS for preparation of extracts from Fenugreek (Methi) seeds having anti-diabetic properties has already been transferred to a company.

Our institute has completed 20 years. In the next phase of growth we would like to broaden our research areas to include regenerative, neuro- and developmental biology. We are in the process of recruiting additional scientific personnel in these areas. To facilitate this expansion, we have added a new building, which will have a modern auditorium, a new library and additional research laboratories. NCCS provides state of the art infrastructure facilities to its scientists. As a part of this process, in the last year we have procured LC-MS and MALDI-TOF.

We have excelled in pursuing basic research in different aspects of biology, which is reflected in our publication over last several years. In the reporting year, we have published over 50 scientific papers in peer reviewed journals. Our scientific activity is augmented by funding from various national and international agencies.

In a nutshell, NCCS has been focusing on understanding the molecular details of biological processes that are critical to human growth, development and homeaostasis. This approach has helped us not only to understand the fundamental mechanisms of important cellular processes but also to develop startegies for better management of diseases caused due to the impairment of these functions. We hope to contribute much more significantly in this direction in the future by coordinating our efforts using diverse research approaches.

G. C. Mishra
Director

Human Resource Development

During 2007-2008, 18 students joined for pursuing Ph.D under various Scientists. All student presentations were completed & their admission is confirmed by University. The total number of Ph. D students as on 31.08.08 was 139.

The Project Training programme is conducted twice in a year i.e. during January-June and July-December, while summer training programme is conducted during the month of May every year. The number of students attended these courses in the last year are:

Project Training - 29

Summer Training - 11

During this year 39 Research Fellows attended seminars/ conferences / symposium conducted by various reputed organizations.

Workshops on Animal Tissue Culture

One of the main objectives of the center is to enhance human resources by way of conducting symposia, workshops and tailor-made programmes for individuals.

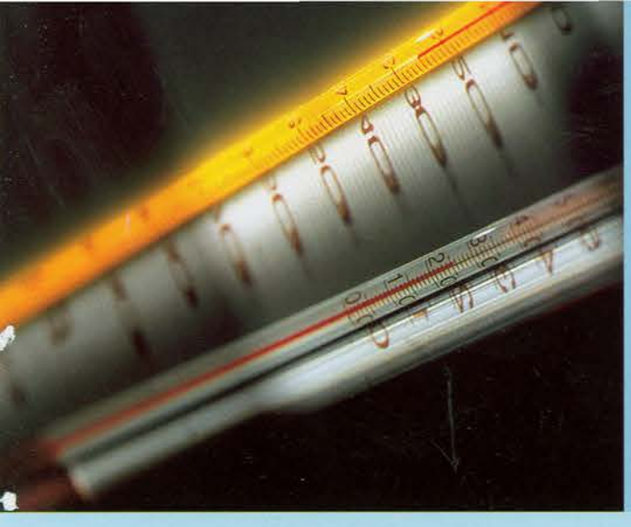
This year, fourteen people from different institutes such as M.S.University of Baroda, KEM Hospital, J.J. Hospital, Mumbai and IBB, Pune University Campus were trained in animal tissue culture techniques.

Moving acadamy of Medicine and Biomedicine and NCCS Jointly conducted Basic Tissue Culture Training Programme for Post graduate college teachers, students (number of participants -15) held during June 15 30, 2008.

Training for two members of Indu research foundation, Gujrat, was conducted at NCCS for five days in Oct. 2007, on cryopreservation of cord blood cells.

Repository

National Centre for Cell Science serves as a National Cell Bank for animal cell lines. The repository manages cell line procurement, expansion, cryopreservation and distribution. In this year, we have procured different cell types from different repositories. The list of cell lines, with details such as media requirement, growth conditions and its use, is available now on demand. In 2007-2008, we have supplied 1800 cell lines to 138 research institutions in the country.



Research Reports

Cell Biology

Studies on nuclear localization of eNOS in mammalian cells

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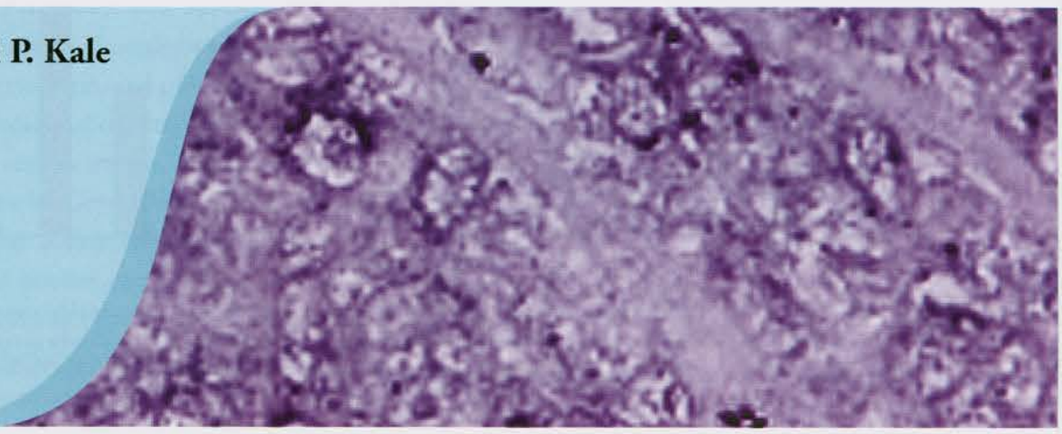

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Background

Nitric oxide (NO) is a multi-functional gaseous signaling molecule that has been shown to play important regulatory roles in physiological processes like regulation of blood pressure, vascular smooth muscle tone, platelet aggregation, cell proliferation, immune function and inflammation. It is generated from a five-electron and a two-step oxidation process of the amino acid, L-arginine, catalyzed by all members of nitric oxide synthase (NOS) family. There are at least three distinct isoforms of this family known to date; termed as neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and endothelial NOS (eNOS/NOS3). The sub-cellular localization of eNOS significantly influences the biological role and the chemical fate of the NO produced by the enzyme. In the endothelial cells and also in the cardiac myocytes, the eNOS is targeted to specialized plasmalemmal signal-transducing regions, termed as "caveolae", which are flask -shaped plasma membrane invaginations of 50-100 nm in diameter that are present in a majority of cell types, including endothelial cells, adipocytes, pneumocytes etc. Acyl modifications of the enzyme by the fatty acid, myristic acid, at Glycine-2 is critical for its membrane localization, and reversible palmitoylations at cysteine residues of position 15 and 26 contribute to the efficient targeting of this protein to the membranes of both Golgi complex and caveolar space. eNOS may be quantitatively associated with caveolin-1 in endothelial cells and with caveolin-3 in ventricular myocytes. The caveolar interaction with eNOS is promoted by the 20-amino acid-long scaffolding domain of caveolin-1 (residues 82-101), through direct protein-protein interactions. An inhibitory effect on eNOS enzymatic activity results from the interaction of caveolin-1 with eNOS that can almost be completely reversed by

Studies on expansion, cryopreservation and differentiation of hematopoietic stem cells

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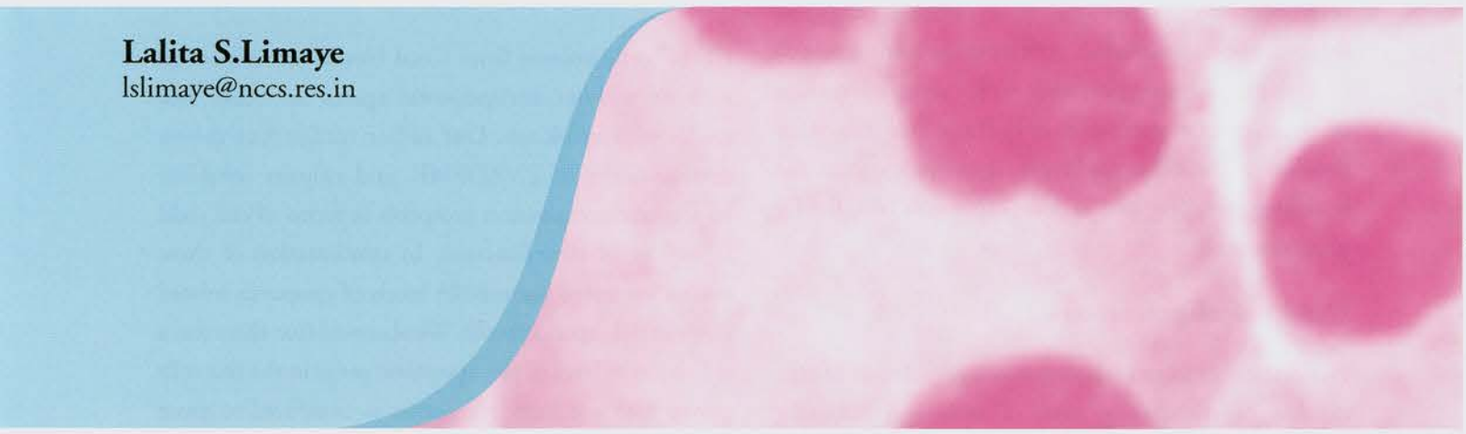

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Background

In vitro expansion and cryopreservation of hematopoietic stem cells:

In vitro expansion of $CD34^+$ cells is being extensively attempted to overcome the limitations of the small sample size, especially of cord blood and pediatric marrow harvests. Optimal storage of these expanded cells is also crucial. There is a possibility that during such in vitro manipulations the cells undergo apoptosis. There are reports that functional expression of Fas-Ag is induced on $CD34^+$ cells expanded *in vitro* in presence of haematopoietic growth factors. We used antiapoptotic agents like ZVAD FMK and calpain inhibitors as additives in expansion and freezing media and found that they indeed were beneficial. The engraftment ability of expanded cells was tested in NODSCID mice. Thus, detection of the level of apoptosis during preservation and expansion, and development of ways and means to prevent it are important aspects of haematopoietic stem cell research.

Megakaryocyte generation:

Umbilical cord blood (UCB) provides an alternative source of hematopoietic progenitor cells for transplantation. However prolonged thrombocytopenia remains a major obstacle due to the lower number of megakaryocyte (MK) progenitor cells and their subsequent delayed engraftment. Efforts are currently underway to expand the number of cord blood (CB) stem cells and MK progenitor cells *ex vivo,* which may facilitate platelet production during post transplantation, and decrease the time of thrombocytopenia. The

optimal conditions for *ex vivo* expansion and cryopreservation of MK progenitor cells have not been established. We used two nutraceuticals, omega 6 and omega 3, as supplements in serum free media for generating megakaryocytes from cord blood $CD34^+$ cells and found that the two additives favoured megakaryocyte generation. Attempts are being made to optimize the freezing protocols for the cryopreservation of these *ex vivo* generated cells. Thus our aim is to develop a clinically applicable improved system for enriching, freezing and *ex vivo* expansion of CB-MK progenitor cells.

Dendritic cell generation

Dendritic cells form a heterogeneous population of cells capable of stimulating naïve T cells and initiating primary immune response. This well known function of DCs has offered the possibility of developing clinical protocol for their use in immunotherapy to tumors. DCs may also play a critical role in the induction of peripheral immunological tolerance, which could have important implications in the treatment of autoimmunity or in the outcome of clinical transplantation. In recent clinical studies, DCs were shown to have anti-tumour effect and were used as vaccine for cancer patients. The total number of DCs available for immunotherapy remains limited. DCs have been shown to be generated from $CD34^+$ cells from bone marrow (BM), UCB and GCSF mobilized PBSC. Cryopreservation of $CD34^+$ cells is important to extend the availability of cellular therapy with DC. However, little is known about the effect of cryopreservation on the functional maturation of DCs. Ex vivo generation of this cell type and its efficient cryopreservation will have direct application in the clinics. In this project our objective will be to explore a culture method to generate a large number of functional and mature DCs from human $CD34^+$ hematopoietic progenitor cells and standardize methods for their efficient freezing.

Aims and Objectives

1. *In vitro* expansion and cryopreservation of $Cd34^+$ haematopoietic stem cells
2. *In vitro* generation of megakaryocytes and dendritic cells

Work Achieved

Expansion of haematopoietic stem cells:-

$CD34^+$ cells isolated from Cord blood were expanded with or without antiapoptotic agents in serum free media with cytokines. Our earlier studies had shown that addition of ZVADFMK and calpain inhibitor improved the expansion protocols in terms of cell yield as well as *in vitro* function. In continuation of these studies we tested the mRNA levels of apoptosis related genes in the expanded cells. We observed that there was a reduction in level of pro-apoptotic genes in the test cells grown with antiapoptotic agents as compared to those grown without them. On the other hand Notch ligand Jagged 1 which is associated with expansion showed improved expression in test cells. (Fig.1 a, b).The engraftment ability was tested by infusing the cells in NODSCID mice and detecting levels of human CD45. We found that this was improved by additives (Fig.1c). We cryopreserved the expanded cells in conventional freezing medium and found that the functionality of expanded cells as analysed by CFU assay was better with additives (Fig. 1d). Thus we see that antiapoptotic agents improve expansion and freezing protocols.

Megakaryocyte generation:

Our previous studies had shown that the nutraceutical Omega 6 favours generation of megakaryocytes. On similar lines we tested the effect of yet another nutraceutical omega 3 and found that it was also indeed beneficial for megakaryocyte generation from cord blood $CD34^+$ cells.

The megakaryocytes were identified by morphology (Fig. 2a) and phenotypic analysis (Fig. 2b). Their functionality was tested by CFUMeg assay (Fig. 2c) and platelet activation (Fig. 2d). Thus our results strongly indicate that the nutraceuticals such as arachidonic acid and docosahexanoic acid are useful additives for megakaryocyte generation.

Dendritic cell generation

In continuation of our efforts to design a simple method for generation of dendritic cells, we first expanded the $CD34^+$ cells isolated from cord blood and then separated $CD14^+$ cells either by plastic adherence or by FACS sorting. The $CD14^+$ cells so obtained were then used to produce dendritic cells and the two methods were compared. It was found that the dendritic cells so generated showed typical morphology (Fig. 3a) and phenotype (Fig. 3b). The functional characteristics like chemotaxis (Fig. 3c), MLR (Fig. 3d) and IL12 secretion were also compared (Fig. 3e). Our results suggest that it is possible to generate large quantities of dendritic cells by simple plastic adherence method. These results may have a significant implication in clinical settings.

Future Work

As cryopreservation is an important aspect in banking of cells, we will optimize protocols for freezing of expanded cells, dendritic and megakaryocytic cells. We will also standardize CTL assays using the in vitro generated dendritic cells.

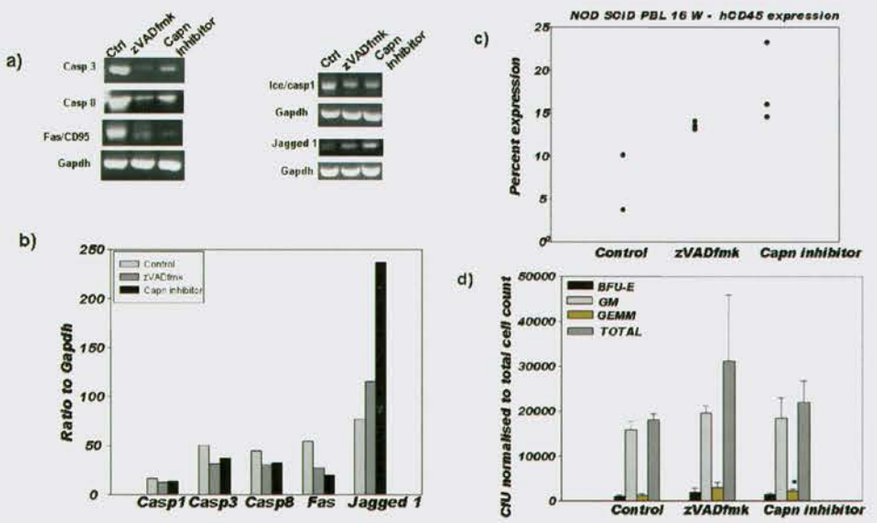

Fig 1. *Ex vivo* expansion of HSPC's using anti apoptotic agents: a) The cells expanded with the two protease inhibitors showed increased survival as indicated by the low mRNA level of Casp1, Casp3, Casp8 and Fas antigen. Notch ligand jagged 1 was analysed to indicate the expansion of stem cell compartment which is found to be higher in the test cells. b) Densitometric values normalized to Gapdh plotted. c) The engraftment of expanded human cells after 16th week post transplant in PBL of NOD/SCID showed a marked increase in the expression of human CD45 antigen in the sets infused with the test cells indicating improved engraftment. d) The expanded cells were efficiently cryopreserved in conventional freezing medium (10%D), the test cells showed improved functionality after revival, which optimizes the protocol for freezing as well.

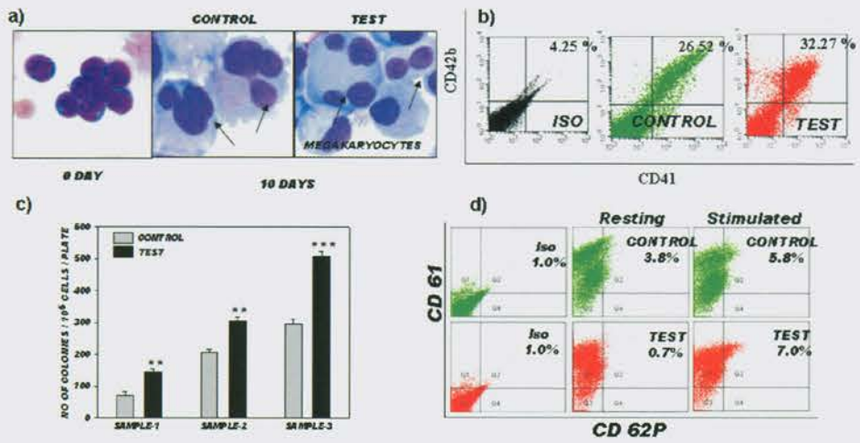

Fig 2. *In-vitro* generation of $CD34^{+}$ cells towards Megakaryocyte lineage using Omega 3 as a nutraceutical. a) Morphological analysis of megakaryocytes by W&G staining, bright field microscopic image of cytospin smears of cultured cells. Megakaryocytes are indicated by arrows (600X magnification). b) Quantitation of late MK progenitors: Marked increase in the expression of CD41 and CD42b dual positive cells are seen in test cells as compared to control. c) Cells cultured with Omega 3 showed a significantly higher MK colony forming ability when compared with control, p = 0.001***. d) Dual colour analysis of platelet activation: Expression of activated platelets was more in Test set.

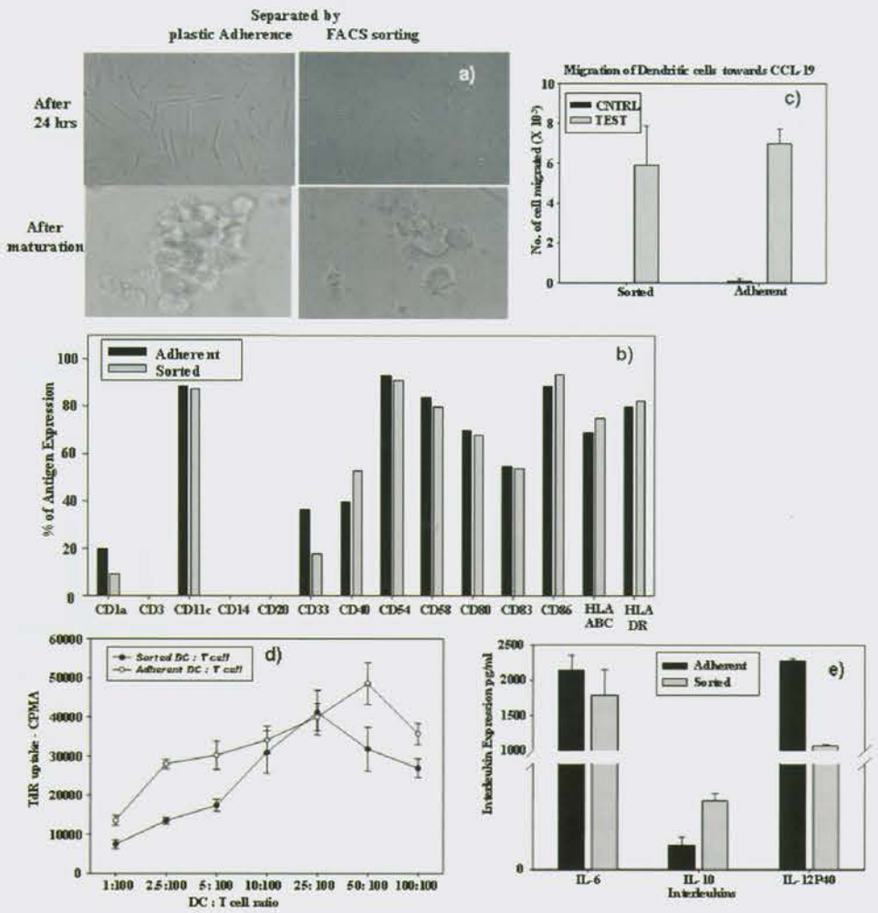

Fig 3. Comparison of dendritic cells generated by plastic adherence vs FACS sorted CD14 cells: a) Phase contrast images of dendritic cells. b) their Phenotypic characterisation. c) Chemotactic migration towards CCL19. d) Allogenic T cell stimulation. e). Secretion of different interleukins by the *in vitro* generated dendritic cells.

Maintenance of self-renewal and pluripotency in ES cells in response to Wnt signaling.

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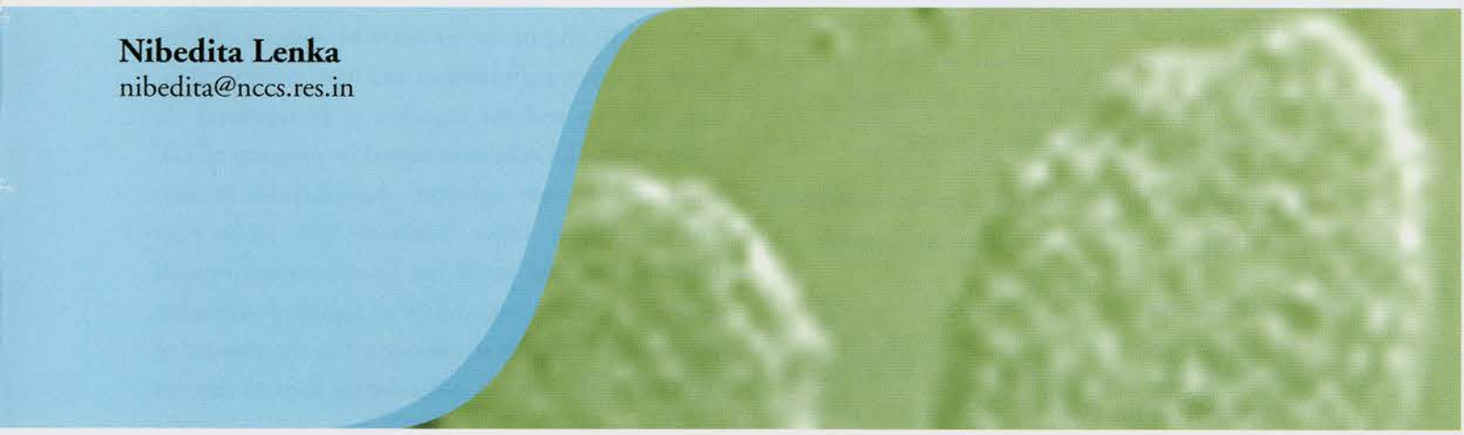

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Background

The embryonic stem (ES) cells are pluripotent cells possessing the characteristics of self renewal and differentiation into derivatives of all three germ layers. In fact, the maintenance of undifferentiated state in culture during propagation is crucial for indefinite self-renewal as well as for the retention of pluripotency, and undoubtedly this is tightly regulated. As a routine practice, the cells are maintained either by culturing on mitotically inactive fibroblast feeders or by supplementing the culture medium with cytokine LIF. However, LIF does not seem to be required for human ES cells maintenance unlike that of murine ones. In fact, nanog has been identified to support ES cell maintenance in a LIF independent fashion. Moreover, in addition to LIF-STAT3 pathway, several other pathways such as BMP, MAPK, Wnt etc, have been reported to be involved during this process. Our earlier findings showed that Wnt influences cardiomyogenesis during differentiation of ES cells. We extended our investigation to decipher whether Wnt signaling would help maintaining the unlimited self-renewal and pluripotency in ES cells and if so, to understand the mechanistic basis, particularly by determining whether it would work independent of or in concert with LIF-STAT3 pathway.

Aims and Objectives

The major focus of our group has been,

1. The maintenance of ES cells in undifferentiated state.
2. The differentiation of ES cells into various lineages such as neural, cardiac etc. and understanding the underlying molecular basis of lineage commitment and specification.

3. Establishment of stable transgenic ES cell clones and promoter/enhancer mediated cell trapping to demarcate the cells of interest during differentiation of ES cells and their subsequent characterizations.

4. Manipulation of extrinsic factors for the efficient generation of functional cardiomyocytes, proliferative neural progenitors and differentiated neurons with special reference to the dopaminergic neuronal subtypes from ES cells *in vitro.*

5. Exploration of the efficacy of *in vitro* generated cells in cell replacement therapy using animal models.

Work Achieved

While studying the influence of Wnt during cardiomyogenic differentiation of ES cells *in vitro*, we observed the attenuation in cardiomyogenesis in response to Wnt activation. However, it retained/induced the expression of undifferentiated ES cell markers such as OCT4 and nanog in the differentiating embryoid bodies (EB). This led us to presume that Wnt probably would help in the maintenance of ES cells in undifferentiated state. Accordingly, the ES cells were maintained with or without LIF and in the presence or absence of Wnt signaling activators/inhibitors and were monitored for their stemness and the retention of pluripotency. As expected, the ES cells maintained in presence of LIF could exhibit their inherent characteristics of self-renewal, while those without LIF underwent differentiation and could not be maintained beyond passage 3-4. However, the Wnt signaling activation facilitated ES cell maintenance even in the absence of LIF (Fig. 1A). The ES cell colonies showed distinct spherical and compact morphology indicating the presence of undifferentiated cells, which was further authenticated by OCT4 (Fig. 1B, C) and nanog

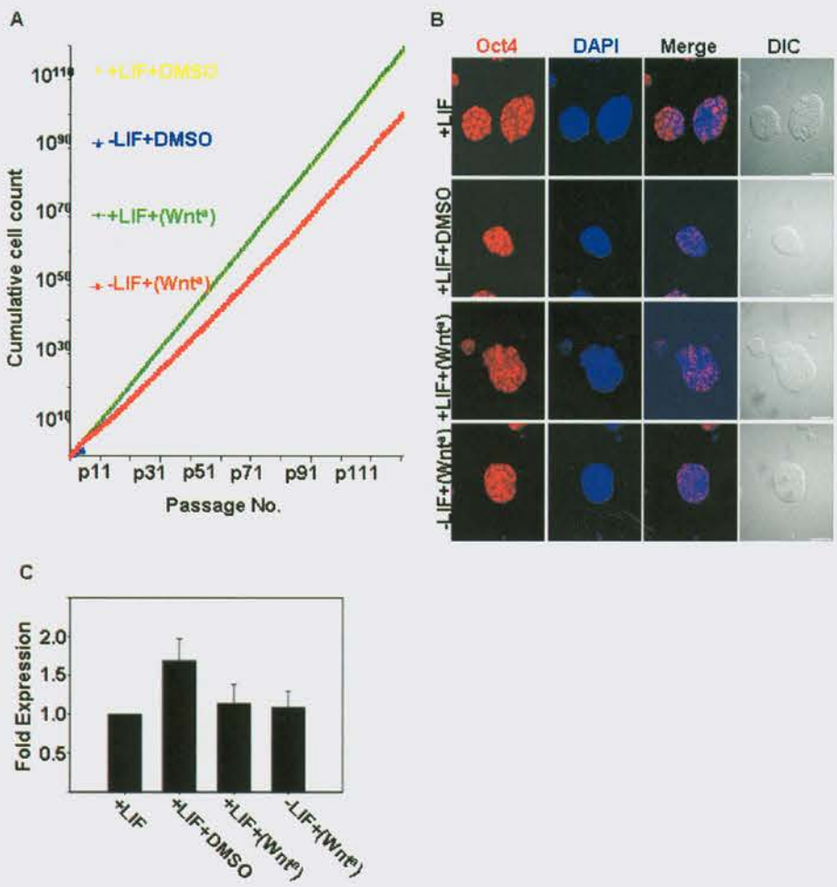

Fig. 1 : The ES cells growth curve during propogation with or without Wnt activation (A). The OCT4 expression implying the undifferentiated state of ES cells that were cultured with or without Wnt activators in the medium as ascertained by immunocytochemistry (B) and quantitative RT-PCR (C).

staining, the ES cell markers. Although the cell growth during passaging with Wnt activation was exponential, it remained lower compared to the ones grown in presence of LIF. The cumulative cell count was determined to be 10^{100} times that of the initial cell density plated when grown for more than 100 passages under the stated conditions (Fig. 1A). Conversely, when Wnt inhibitors were used in the medium along with LIF, the cells retained the expression of OCT4 and nanog similar to the control as determined by immunocytochemistry and qPCR without any apparent differentiation. Hence, Wnt though promoted ES cells maintenance in the absence of LIF, it might not be considered indispensable for the same and LIF signaling might be overriding the effect of Wnt inhibition.

Next we sought to verify whether the ES cells maintained in long term culture with Wnt activators and without LIF retained pluripotency both *in vitro* and *in vivo*. Accordingly, the ES cells were subjected to differentiation *in vitro* into cardiac and neural lineages. While, the cardiac differentiation was ascertained by monitoring spontaneous pulsating activity and the EYFP expression in the βMHC transgenic ES cell clones cultured under the stated conditions, the neural differentiation was determined by immunostaining using neural cell specific antibodies. Interestingly, the ES cells could differentiate into both cardiomyocytes and neurons with equal efficiency when compared with control (Fig. 2A, B). Similarly, upon injection of the respective ES cells to SCID mice for demonstrating their *in vivo* pluripotency, teratoma development was

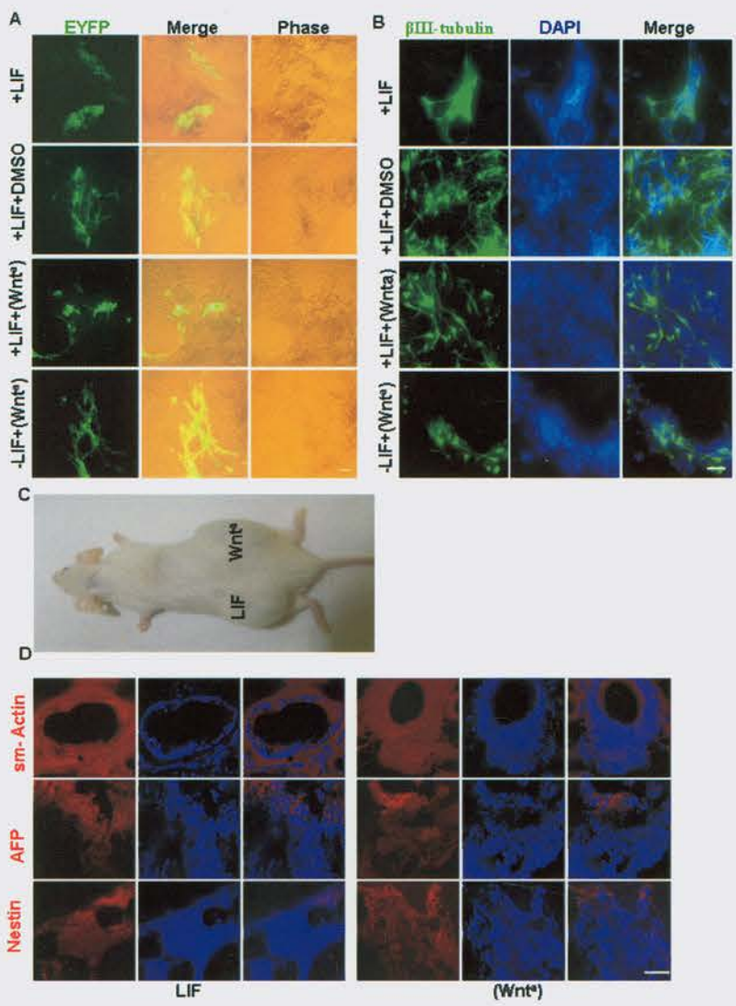

Fig. 2: The *in vitro* differentiation of ES cells maintained with or without Wnt activators into cardiomyocytes (A) and neurons (B). The teratomas developed in SCID mice following injection of ES cells from both LIF and Wnt groups (C) showed differentiation into all the three germ layers (D).

observed in both control (LIF) and test (Wnt) groups (Fig. 2C). The teratomas indeed contained cells of all the three germ layers as ascertained by immuno-histochemistry (Fig. 2D) suggesting the efficacy of Wnt in maintaining the undifferentiated state in ES cells even when cultured in the absence of LIF and retaining their pluripotency both *in vitro* and *in vivo*. Together our investigation delineated an interesting paradigm of multifaceted Wnt influence during ES cells maintenance and differentiation.

Future Work

Preliminary evidences indicated the mechanistic basis of Wnt mediated ES cells maintenance to be different from that of LIF-STAT3. We would like to pursue the same in detail.

Translational Regulation of Insulin mRNA

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Background

Pancreatic β cells regulate insulin production to control blood glucose levels. These cells contain a large pool of cytoplasmic insulin mRNA (10-15% total mRNA), which is translationally quiescent at hypoglycemic (<3 mM glucose) concentrations. Recruitment to polysomes and activated translation of this mRNA occur in response to higher glucose levels, leading to about 50-fold increase in insulin biosynthesis within an hour. The level of insulin mRNA does not alter significantly during this period of glucose stimulation and transcription inhibitors do not affect this early increase in insulin biosynthesis, indicating the predominance of the posttranscriptional events in enhancing insulin biosynthesis.

Glucose induced translation of insulin in pancreatic beta cells is mediated by the 5'UTR of insulin mRNA. We have previously reported the minimal sequence/structure in the 5'UTR of rat insulin gene1 required for this regulation. We had shown that specific factors in the pancreatic islets bind to the 5'UTR of the insulin mRNA upon glucose stimulation. A minimal 29-nucleotide element in the 5'UTR was shown to be sufficient for the glucose mediated translation activation of insulin mRNA. Conserved residues in the predicted stem loop region of the UTR seem to be important for the complex formation and translational regulation. Using RNA affinity pull down method, we isolated and identified the 5'UTR binding protein, which is referred to as ITR (for, Insulin translation regulator). We verified the presence of this protein in the activation complex through super shift RNA EMSAs (electrophoretic mobility shift assays) before and after immunodepletion of ITR from the reticulocyte lysate, using specific antibodies. The Ins-5' UTR binding activity of ITR was confirmed

further using a yeast three hybrid assay. Co-immunoprecipitation of insulin mRNA with ITR from βTC-6 cell extract suggested that ITR binds to insulin mRNA *in vivo*. Using immunoprecipitation assays, we showed that glucose stimulation increased association of ITR with the Ins-5' UTR in islet cells. We also demonstrated that ITR activated insulin mRNA translation using an in vitro translation assay, in which the functional ITR was blocked by the addition of neutralizing antibodies. Further characterization of the ITR and its role in translation regulation are in progress.

Aims and Objectives

1. Isolation and characterization of the insulin mRNA UTR binding protein or protein complex by RNA affinity chromatography.
2. To understand the basic mechanism of translational regulation of insulin mRNA and the role of RNA binding proteins in this regulation.

Work Achieved

Major regulation of insulin biosynthesis occurs at the secretion and the translational level in β-islet cells. Glucose stimulates the beta cells to increase the translation of insulin mRNA but the mechanism is not completely understood. The un-translated regions (UTR) of the insulin mRNA is thought to be essential for this regulation. Rat insulin mRNA has 57 base 5'-UTR. We synthesized radio-labeled wild type rat insulin 5' UTR and performed the RNA gel shift assay using RIN cell extracts to identify specific RNA-protein complexes. A similar specific complex was also formed with extracts from rat pancreatic islets. The complex formation was induced by high glucose treatment of the islets. We identified a minimal 29 nucleotide element that is necessary and sufficient for the complex formation. Our analysis also revealed that the predicted stem loop structure is important for the complex formation. We synthesized biotinylated insulin 5'UTR and purified the binding factor(s) associated with the RNA.

Identification of insulin 5'UTR binding Protein

The insulin 5'UTR mRNP was isolated by RNA-affinity chromatography using biotinylated 5'UTR element as ligand. We took advantage of a mutant 5'UTR (Δ14-16; 41-43) that possessed lower capacity to assemble the mRNP. Cytosol from glucose stimulated cells was pre-cleared with 5'-biotinylated mutant and scrambled oligonucleotides, and then applied to affinity columns containing either biotinylated wild-type or mutant 5'UTR as ligand. The eluates were resolved by SDS-PAGE and stained. Protein band preferentially eluted from the wild-type column was subjected to tryptic digestion and sequencing by liquid chromatography-tandem mass spectrometry. Protein Disulfide Isomerase (PDIA1) was identified as one of the proteins and was confirmed by Western analysis of the eluates.

PDI specifically binds to the insulin 5'UTR

We performed RNA-EMSA in the presence of specific PDI antibodies to show that the complex formed is specific for PDI. Anti-PDI antibody completely abolished the formation of the RNA-protein complex with the 5'UTR RNA and we could also detect a specific shifted band in the presence of antibody in case of the Con1 probe (29 nt minimal sequence capable of binding to the protein) (Fig 1A). We tested the RNA binding activity of PDIA1 in yeast three hybrid assay and showed that PDIA1 specifically binds to the insulin 5'UTR (Fig 1B)as assessed by the ability to grow in media without histidine (his-) as well as by beta-galactosidase activity.

We also showed that recombinant PDIA1 was able to neutralize the anti-PDI antibody indicating the specificity of the antibody for PDIA1. However recombinant bacterially expressed PDIA1 was unable to bind to insulin 5'UTR and cause translation regulation suggesting a role for post translational modification. Immunodepletion of cytosol with PDI specific antibodies almost completely removed the RNA-protein complex forming activity; control antibodies were ineffective (Fig. 2A). We then tested the *in vivo* binding of insulin RNA to PDI by RNA-Immunoprecipitation (RIP). The anti-PDI antibody specifically pulled down insulin mRNA while the control IgG fails to do so, suggesting a complex formation between PDI1A and the insulin mRNA *in vivo*. This association of PDI with insulin mRNA was induced by glucose (Fig. 2B).

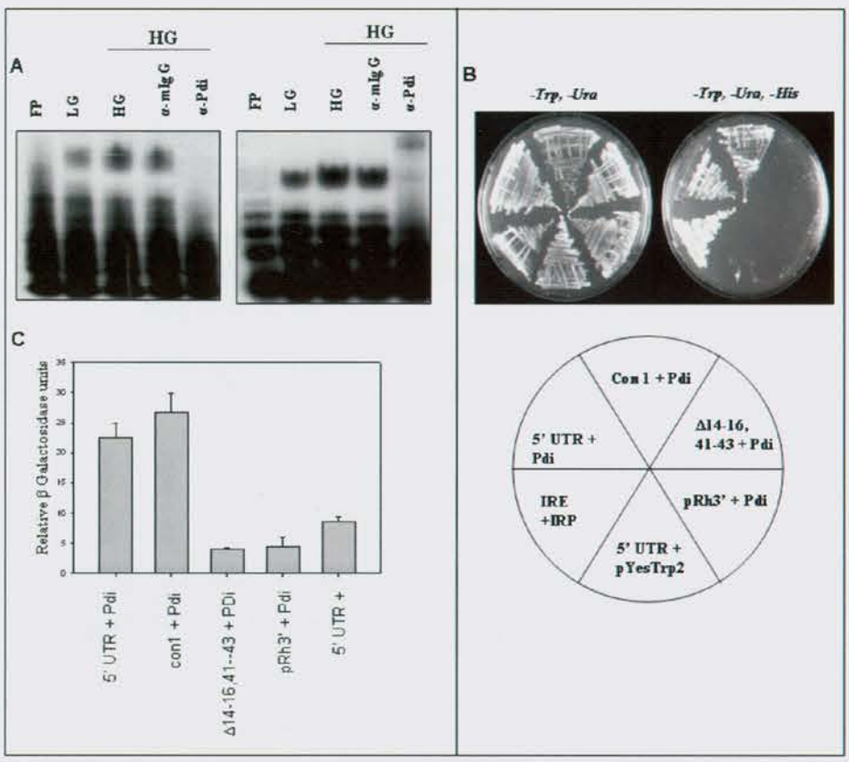

Fig. 1: PDI binds to insulin mRNA 5'UTR. RNA-EMSA was performed with the beta islet extracts in the presence of either PDI specific antibody or mouse IgG. LG and HG indicate the low and high glucose treated extracts. EMSA with the full rat insulin 5'UTR (Left) or the 29 nucleotide minimal sequence (Right panel) A. insulin 5'UTR, 14-16 deletion, and the minimal sequence were cloned into the yeast vector to produce an MS2 fusion RNA. PDI was cloned as a fusion with B42 activation domain. The yeast three hybrid assay system was used for confirming the RNA binding activity of PDI. The interaction between the insulin RNA and the PDI protein will result in activation of his3 gene and beta galactosidase which is assessed by growth of yeast in media lacking histidine (B). or by measuring the beta galactosidase activity using ONPG as substate and measuring the change in OD (C).

PDI is necessary for the translation activation by glucose

We tested the translation responsiveness of the glucose treated extracts in the presence or absence of the PDI. We immunodepleted the beta cell lysates with anti-PDI antibody and the PDI depleted lysates failed to activate translation of a heterologous reporter transcript in an *in vitro* translation assay (Fig. 3). These results show that PDIA1 is a functional translation activator of insulin mRNA.

Future Work

The mechanisms that underlie nutrient-induced translational regulation of insulin biosynthesis are likely to be crucial for understanding wider aspects of β-cell physiology and metabolic homeostasis, because this is the major control of insulin production in mammals under normal physiological conditions. Indeed, there is dysregulation of insulin biosynthesis in an animal model of type II diabetes, which contributes to cell dysfunction and decreased availability of insulin. We have identified a protein that binds to the insulin 5'UTR and regulates its translation in response to glucose. We plan to further characterize the 5' UTR complex and its interaction with the basic translation machinery of the cell. The specific post translation modification that is responsible for the regulation of insulin translation will also be investigated.

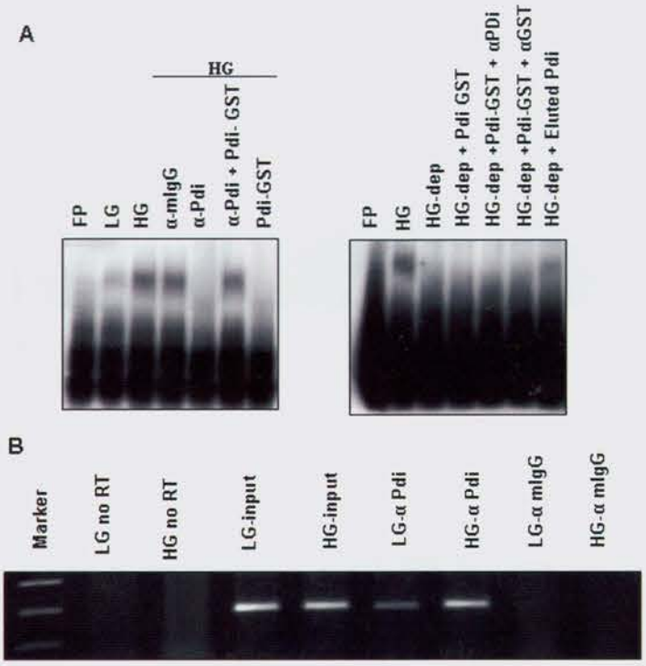

Fig. 2: PDI binds to insulin mRNA 5'UTR. RNA-EMSA was performed with the beta islet extracts in the presence of either PDI specific antibody or mouse IgG. The PDI specific antibody was titrated with either recombinant GST-PDI or GST. LG and HG indicate the low and high glucose treated extracts (A, Left). RNA EMSA was done with extracts immuno-depleted using PDI antibody. Recombinant GST-PDI was added and was found to be inactive as RNA binding protein, while the RNA affinity purified endogenous PDI was active as RNA binding protein (A Right). Beta cells were stimulated with glucose and the cells were treated with formaldehyde to crosslink and capture the in-vivo RNA-protein interaction. Cell lysates were prepared and the immunoprecipitation was performed with either IgG or Anti PDI antibody. RNA was prepared from the immunoprecipitates and analysed for the presence of insulin RNA by RT-PCR (B). Specific association of insulin mRNA with PDI was seen (compare IgG lanes with anti-PDI) which was induced upon glucose stimulation (Compare LG vs HG).

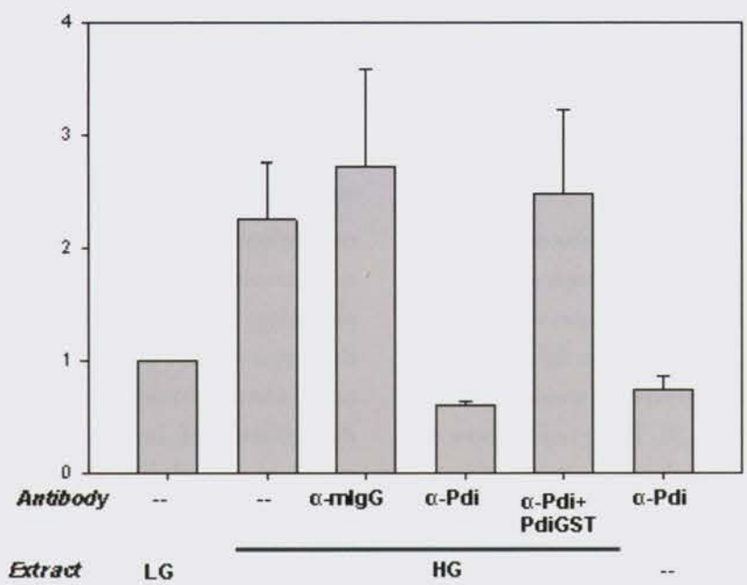

Fig. 3: PDI regulates insulin translation. *In vitro* translation of the chimeric ins 5'UTR-Luc-ins3'UTR in the presence of extracts from low (LG, 2.7 mM) or high glucose (HG, 16.7 mM) treatment was performed in RRL. The translation efficiency was measured by luciferase assay. Anti PDI antibody but not the mouse IgG was able to inhibit the glucose induced translation significantly. Addition of recombinant PDI neutralizes the antibody and thereby restores the translation activity. The experiments were performed with three different sets of extracts and Renilla luciferase RNA was used to normalize the translation activity.

Studies on regulation of human osteoclast differentiation and activation by IL-3

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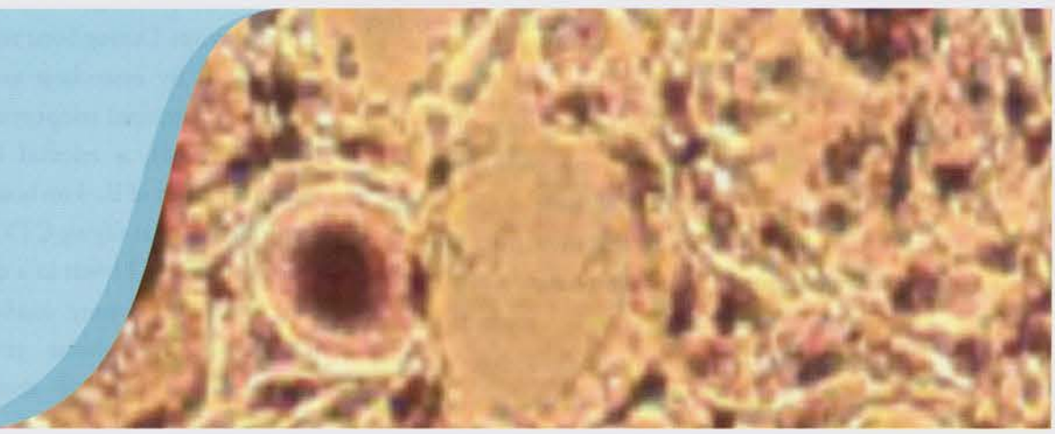

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Background

Osteoclasts are bone resorbing cells that differentiate from hemopoietic precursors of monocyte/macrophage lineage. Increased osteoclast activity is a major cause of bone loss in osteoporosis, rheumatoid arthritis and bone metastasis of breast and prostate cancers. Osteoclast differentiation is regulated by osteoblast-derived factors, receptor activator of NF-κB ligand (RANKL) and M-CSF. Osteoclast differentiation and activation are also regulated by various osteotropic factors including cytokines. IL-3, a cytokine secreted by activated T lymphocytes regulate the proliferation, differentiation and growth of hemopoietic cells. In previous studies we have shown that IL-3 acts directly on mouse osteoclast precursors and irreversibly inhibits RANKL- and TNF-α-induced osteoclast differentiation. IL-3 also significantly inhibited pathological bone resorption. Furthermore, the inhibitory action of IL-3 on osteoclast differentiation and bone resorption was seen in the presence of other inflammatory cytokines such as IL-1α, TGF-β_1, TGF-β_3, IL-6 and PGE_2. Moreover, IL-3 prevented the development of inflammatory arthritis induced by a mixture of anti-type II collagen mAbs and LPS. This study in mice suggests the potential of IL-3 in prevention of pathological bone resorption and inflammatory arthritis. However, the role of IL-3 in human osteoclast differentiation and bone resorption is not yet known.

Aims and Objectives

1. To investigate the role of IL-3 in regulation of human osteoclast differentiation and activation.

2. To understand the mechanism(s) of IL-3 action on human osteoclast differentiation and bone resorption.

Work Achieved

IL-3 inhibits RANKL-induced human osteoclast differentiation

Osteoclasts precursors isolated from peripheral blood of healthy donors were used to generate human osteoclasts. RANKL induced formation of large multinucleated giant cells with ruffled border that were strongly positive for tartrate-resistant acid phosphatase (TRAP) and human $\alpha_v\beta_3$ integrin 23c6. IL-3 in a dose-dependent manner inhibited formation of multinuclear osteoclasts. In the presence of IL-3 few multinuclear cells were present that were round, small in size and without ruffled borders. IL-3 significantly inhibited the number of TRAP positive osteoclasts (Figure 1). The inhibitory effect of IL-3 on osteoclastogenesis was not caused by induction of cell death.

IL-3 inhibits bone resorption through inhibition of human osteoclast differentiation

We next examined whether the decrease in osteoclast number by IL-3 was accompanied by a decrease in the resorptive capacity of osteoclasts. Interestingly, IL-3 inhibited RANKL-induced bone resorption in a dose-dependent manner and complete inhibition of bone resorption was seen at 100 ng/ml. IL-3 also inhibited expression of 23c6 (Figure 2). Thus, IL-3 inhibits bone resorption through inhibition of osteoclast differentiation. During bone resorption, type I collagen is degraded by osteoclasts and a small degradation product, C-terminal telopeptide fragment of collagen type I (CTX-I), is released into the medium. The inhibitory effect of IL-3 on bone resorption was further confirmed by quantifying CTX-I. It was found that IL-3 decreases CTX-I levels in a dose-dependent manner. This quantitative assay confirms that IL-3 inhibits RANKL-induced bone resorption. In further investigation we found that IL-3 down-regulates expression of osteoclast specific genes such as integrin β_3, CTR, MMP-9 and cathepsin K. In preliminary studies, we found that IL-3 inhibits osteoclastogenesis through inhibition of c-fms, a receptor for M-CSF.

Future Work

We are further investigating in detail the mechanism(s) of inhibitory action of IL-3 on human osteoclast differentiation and activation.

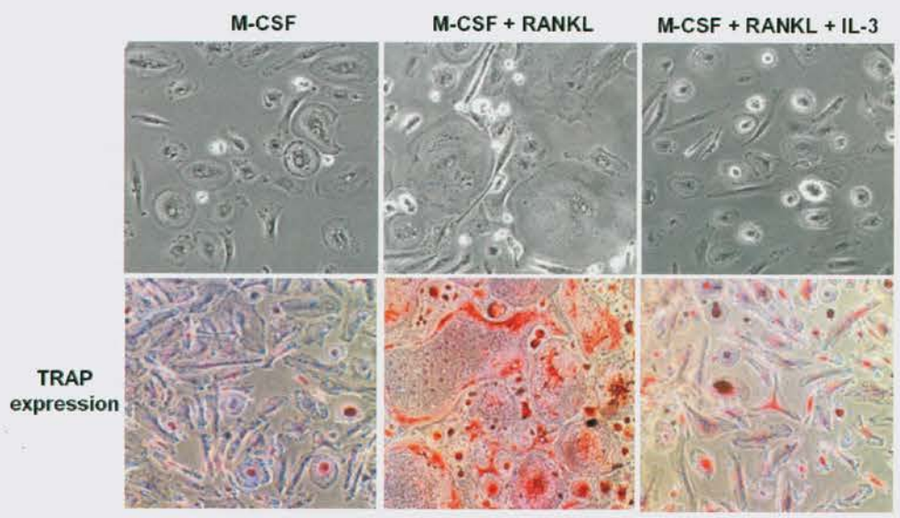

Fig. 1: IL-3 inhibits human osteoclast formation. Osteoclast precursors isolated from human peripheral blood were incubated on glass coverslips with M-CSF (25 ng/ml) or M-CSF and RANKL (40 ng/ml) in the absence or presence of IL-3 (100 ng/ml). After 21 days cells were stained for TRAP expression.

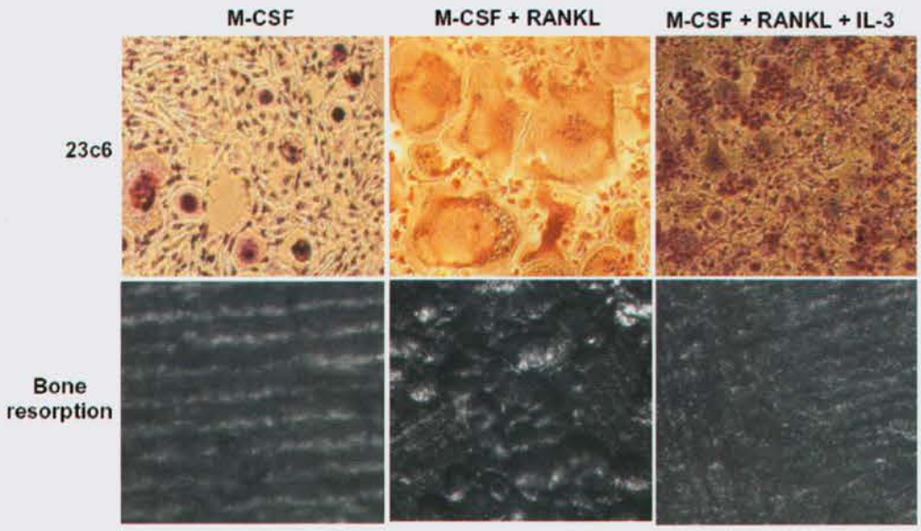

Fig. 2: IL-3 inhibits RANKL-induced bone resorption. Human osteoclast precursors were incubated on bovine cortical bone slices with M-CSF or M-CSF and RANKL in the absence or presence of IL-3. After 21 days cells were stained for 23c6, and bone resorption pits were observed under reflected light microscopy.

Non-traditional roles of nucleoporins: Nup358 regulates cell polarity

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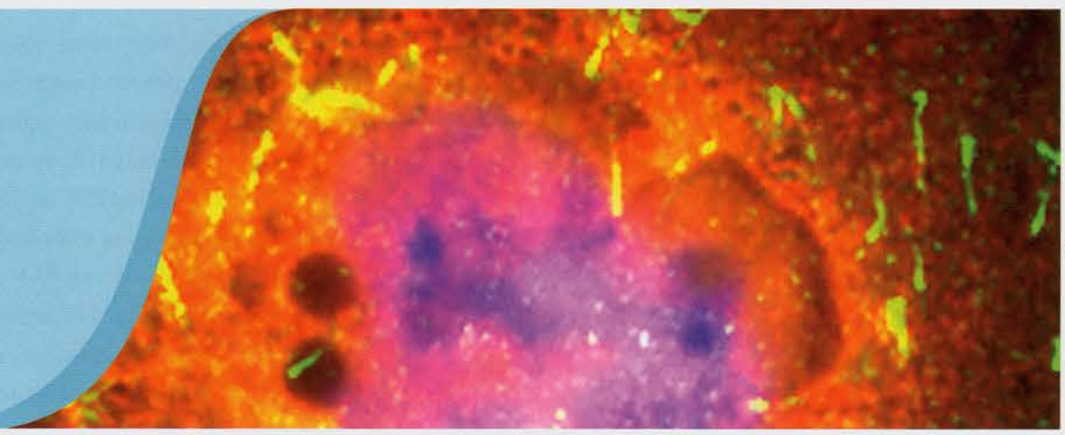

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Background

Adenomatous polyposis coli (APC), a tumor suppressor involved in Wnt signaling and cell polarity, is mutated in most colorectal cancers. APC is a microtubule plus-end-tracking protein (+TIP), which accumulates at the tips of a subset of microtubules that are present at the leading edges of migrating cells. This asymmetric distribution of APC is critical for the capture and stabilization of microtubules at the cell cortex to control polarity during migration. Although previous studies have implicated a role for the plus end directed motor Kinesin-2 in the polarized accumulation of APC at the cortex, the mechanism is not well understood. APC is known to associate with microtubules in three ways: through interaction with another +TIP (EB1), direct binding through a basic region within its carboxy terminus and through interaction with the plus end motor Kinesin 2.

In migrating cells, APC-mediated stabilization of microtubules at the leading edge is regulated by the small GTPases Cdc42, Rac1 and Rho, which act through downstream effectors that include the Par3/Par6/aPKC polarity complex, IQGAP1 and mDia. Recently, Wnt signaling has also been reported to control the localization of APC and cell polarity. During mitosis, APC localizes to kinetochores in a microtubule-dependent manner to mediate proper kinetochore-microtubule interactions. Nup358, a Ran-binding nucleoporin with SUMO E3 ligase activity, has a similar microtubule-dependent localization and a regulatory function at the kinetochores. Recently, we reported that Nup358, through its N-terminal leucine-rich-region, associates with and regulates interphase microtubules to control cell migration. Owing to the similarities between APC and Nup358 in the

regulation of microtubule dynamics both in interphase and mitosis, we hypothesized that these two proteins are functionally related.

Aims and Objectives

1. Study the interaction between Nup358 and the tumour suppressor APC
2. Regulation of APC by Np358 and its functional relevance to cell polarity

Work Achieved

Co-immunoprecipitation of HEK293 lysates using Nup358 antibodies, followed by Western analysis of APC indicated the *in vivo* interaction between these two proteins. Further biochemical studies demonstrated that Nup358 interacted with the middle region of APC (1211 to 1859 amino acids), hereafter called APC-M. Notably, transient expression of RFP-APC-M in cells resulted in its localization to distinct comet-like structures in the cytoplasm. To test if they represented the plus ends of growing microtubules, co-staining with antibodies against EB1, a prototypical +TIP, was performed. The result confirmed that APC-M partially co-localized with EB1 at the tips of growing microtubules, suggesting that this region has an independent plus-end-targeting function (Fig. 1A, arrows). As the middle region is also involved in interaction with Nup358, we examined the localization of endogenous Nup358 in RFP-APC-M expressing cells. Immunostaining analysis showed that endogenous Nup358 co-localized with RFP-APC-M at the plus ends of microtubules (Fig. 1B, arrows), indicating that this region of APC is sufficient to recruit endogenous Nup358 to microtubule plus ends.

Previous studies have shown that APC requires interaction with Kinesin 2 for its localization to clusters. Whereas C-terminal basic and EB1 binding regions are

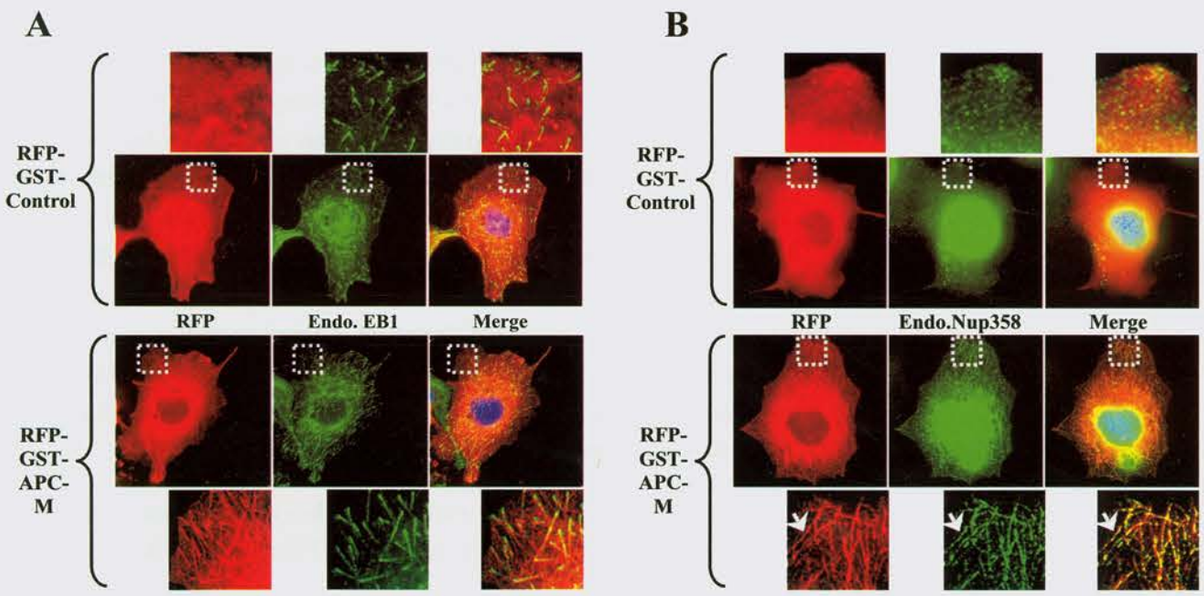

Fig. 1. Middle region of APC has a microtubule plus-end targeting function and is sufficient to recruit endogenous Nup358 to the plus ends. (A) APC-M localizes to the growing plus ends of microtubules. COS-7 cells transfected with RFP-GST or RFP-GST-APC-M (red) were fixed and stained for endogenous EB1 (green) using antibodies. DNA was visualized by Hoechst staining (blue). Closer view of the marked region (bottom panel) reveals partial co-localization of RFP-GST-APC-M with EB1 at the microtubule tips (arrow). (B) Endogenous Nup358 co-localizes with APC-M at the plus ends of microtubules. RFP-GST or RFP-GST-APC-M (red) transfected COS-7 cells were analyzed for endogenous Nup358 (green) localization. DNA was stained with Hoechst. Bottom panel shows the magnified view of the marked region and arrow indicates co-localization of Nup358 and APC-M at microtubule plus ends.

dispensable for the APC clustering at the cell cortex, Kinesin II-binding is essential but not sufficient for the localization. In addition to the N-terminal ARM repeats through which APC interacts with Kinesin 2, the middle region has also been shown to be essential for APC's cluster localization. As APC interacts with Nup358 through its middle region, we investigated whether this association is critical for APC to accumulate at the cell cortex. Our results suggested that Nup358 may exist in a larger complex containing APC and Kinesin 2. Further, using Nup358 RNA interference (RNAi) and cell migration assays, we demonstrate that Nup358 functions in cell polarity. Nup358 RNAi also impaired the APC cluster formation in vivo, suggesting that Nup358 plays an essential role in localization of APC and therby cell polarization during migration.

Based on the results, we propose a model wherein Nup358, in co-ordination with Kinesin-2, targets APC to the plus ends of microtubules (Fig. 2). The Nup358-APC-Kinesin-2 complex may regulate microtubule dynamics and aid in establishing cell polarity by mediating the capture and stabilization of microtubules at the cell cortex. Consistent with this function, both APC and Nup358 have been independently reported to be involved in polarized stabilization of microtubules in migrating cells. Nup358 could act as a scaffolding protein to localize and/or stabilize APC at the ends of those microtubules that are anchored at the cell cortex, after which Nup358 is dissociated. It is possible that the Ran-binding as well as SUMO E3 ligase functions of Nup358 contribute to its role in regulating APC localization and cell polarity. Our observations also raise the possibility that both Nup358 and APC have a similar interdependent role in mediating stable kinetochore-microtubule interactions during mitosis.

Why a nucleoporin such as Nup358 would be involved in the functioning of APC at the clusters? Recently, a role for APC in the targeting of a set of specific mRNAs to the cell cortex during cell migration has been identified. How APC may regulate this process is not clear. Given that Nup358 has been implicated in the export of mRNAs from the nucleus, we speculate that Nup358 functions with APC to couple the export of mRNAs to their polarized accumulation at the cell cortex. However, future studies are needed to address the role of Nup358

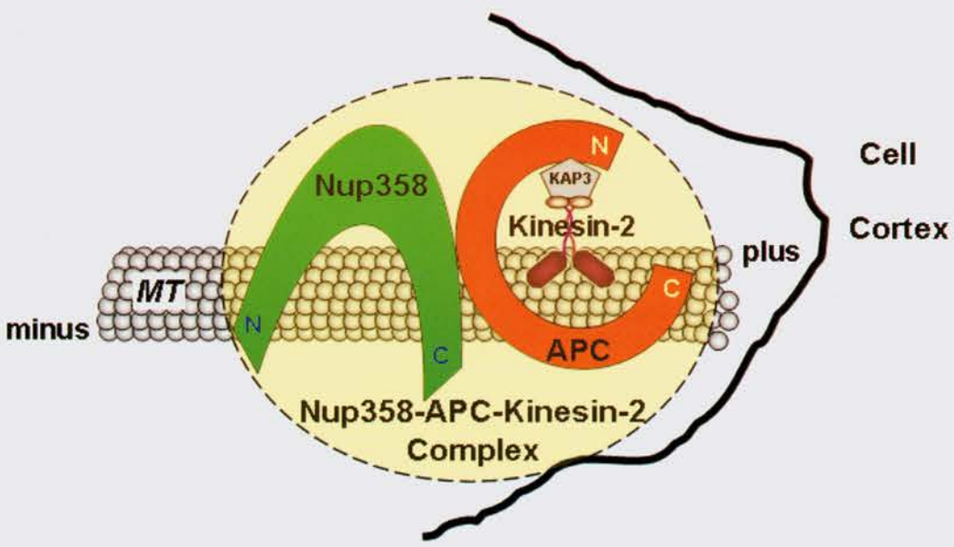

Fig. 2: A model for the role of Nup358 in cell polarity through regulation of APC localization. With the aid of Kinesin-2, Nup358 and APC move to the plus ends of growing microtubules (MT). The Nup358-APC-Kinesin-2 complex regulates microtubule plus end dynamics and mediates the capture and stabilization of microtubules by the cortex, after which Nup358 is released.

in this process. Although a few nucleoporins have been previously implicated in cell polarity, the molecular mechanism of their action remained elusive. The data presented here, combined with our previous results, reveal a novel function for the nucleoporin Nup358 in regulating cell polarity through modulation of microtubule dynamics and APC localization to the cell cortex.

It is intriguing to note that the additional plus end-targeting and the Nup358 interacting functions identified here are mediated by the middle region of APC that is deleted in most colorectal cancers due to mutations. This indicates that these functions could possibly contribute to the tumour suppressor activity of APC. However, further insights into the molecular details of how Nup358 regulates APC may have far reaching implications in understanding the biology of APC in health and disease.

Research Reports

Cancer Biology

Identification of Gene Targets of the Transcriptional Repressors Snail and Slug in the Context of Ovarian Cancer

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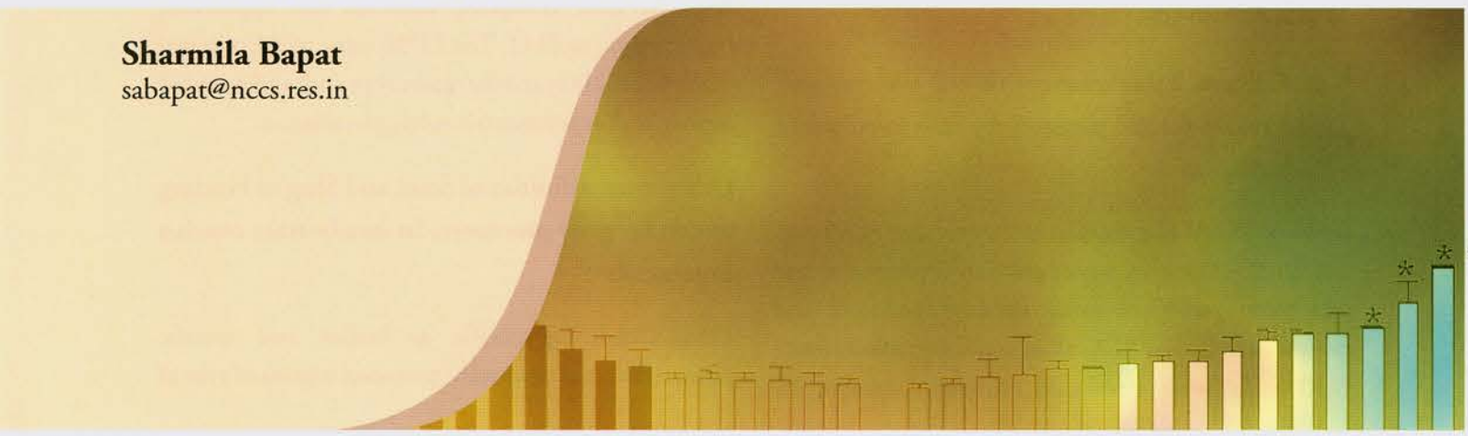

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Background

The transcriptional factors (TFs) Snail (*SNAI1*) and Slug (*SNAI2*), known to be involved in embryonic development, are also implicated in cancer progression. They recognize a consensus sequence (-CANNTG-) in gene promoters to repress target genes. Earlier work in our lab showed that the aberrant expression of Snail and Slug in epithelial ovarian cancer cells mediates Epithelial-Mesenchymal-Transition (EMT), a key event underlying invasion and metastasis. Further, we identified that both Snail and Slug were activated in response to hypoxia, wherein a protective role of these molecules was suggested.

Although a role of Snail and Slug in carcinogenesis is strongly implicated, it is not known whether each of these repressors play distinct or redundant roles in the tumorigenic process. Very few direct targets of Snail and Slug have been identified and include *CDH1, KRT17, KRT18, OCLN* and *PUMA*. Several of these targets are repressed by both transcription factors. However, it seems likely that Snail and Slug may regulate these targets under different conditions. The present report is a brief description of such a resolution of target genes of the two transcription factors in ovarian cancer cells at steady-state and in association with radiation resistance.

Aims and objectives

1. Identification of the targets of Snail and Slug in steady-state ovarian cancer cell line A4 that was previously established in our lab.

2. Studying the modulation of gene targets on exposure to LD50 dose of γ-radiation.

3. Investigating the role for these TFs in radioresistance.

Work Achieved

Cell Culture, Establishment of cell systems and LD50 values for the parental A4 cells in response to γ-Radiation

Stable Snail and Slug expressing transfectants of A4 cells (an epithelial ovarian cancer cell line developed in our lab) were established using standard protocols and termed as SNA and SLA respectively. A4 radioresistant (AR) cells were developed through exposure to incremental doses of -radiation using Gamma Chamber (^{137}C source). Cells resistant to 10Gy were termed as AR and used for further studies. The three cell systems, viz. AR, SNA and SLA transfected cells, demonstrated a remarkable morphological difference from the parental A4 cells with elongated fibroblast-like appearance characteristic to EMT. The LD50 value of A4 cells was identified as 20Gy at 36hr recovery period, and was used further in all experiments involving irradiation.

Differential affinities of Snail and Slug in binding to certain gene promoters in steady-state ovarian cancer cells

Using Snail/Slug-specific antibodies and specific primers designed against the promoter regions of a list of

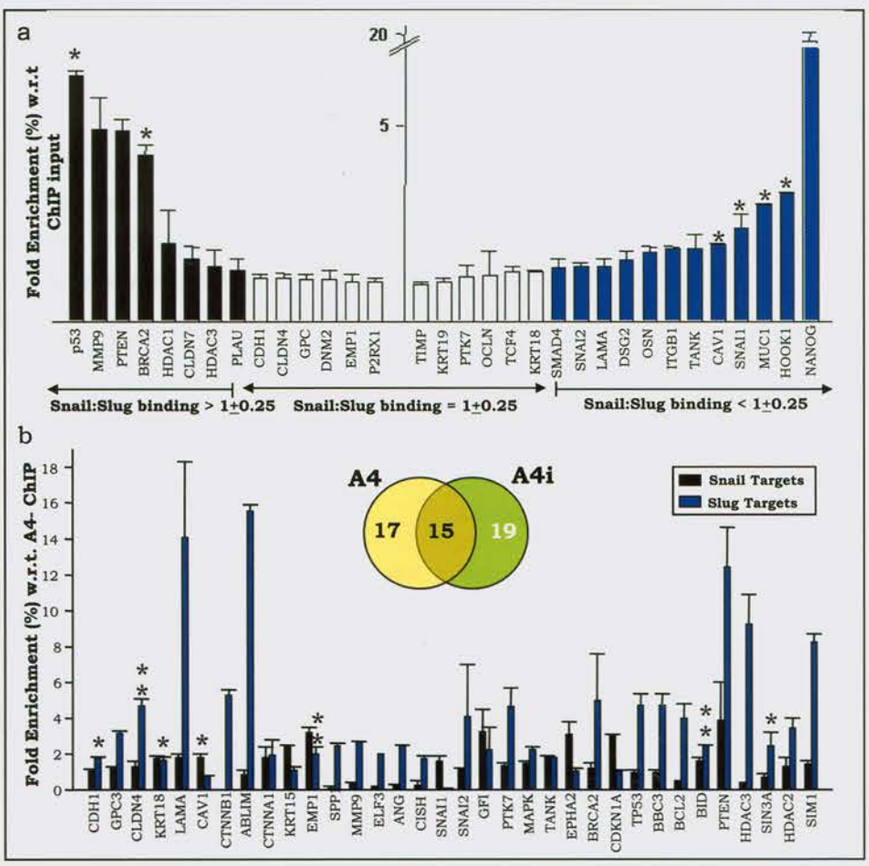

Fig. 1. Binding affinities of Snail and Slug to target-gene promoters in A4 cells. (a) Binding of Snail and Slug to target gene promoters. Fold enrichment refers to ratio of PCR amplification in Snail-immunoprecipitated DNA to that in Slug-immunoprecipitated DNA for the same gene promoter. The ratio is plotted as mean ±SE of triplicate values analyzed for statistical significance using paired t test (p-value < 0.05). (b) Alteration in binding to gene promoters by Snail and Slug in A4 cells exposed to LD50 dose of γ-irradiation. Genes where the fold change was more than/equal to that in A4 were considered as targets under irradiated conditions. Fold enrichment is plotted as mean±SE of triplicate values; analyzed for statistical significance using paired t test (p-value < 0.05).

putative target genes identified on the basis of the presence of the consensus sequence, we probed for the direct binding of these TFs to their targets in the parental ovarian cancer cell line A4. Promoter amplification in the immunoprecipitated DNA (ChIP-PCR) from these steady-state A4 cells was achieved for 32 genes that encode crucial proteins involved in angiogenesis; cell adhesion, division and differentiation; cytoskeletal organization; chromosomal segregation and DNA machinery (chromatin modification); apoptosis etc. (Fig.1a). The binding affinities of the individual TFs in targeting promoters were nearly identical for 12 gene promoters including the classical targets *CDH1*, *CLDN4, OCLN, KRT18, KRT19*, etc. A novel finding revealed through statistical analysis of the differential binding affinities (ratio of Snail : Slug binding < or > 1±0.25) is the identification of an increased affinity of Snail for *TP53* and *BRCA2* promoters as opposed to a preferential binding to *ITGB1*, *CAV1, MUC1*, and *HOOK1* promoters by Slug (Fig.1a).

Modulation of gene targets in ovarian cancer cells in response to -irradiation

Since additional roles for these TFs besides EMT have been recognized in cell survival - specifically in radioresistance, we probed if our cell system would express an altered target profile on exposure to γ - irradiation. Using ChIP-PCR assays, a total of 34 targets were identified in the irradiated A4 cells (termed as A4i); 15 targets were the same as in steady-state A4 cells, but were enriched further in ChIP indicating a stronger affinity, 17 were lost and 19 new ones were acquired (Fig. 1b). The more significant of the repressed genes included *CDH1, CLDN4, KRT18, CAV1, EMP1, BID* and *SIN3A*; de-repression of others such as *NANOG, KRT19, MUC1, HOOK1*, etc. was also quite marked.

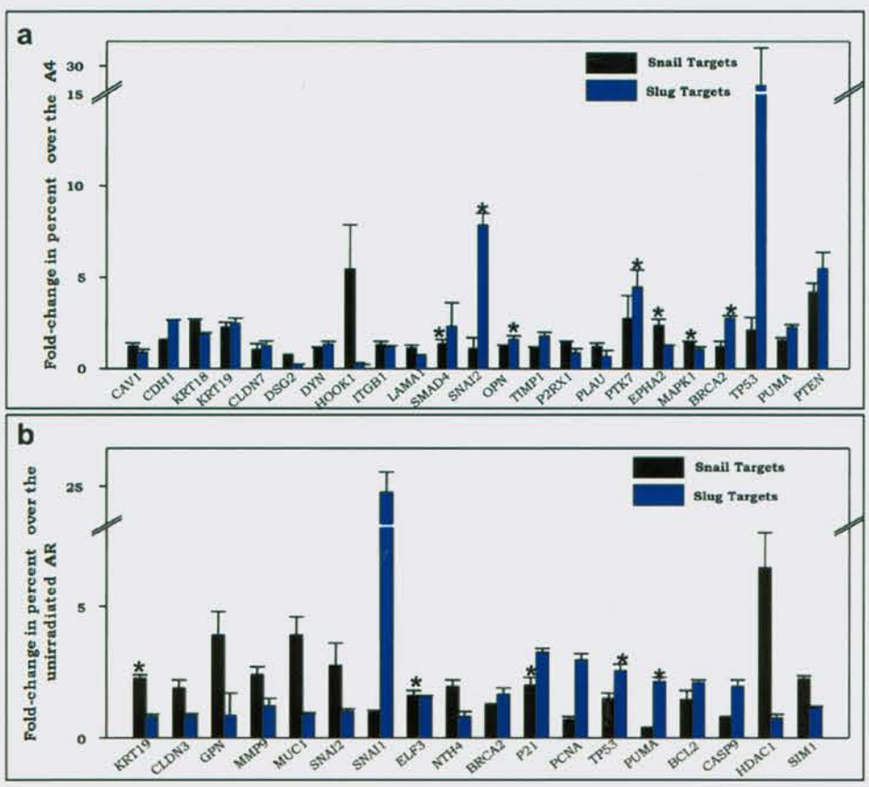

Fig. 2. Target-gene profiles of Snail and Slug in radioresistant AR cells. (a) Fold-change in gene targets of Snail and Slug in the steady-state radioresistant AR cells identified in comparison with parental A4 cells. Fold change is plotted as mean±SE of triplicate values; analyzed for statistical significance using paired t test (p-value < 0.05). (b) Snail and Slug targets identified in irradiated AR cells. Fold change was calculated by dividing the expression value obtained in irradiated AR to it's corresponding expression in non-irradiated AR cells for that particular gene. Fold change is plotted as mean±SE of triplicate values; analyzed for statistical significance using paired t test (p-value < 0.05).

Role for Snail and Slug in radioresistance

Enhanced levels of Snail and Slug are evident in the radioresistant cells AR (data not shown). Snail strongly binds to *CDH1, KRT18, KRT19, HOOK1, EPHA2* and *PTEN*; whereas Slug was strongly associated with *CDH1, SMAD4, PTK7, BRCA2, PTEN, PUMA* and *P53*. There was also a moderate increase in the binding of these two TFs to the promoters of *ITGB1, DNM2, CLDN7, TIMP1 and MAPK1* genes; and a common strong affinity to *CDH1, KRT18, KRT19, TP53, PUMA* and *PTK7* promoters. *EPHA2*, a receptor tyrosine kinase, emerged as a novel target in the radioresistant cells (Fig. 2a). On irradiation of these cells, a clear distinction between the genes targeted by Snail and Slug was evident. Snail binding affinity increased to the promoters of EMT-related genes such as *KRT19, GPN, CLDN3, MMP9, MUC1* and *SNAI2* (Fig. 2b); whereas Slug targeted cell-cycle related genes including *BRCA2, P21, PCNA;* and apoptosis related genes such as *TP53, PUMA, BCL2* and *CASP9*. Snail binding to the promoters of some signaling molecules such as *PLAU, P2RX1, PTK7, MAPK* and *EPHA2* was seen to decrease as was Slug binding to metastatic proteins *PTK7, SNAI2, OPN, SMAD4* and *TIMP1*.

The above results indicate:

1. At steady state, Snail and Slug primarily mediate EMT. Despite the overlap in their targets, a preferential repression of different gene promoters is evident. Further, these targets are differentially modulated under conditions of stress such as γ-irradiation.

2. In radioresistant clones, Slug primarily mediates cell survival through repression of pro-apoptotic molecules, whereas Snail continues to target the junction component elements to mediate cell migration and stress evasion, both processes; suggesting that under stress, Slug may be more important for cancer cells to survive environmental stress.

Future Work

Genome-wide identification of putative gene targets of these transcription repressors that mediate metastases.

Chemosensitivity of cancer cells to drugs: Elucidation of mechanism of cell death and Role of tumor suppressor p53

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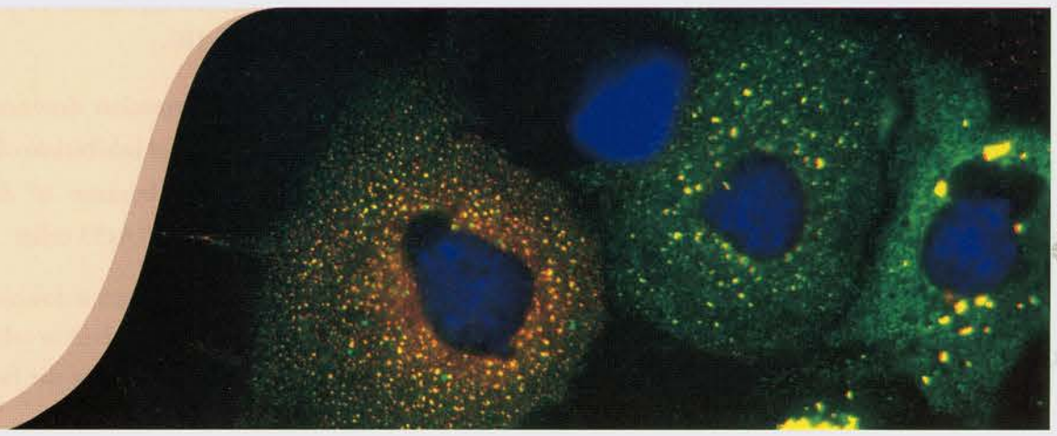

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Background

There is a medical need for advances in cancer treatment since surgery, radiotherapy and conventional cytotoxic chemotherapy have made only a modest overall impact on mortality. Hence, the significance of discovering new targets, pathways and strategies for therapeutic intervention in cancer is extremely important. Therefore, understanding the molecular events that contribute to cancer development as well as those which enhance drug-induced cell death will not only help in explaining the relationship between cancer genetics and chemotherapeutic drugs but also will improve sensitivity and specificity of the treatment. Cancer appears at a substantial frequency in the human populace-implicating malfunction of specific components of genomic "care taker" systems leading to increased mutability of the human genome. The most prominent member of these systems is the p53 tumor suppressor protein. p53 plays a pivotal role in the control of normal cell growth and survival. The loss of p53 function is an important event in breast tumorigenesis as documented in both human and murine systems. However, the exact mechanisms by which such lack of normal gene function leads to cancer formation and its progression are only beginning to be understood. Moreover, the downstream signaling pathways influenced by p53 remain to be clearly discovered. In cancers, it is clear that not all p53 mutations have equal effects; some have a dominant-negative effect (such as transdominant suppression of wt p53 or oncogenic gain of function) or loss of function, whereas others show only a partial loss of function where, for example, only a fraction of p53 target genes are deregulated. Therefore, elucidation of the role of tumor suppressor p53 is vital to rational

understanding of its involvement in cell cycle checkpoints, DNA repair, senescence, apoptosis, angiogenesis, surveillance of genomic integrity, signaling networks and sensitivity to drugs.

Aims and objectives

Role of p53 in cell growth, signaling and drug sensitivity of breast cancer cells

Work Achieved

p53 depletion promotes proliferation by upregulating cyclin D1:

Breast cancer cells, in which p53 was depleted (MCF-7As53), were identical to parental MCF-7 cells except for the growth pattern. To investigate whether this phenotype is due to any alteration in the status of cyclins that control cell cycle phase transition and progression, we analyzed the levels of cyclin D1 and cyclin E. As shown in Fig. 1A, cyclin D1 was barely detectable in MCF-7 cells whereas in MCF-7As53 cells, significantly increased expression of cyclin D1 was detected. Moreover, at any given time point cyclin D1 levels in MCF-7As53 cells are much higher than those in MCF-7 cells. This was further confirmed by confocal microscopy studies (Fig 1C). Under similar experimental conditions no significant alterations in either cyclin E or β-actin levels were detected in both the cell lines. Re-expression of p53 decreased cyclin D1 levels (Fig. 1B).

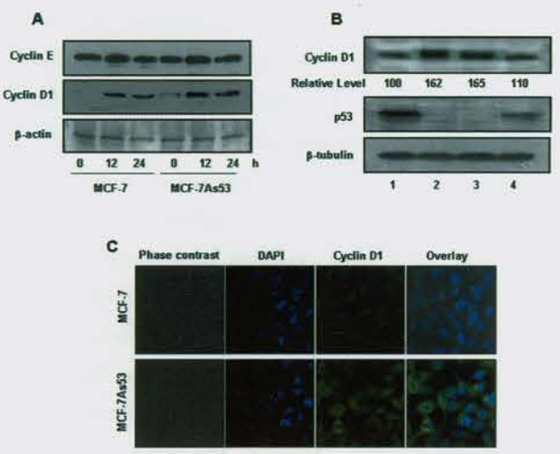

Fig. 1: Upregulation and stabilization of Cyclin D1: MCF-7 and MCF-7As53 cells were serum starved for 24 h and then stimulated with serum for the indicated times. (A) Cell lysates were then resolved on gel, transferred onto membrane and probed with anti cyclin D1 and cyclin E antibodies. β-actin was detected as an internal control. (B) Overexpression of p53 in MCF-7As53 cells abrogated cyclin D1 stabilization. Shown here (1) MCF-7 cells, (2) MCF-7As53 cells, (3) MCF-7As53 cells subjected to mock transfection and (4) MCF-7As53 cells transfected with p53 expression vector. (C) Immunofluorescence studies showing staining of cyclin D1 in MCF-7 and MCF-7As53 cells. In lower panel, increased immunostaining in MCF-7As53 cells is seen.

p53 overexpression downregulates constitutively active Akt, and inhibition of Akt activity decreases proliferation because of diminished cyclin D1 levels in MCF-7As53 cells:

Akt activation, which is downstream of PI3-K pathway, is known to be involved in cell growth and survival. In our quest to investigate the factors responsible for the proliferative phenotype, we found that Akt is constitutively activated and pAkt levels are high in MCF-7As53 cells (Fig. 2A and Fig. 2C). To ascertain that the activation of Akt is a direct consequence of decreased p53 levels, MCF-7As53 cells were either mock transfected or transfected with the wild-type p53 expression vector. Interestingly, expression of p53 results in decrease in pAkt levels whereas, basal Akt levels

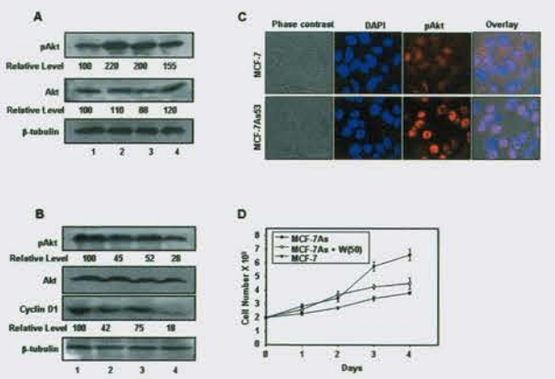

Fig. 2: Constitutively enhanced Akt activity can be abrogated by overexpression of p53. (A) Levels of Akt and pAkt in MCF-7 (1), MCF-7As53 (2), and MCF-7As53 (3) cells subjected to mock transfection, and MCF-7As53 cells (4) transfected with p53 expression vector. Densitometry was normalized with β-tubulin. (B) Akt phosphorylation and cyclin D1 levels in MCF-7As53 cells (1), MCF-7As53 cells (2) after wortmannin treatment, MCF-7 cells (3), MCF-7 cells (4) after wortmannin treatment. (C) Immunofluorescence studies showing staining of pAkt in MCF-7 and MCF-7As53 cells. In lower panel, MCF-7As53 cells show increased immunostaining. (D) Cells were evaluated for proliferation after wortmannin treatment (50 nM). Cell growth assay was performed and results are shown in growth curve.

remained unaltered (Fig. 2A, lane 3 vs. lane 4). These results clearly suggest a direct correlation between p53 levels and Akt activation. Also, the phosphoinositide 3-kinase (PI3-K) signaling pathway plays a pivotal role in intracellular signaling involved in cell growth, cellular transformation and tumorigenesis. Activation of Akt contributes to various malignant phenotypes in human cancers, including breast tumors. Our results indicate that in MCF-7As53 cells cyclin D1 is significantly upregulated and it plays a role in cell proliferation. Thus, we next probed whether Akt activation and cyclin D1 are interrelated. MCF-7As53 and MCF-7 cells were treated with the pharmacological inhibitor of PI3-K, wortmannin. pAkt and cyclin D1 levels are elevated in MCF-7As53 cells in comparison with MCF-7 cells (Figs 2B and 2C). Treatment of cells with wortmannin not only decreases pAkt levels, but also diminishes cyclin D1 levels. Moreover wortmannin treatment also inhibits Akt activation, resulting in decreased cell proliferation rate (Fig. 2D). These data imply that constitutive activation of PI3-K/Akt results in faster G1 to S cell cycle entry due to increase in cyclin D1 levels in MCF-7As53 cells.

p53 is a negative regulator of Cav-1/Akt regulated signaling in breast cancer cells:

To identify the upstream regulator of activated PI3-K/Akt in MCF-7As53 cells, we probed for Cav-1 as well as pCav-1 levels in these cells, since previous studies have indicated that Cav-1 is a potent activator of PI3-K/Akt pathway. In MCF-7As53 cells, we detected significantly higher levels of Cav-1 as well as pCav-1, in comparison to those present in parental MCF-7 cells (Fig. 3A, lane 1 vs. lane 2). p53 overexpression in these cells decreases Cav-1 as well as pCav-1 levels, which is indicative of a direct correlation between p53 levels and Cav-1 expression, as well as its activation (Fig. 3A, lane 2 vs. lane 3). Additionally, immunofluorescent studies also confirm that Cav-1 is overexpressed and its enhanced localization could be detected on the cell membrane in MCF-7As53 cells, as compared to MCF-7 cells (Fig. 3B). To investigate whether constitutively upregulated Cav-1 activity is indeed responsible for activation of Akt, we treated the cells with cholesterol depleting agent Methyl-β-cyclodextrin (MCD). Following MCD treatment, we observed that the decrease in Akt activity

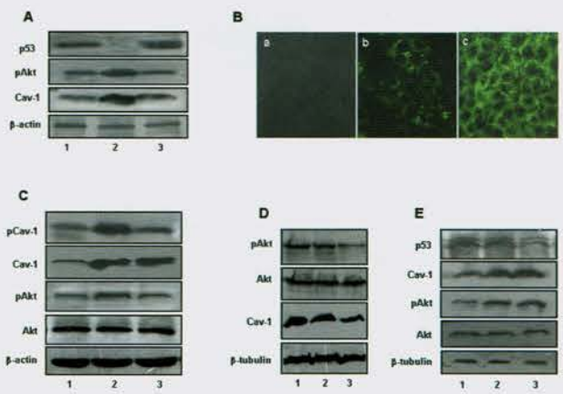

Fig. 3: p53 is upstream to Cav-1 expression in MCF-7As53 breast cancer cells. (A) MCF-7As53 cells were either mock transfected or transfected with p53 expression vector. The lysates were prepared and immunoblotted for p53, pAkt and Cav-1. Protein levels were normalized with β-actin. (B) Immunofluorescence studies for Cav-1 on MCF-7 and MCF-7As53. Panel, (a) shows cells probed with only secondary antibody. Panel (b) shows staining of Cav-1 in MCF-7 cells. Panel (c) depicts the staining pattern of membrane localized Cav-1 in MCF-7As53. (C) Immunoblot analysis for pCav-1, Cav-1, pAkt and Akt on lysates of MCF-7 cells (1), MCF-As53 cells (2) mock treated, and MCF-As53 cells (3) treated with cholesterol depleting agent MCD. β-actin was used as protein loading control. (D) Immunoblot of pAkt, Akt and Cav-1 in MCF-7As53 cells; Lane 1, control cells, Lane 2, cells transfected with control siRNA, and Lane 3, cells transfected with Cav-1 siRNA. (E) Immunoblot for p53, Cav-1 and Akt in MCF-7 cells (1), MCF-7 cells (2) treated with PFT-α and MCF-7 cells (3) transfected with p53 siRNA. β-tubulin was used as a control for loading.

correlated with the decrease in phosphorylation of Cav-1 (Fig. 3C). Further, direct correlation between Cav-1 and Akt activation was verified by utilization of specific siRNA (Fig. 3D & 3E). We also performed the experiment in MCF-7 in which p53 activity was inhibited either by pifithrin-α (PFT-α) treatment or by silencing the p53 message using p53 siRNA. As expected p53 siRNA expression decreases p53 protein levels (Fig. 3E, lane 1 vs. lane 3). We observed that Cav-1 as well as pAkt levels increased in the cells in which p53 was inactivated by PFT-α and, also in the cells which were transfected with p53 siRNA, as compared to mock transfected MCF-7 cells (Fig. 3E). The proposed model for regulation of cyclin D1 by p53 is depicted in Fig. 4.

In summary, with MCF-7As53 cells, we have established an experimentally amenable system to analyze how the absence of p53 promotes genomic instability, which in turn may result in molecular alterations in signaling pathways in the breast cancers. Our studies for the first time indicate the significance of

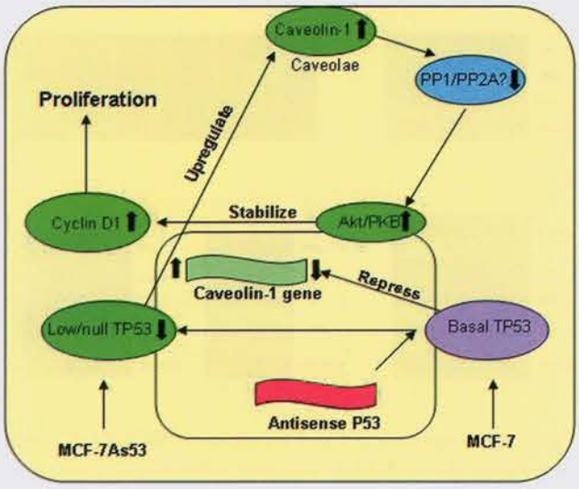

Fig. 4: Proposed model for regulation of Cyclin D1 by p53

p53 in modulation of signaling for cell growth and also points towards the scope for exploring these pathways either to increase cancer cell killing in future therapeutic interventions or for better understanding of factors regulating cancer cell growth.

Future work

Studies on effects of various chemotherapeutic drugs and role of p53 in breast cancers cells will be continued. These investigations will be expanded to other cell lines derived from various human solid tumors.

Cell death receptor-mediated signaling pathways in human gliomas - molecular and cellular mechanisms

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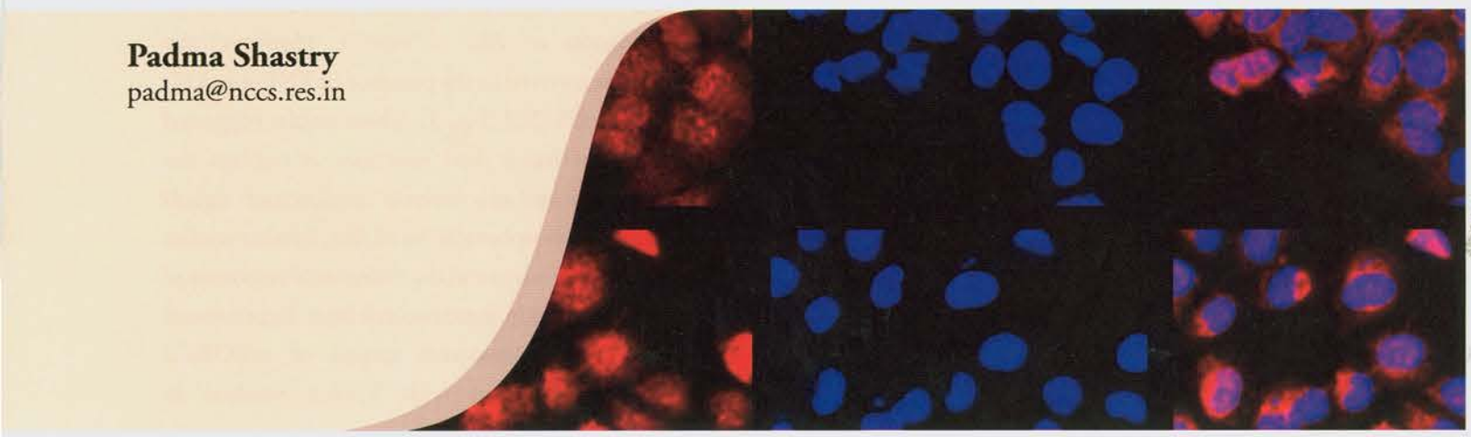

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Background

Gliomas are highly invasive brain tumors resistant to most conventional therapies like radiotherapy and chemotherapy. Macrophages comprise an important component of the immune system against tumors. In gliomas, macrophages are recruited and remain at the site of tumor (referred to as Tumor Associated Macrophages-TAMs) constituting a major proportion of the tumor mass. The TAMs exert influence on tumors by secretion of a variety of molecules such as chemokines, growth factors, Matrix Metallo-proteinases and cytokines like the Tumor Necrosis Factor (TNF-α), IL-1, IL-6. Studying the microenvironment of the tumor is therefore important in understanding gliomagenesis. TNF-α is an activator of the major survival pathways NF-κB and PI3K/Akt. Malignant gliomas constitutively express high levels of activated Akt and NF-κB which correlate with the aggressive nature and resistance of these tumors. While PI3K/Akt is known to regulate NF-κB pathway in many systems, in some cell types and under specific conditions NF-κB pathway is demonstrated to be upstream of Akt. In our previous studies with TNF-α, we found that these two pathways act independently in gliomas and confer resistance to TNF-α-mediated cytotoxicity.

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 resistance in these tumors with focus on activation of Akt/mTOR in gliomas.

Work Achieved

In continuation of our earlier studies, the mammalian Target of Rapamycin (mTOR) pathway was explored as the major downstream target of Akt. mTOR forms two complexes- mTORC1 with Raptor and mTORC2 with Rictor. The mTORC1 is sensitive to Rapamycin, while mTORC2 is not sensitive to Rapamycin treatment and is known to be important for phosphorylation of Akt at ser473. Our data revealed that stimulation with TNF-α resulted in overall activation of the mTOR pathway in human glioma cell lines- LN-18 and LN-229. The components of the pathway pmTOR, Raptor, phospho-p70S6kinase and Rictor were upregulated (Fig. 1) suggesting that TNF-α activates both rapamycin-sensitive and insensitive targets of the mTOR pathway. Rapamycin, the inhibitor of mTOR, inhibited proliferation of LN-18 and LN-229 cells. In these experiments, TNF-α did not affect the arrest in growth induced by rapamycin (Fig. 2). Our studies also showed that prolonged exposure to rapamycin downregulated rictor activity in LN-18 and LN-229 cells, as assessed by phosphorylation of Akt at ser473. However, the reduction was reverted in the presence of TNF-α in LN-18 but not in LN-229 (Fig. 3). These results suggested that rapamycin has a dual function- it inhibits the mTOR pathway and also controls the upstream signals by affecting the phosphorylation of Akt. Further studies are under way to understand the differential responses of Rictor to rapamycin in these two cell lines. Experiments to identify the downstream targets of mTORC2 revealed that activation with TNF-α resulted in

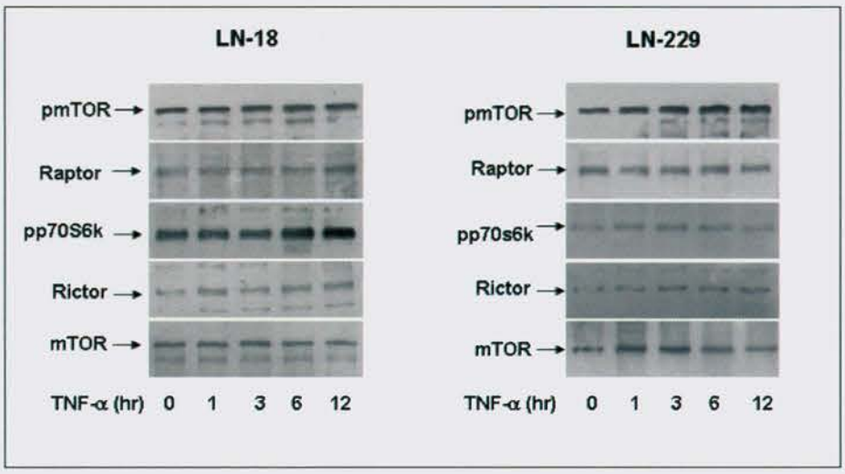

Fig. 1: TNF-α activated mTOR pathway components. LN-18 and LN-229 cells were cultured for 24hr and treated with TNF-α (10 ng/ml) for 1, 3, 6 and 12 hr. Whole cell lysates were prepared and subjected to immunoblotting for the expression of mTOR pathway components.

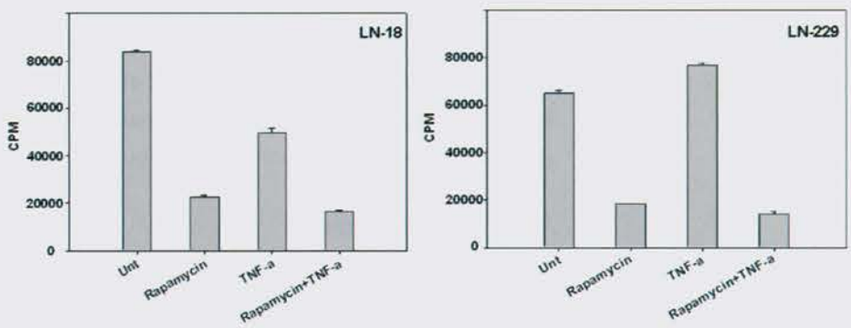

Fig. 2: Rapamycin-inhibited proliferation of LN-18 and LN-229 cells. 10^4 cells were seeded in 96-well plates, treated with rapamycin (25mM) alone or in combination with TNF-a (10ng/ml) for 24hr and subjected to proliferation assay using tritiated thymidine incorporation. The data represents mean CPM values +/- SE of three independent experiments.

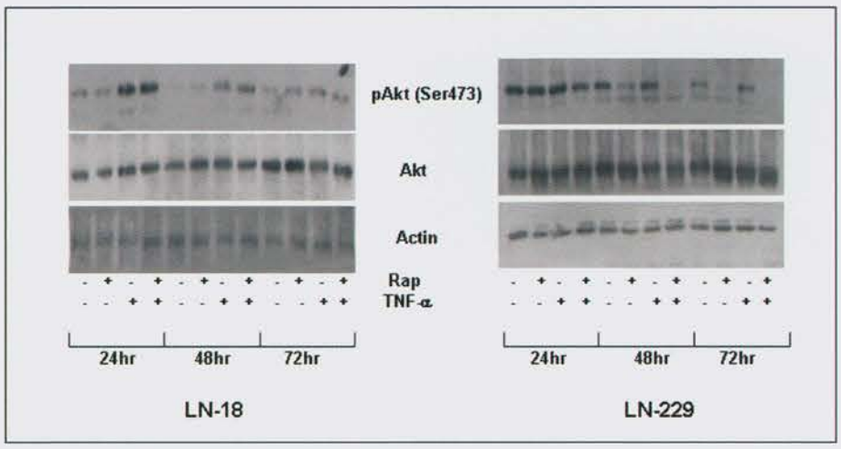

Fig. 3: Prolonged treatment with rapamycin affects phosphorylation of Akt at ser473. LN-18 and LN-229 cells were cultured for 24hr and treated with rapamycin (25μM) alone or in combination with TNF-α (10ng/ml) for 24, 48 and 72hr. Whole cell lysates were extracted and subjected to immunoblot assay for the expression of pAkt (ser473) and Akt. Actin was used as loading control.

significant increase in the expression of PKC-α with localization restricted to nucleus (Fig. 4A). As Cyclin dependent kinase inhibitor (CDKi)-p21 is regulated by PKC-α, we determined the expression of p21. We found that increase in p21 paralleled that of PKC-α, suggesting that effect of TNF-α on p21 could be due to activity of PKC-α (Fig. 4B). The implications of these finding on proliferation and cell cycle progression are currently under investigation.

Future plan

Study the involvement of mTOR components in biological activity of TNF-α and IL-1, in context of tumor progression and invasive potential of gliomas.

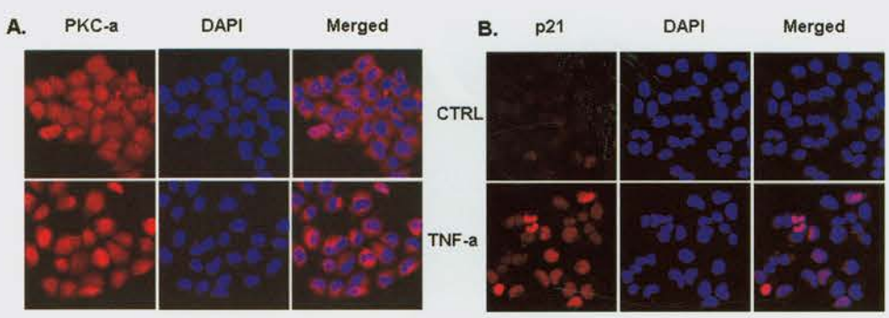

Figure 4. TNF-a upregulated PKC-a and p21. Expression and localization of PKC-α (A) and p21 (B) in LN-18 cells treated with TNF-α (10 ng/ml) for 12 hr. The nucleus was stained with DAPI (blue). The merged images show nuclear localization of proteins (magnification 63X).

Understanding mechanisms of transformation elicited by a novel 600bp non-coding RNA gene - M3TR

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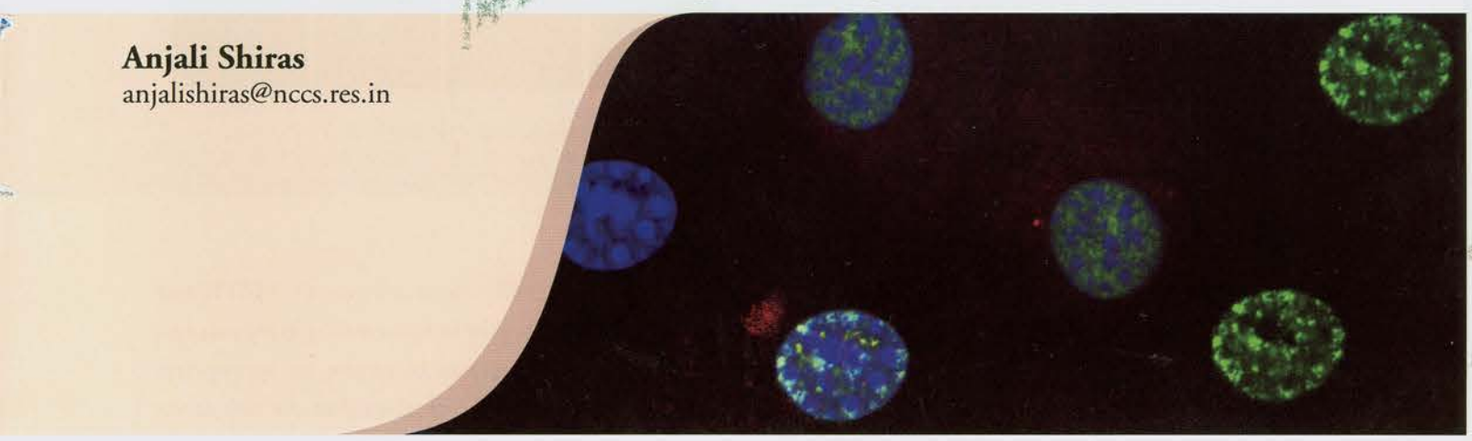

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Background

In our endeavor to study novel genes in melanoma genesis, we generated an expression cDNA library from mouse melanoma cells - Clone M3. From this Clone M3 cDNA a new kind of molecule M3TR was identified that was unconventional in the sense that it was a non-coding RNA (ncRNA). This non-coding RNA obtained through experimental cDNA library screening and bio-computational methods, displays transforming potential on ectopic expression and is expressed as a functional anti-sense transcript complementary to repeats throughout the genome. Interestingly, it seems to be playing important roles during development and neurogenesis as evident from its preponderant presence in embryos and brain. We are using different approaches to look into the role of this RNA during normal homeostasis, development and disease.

Aims and Objectives

Our work aims to address the role of M3TR derived from human neuroepithelioma cell line hM3TR, and study its effects on cell growth and regulation with the following specific objectives.

1. Clone and characterize the non-coding RNA M3TR from human cells
2. Study the biological function of M3TR by ectopic expression studies
3. Understand the molecular mechanisms and signaling pathways elicited by M3TR.

Work Achieved

The human brain is uniquely evolved and constitutes about 30% of the complex gene expression network facilitating intricate signal transmission between the neurons. Nervous system is reported to be a rich source of ncRNAs. These ncRNAs are implicated in neurodevelopment and brain function, through mechanisms such as RNA editing. It was interesting to study if M3TR was present in human neuronal cells. The expression analyses indicated that M3TR was present in fetal brain as well as in cell-lines derived from tumors of the neuroectodermal as well as of neuroepithelial origin. We cloned human M3TR (hM3TR) from a neuro-epithelioma cell line SK-N-MC and characterized it. The sequence analyses showed a total conservation of M3TR gene in human and mouse species. Studies with Northern and RPA showed that hM3TR existed as a natural antisense transcript (NAT) peculiarly, though it was only the sense transcript that possessed high transforming ability as deciphered from its clonogenicity in soft agar and tumorigenicity in nude mice. The hM3TR reverse transcript did not possess transforming potential. The much sensitive, Strand specific RT assay showed expression of M3TR as a pair of natural antisense transcripts. We found that hM3TR was expressed as an unspliced RNA of 557 bp length. However, with northern hybridization it displayed a size of 1.9 kb with polyadenylation signal at the 3' end. Our data illustrated that the transforming ability was manifested by M3TR due to the creation of genomic instability. The cells showed common features of more facile G1•S transition with very little growth arrest despite a robust activation of DNA damage response and accumulation of stalled DNA replication forks (Fig. 1). From our studies it appears that M3TR when added exogenously induces formation of dsRNAs that were capable of generating siRNAs. These siRNAs mimicked also the features of M3TR including their ability to induce genomic destabilization. This ultimately seems to contribute to failure of genomic surveillance mechanisms, loss of cell cycle checkpoint control and continuation of error-prone DNA synthesis leading to loss of homeostasis and favoring cell transformation.

In conclusion, we experimentally demonstrate the role of a pair of convergent natural antisense transcripts

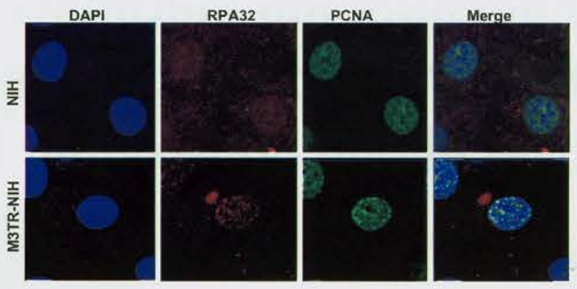

Fig. 1: Stalling of replication fork: detected by presence of a punctate pattern of RPA-32 (red) co-localizing with PCNA (green) only in pCMV- M3TR (lower) cells compared to NIH-empty vector (upper) cells.

(NATs) of M3TR ncRNAs in cell growth. M3TR and M3TR-as transcripts exist in homeostasis in human and mouse cells and co-operate to ensure an appropriate serum-induced G1-S cell cycle transition. An imbalance caused to their homeostasis by ectopic expression of M3TR (sense) but not that of M3TR-as (reverse); or M3TR-siRNAs (eliciting RNAi), induces precocious G1-S transitions and aberrant cell cycle progression characterized by the activation of checkpoint kinases and repair pathways for DNA damage, p53-induced p21cip1/waf1 (cytoplasmic) up-regulation, hyper-phosphorylation of pRb and abrogation of growth arrest leading to uncontrolled cell proliferation.

Future Work

1) Identify the targets through which M3TR functions. These targets could be could either be acting singularly or may act through a multitude of target genes possibly belonging to the family of tumor suppressors.

2) Study the role of this RNA during normal homeostasis, development and disease.

Osteopontin and PGE_2 regulate tumor growth and angiogenesis via multiple signaling cascades in breast and prostate cancers

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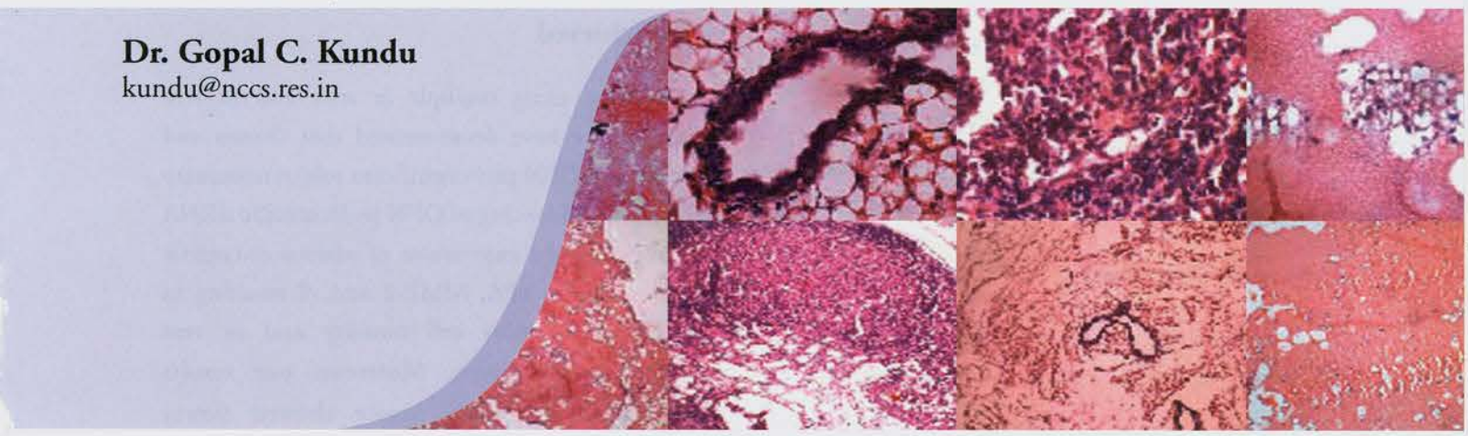

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Background

Migration of malignant cells to adjacent and distant sites is the prerequisite of metastasis. Angiogenesis, the formation of new blood vessels from the existing one, is the key step in tumor growth and metastasis, and various pro-angiogenic factors and their cognate receptors have been identified recently. Osteopontin (OPN), a chemokine like ECM associated Small Integrin Binding LIgand N-linked Glycoprotein (SIBLING) family of protein, plays an important role in determining the oncogenic potential of various cancers. The role of OPN in various pathophysiological conditions, particularly in cancer, suggested that the variation in glycosylation, phosphorylation and sulfation generate the different functional forms that might alter its normal physiological functions. Recent evidences indicated that OPN regulates tumor growth through induction of cyclooxygenase-2 (COX-2), uPA and VEGF and activation of matrix metalloproteinases (MMPs) in various cancer cells. The role of stroma- and tumor-derived OPN in regulation of tumor growth and angiogenesis is under intense investigation. Earlier we have earlier reported that OPN regulates breast tumor angiogenesis through induction of VEGF expression. However, the role of curcumin in regulation of OPN-induced VEGF dependent breast tumor angiogenesis is not well defined.

Recently, COX-targeted approach has shown great promises in anti-cancer therapeutics. Previous studies on several *in vitro* and *in vivo* models have revealed that elevated expression of COX-2 correlates with prostate tumor growth and angiogenesis. However, inhibitors of COX-2 have shown some side effects and therefore, targeting the signaling pathway mediated by COX-2's major metabolite, prostaglandin E_2

(PGE_2) might be a rationale approach for the treatment of cancer. Earlier, we have demonstrated that OPN induces COX-2 dependent PGE_2-mediated prostate cancer progression. PGE_2 interacts with the EP (E prostanoid) family of receptors that consists of four different subtypes (EP-1-4). The enhanced expressions of EP2 and EP4 receptors have been reported in prostate cancer as well as in endothelial cells. However, the molecular mechanism by which PGE_2 and its receptors (EP-2 and EP-4) directly regulate prostate cancer progression and angiogenesis is not well studied.

Aims and Objectives

1. To examine the role of stroma- and tumor-derived OPN in regulation of breast tumor growth in mice
2. To study whether curcumin attenuates OPN-induced VEGF-dependent breast tumor angiogenesis
3. To investigate whether PGE_2 controls EP2/EP4-mediated EGFR/MAPK or β3 integrin dependent ATF-4/AP-1 activation leading to enhanced uPA and VEGF expression which in turn regulate prostate tumor cell motility and angiogenesis

Work Achieved

In this study, using multiple *in vitro* and *in vivo* approaches, we have demonstrated that stroma and tumor derived OPN plays significant role in mammary carcinogenesis. Silencing of OPN by its specific siRNA down regulates the expressions of various oncogenic molecules such as uPA, MMP-2 and -9 resulting in inhibition of *in vitro* cell motility and *in vivo* tumorigenicity in mice. Moreover, our results demonstrated that $OPN^{-/-}$ mice showed slower progression of breast tumor growth and down regulation of expression of various oncogenic molecules as compared to wild type mice (Fig. 1). Furthermore, the data showed that injection of a carcinogenic

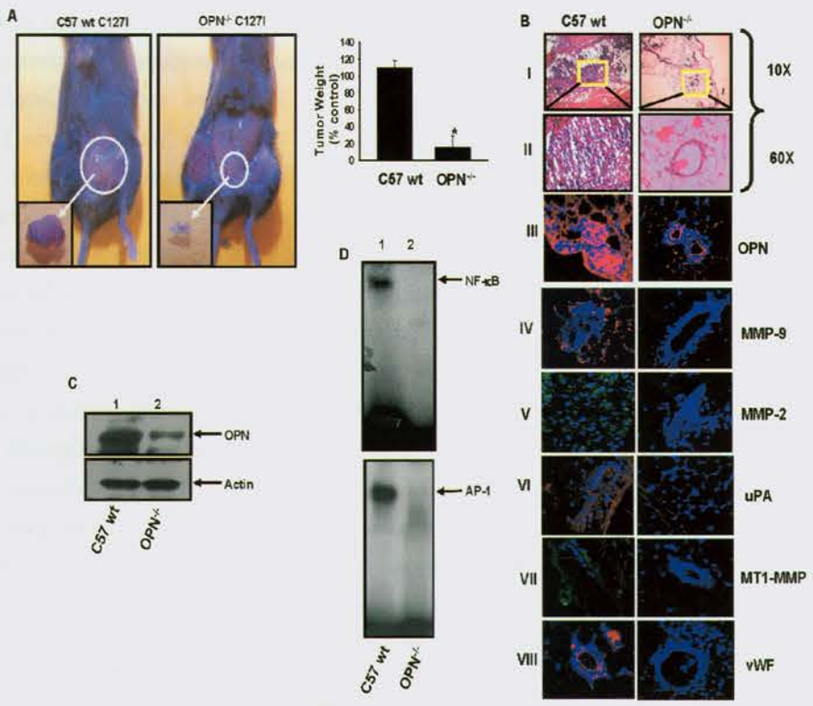

Fig. 1: Deficiency of host OPN reduces breast tumor growth in $OPN^{-/-}$ mice. (A) Typical photographs of orthotopic breast tumors in wt and OPN-/- mice. Isolated tumors are shown in inset. Tumor weights are represented graphically (n=6) (*p<0.04). (B) Histopathological (H&E) analysis of mice tumors. Photographs were taken in 10X and 60X magnifications (panels I and II). The expressions of OPN (panel III), MMP-9 (panel IV), MMP-2 (panel V), uPA (panel VI), MT1-MMP (panel VII) and vWF (panel VIII) in mice tumors were detected by immunohistochemistry using specific antibodies. A higher vWF expression was observed in wild type but not in $OPN^{-/-}$ mice tumors indicated the host OPN deficiency suppresses tumor neovascularization. (C) The expression of OPN in tumor lysates was detected by Western blot. Actin was used as control. (D) NFκB and AP-1-DNA binding in wild type and $OPN^{-/-}$ mice tumors were determined by EMSA.

compound, pristane (2, 6, 10, 14-tetramethylpentadecane), induces breast tumor progression leading to enhanced expression of OPN and other oncogenic molecules in mammary fat pad of nude and wild type mice but not in $OPN^{-/-}$ mice. However, intratumoral injection of OPN siRNA to pristane-induced tumor significantly suppressed these effects. The data revealed that knocking down of OPN effectively curbed breast cancer progression, suggesting that development of OPN based therapeutics might be an emerging approach for breast cancer management.

In another study, we have shown that curcumin (diferuloylmethane) abrogates OPN-induced VEGF expression and curbs OPN-induced VEGF dependent breast tumor angiogenesis *in vivo.* Furthermore, curcumin along with anti-VEGF or anti-neuropilin1 (NRP-1) antibody suppressed tumor angiogenic activity compared to curcumin alone, suggesting that curcumin along with anti-VEGF or anti-NRP-1 antibody synergistically suppressed breast tumor angiogenesis (Fig. 2).

We further demonstrate the in-depth molecular mechanism and show that PGE_2 activates the epidermal growth factor receptor (EGFR) and β3 integrin through EP2- and EP4 receptors-mediated pathways, which leads to the AP-1 activation. Moreover, PGE_2 also induces activating transcription factor-4 (ATF-4), and stimulates the crosstalk between ATF-4 and AP-1, which leads to increased expressions of uPA and VEGF, eventually regulating prostate tumor cell motility (Fig. 3). *In vivo* Matrigel angiogenesis assay revealed that PGE_2 induces angiogenesis through EP2 and EP4 receptors. Human prostate cancer specimen analysis also supported our *in vitro* and *in vivo* studies. Taken together, our data suggest that targeting PGE_2 signaling pathway (i.e. blocking EP2 and EP4 receptors) might be a rational therapeutic approach for overcoming the side effects of COX-2 inhibitors in prostate cancer management.

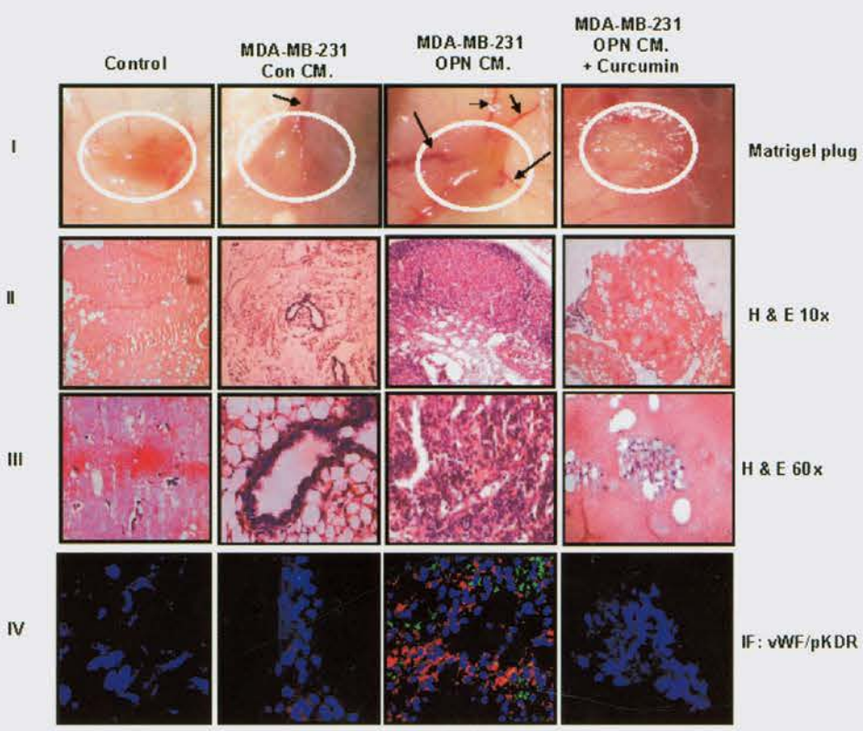

Fig. 2: Curcumin abrogates OPN-induced *in vivo* tumor angiogenesis. Typical photographs of *in vivo* Matrigel plug angiogenesis in nude mice. Angiogenic blood vessels are indicated by arrows. Note the significant reduction of blood vessel formation in curcumin treated plug along with OPN as compared to OPN alone (panel I). Matrigel plugs were analyzed histopathologically by H&E staining and photographs were taken in 10 x and 60 x magnifications (panel II & III). Matrigel plug sections were analyzed immunohistochemically using anti-vWF and anti-pKDR antibodies (panel IV).

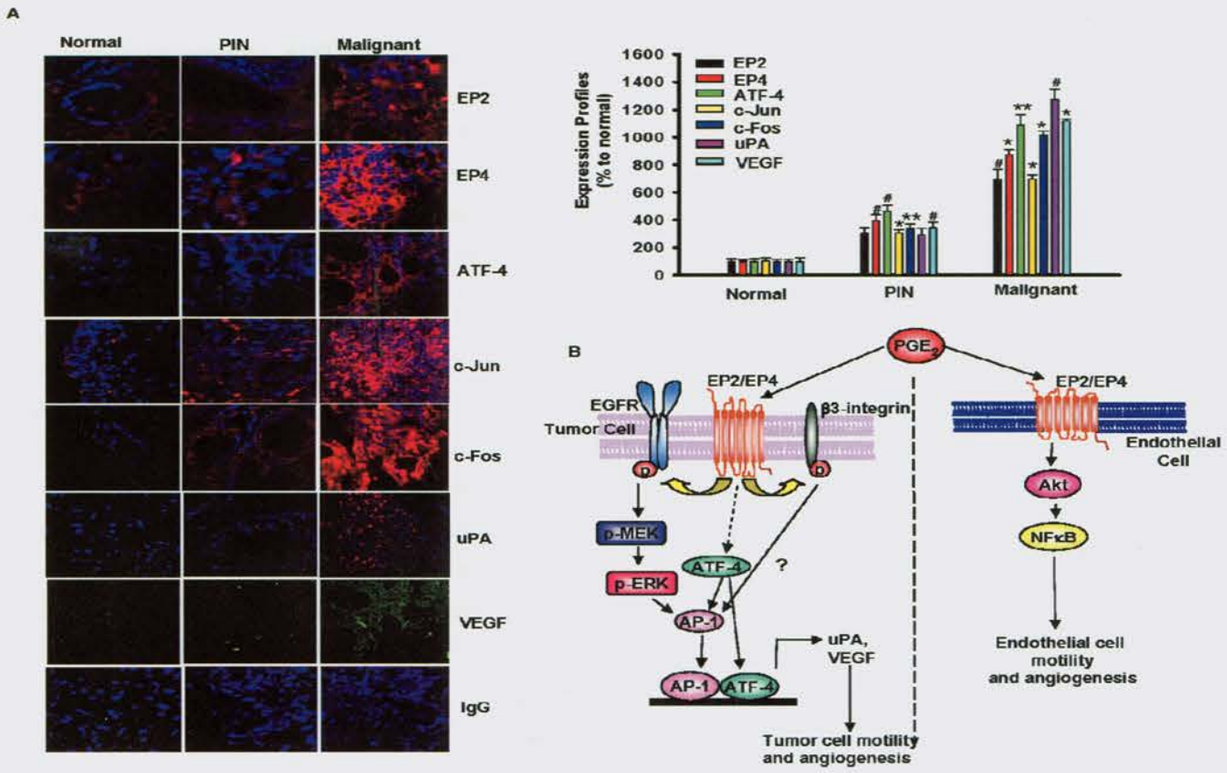

Fig. 3: Expression profiles of EP2, EP4, ATF-4, c-Jun, c-Fos, uPA and VEGF in human prostate cancer specimens and their correlation with human prostate cancer progression in different pathological grades. (A) The levels of EP2, EP4, ATF-4, c-Jun, c-Fos, uPA and VEGF were detected by immunohistochemical studies using specific antibodies. Sections stained with anti-rabbit IgG was used as control. The expression profiles were quantified by Image Pro Plus 6.0 Software and represented in the form of bar graph (*, P<0.003; **, P<0.006 and #, P<0.02). (B) Schematic representation of PGE_2-induced EP2/EP4-mediated EGFR/MAPK or β_3 integrin dependent ATF-4/AP-1 activation leading to enhanced uPA and VEGF expression which in turn control prostate tumor cell motility and angiogenesis. In endothelial cells, PGE_2 through EP2/EP4 receptor stimulates Akt and NF-κB activation leading to enhanced endothelial cell motility and angiogenesis.

Future Work

The therapeutic and diagnostic significance of OPN as well as the role of stroma- and tumor-derived OPN in regulation of tumor growth, metastasis and angiogenesis in various cancer models will be further addressed.

Blockade of caveolae dynamics by an extrinsic means leads to Apoptosis

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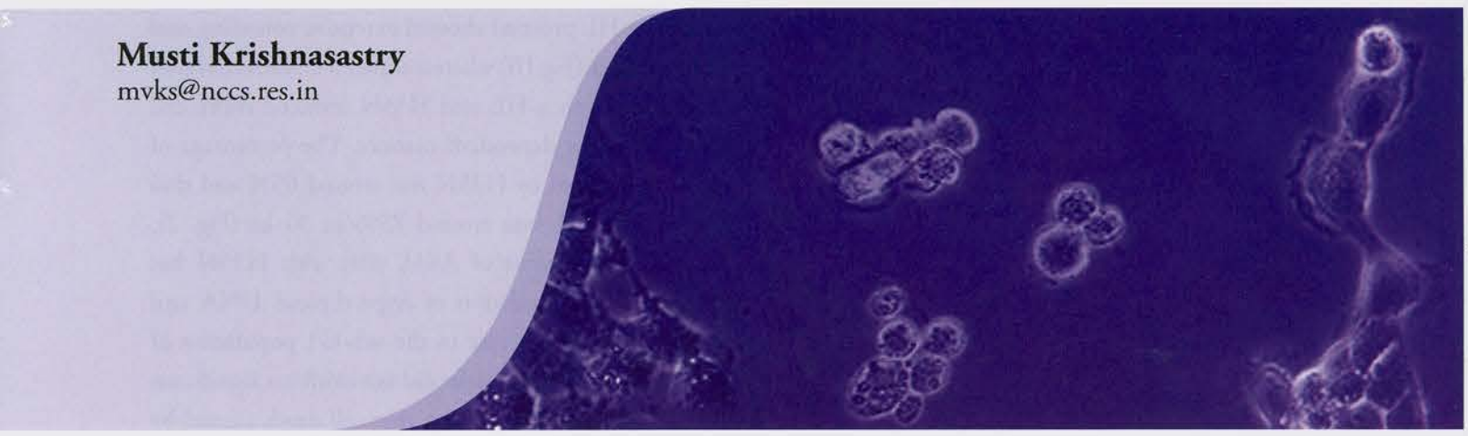

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Background

Binding of pore forming toxins such as staphylococcal α-HL can cause significant changes in cellular signaling of many cell types. α-HL's assembly on Jurkat cells results in necrotic form of cell death even though caspases are active. An intriguing question remained unanswered is how are the caspases activated by α-HL's assembly? The answer probably lies in the nature of the structural form of α-HL present on the cell surface - the presence of functional pore on target cell membrane possibly leads to osmotic imbalance and necrotic form of cell death. Theoretically, when α-HL binds to a target cell, all the three forms i.e. the cell bound monomer, non-lytic pre-pore and lytic pore, can be present on the target cell membranes but only a fraction of bound α-HL undergoes all the conformational changes to form the lytic pore. The precise nature of disturbances in cellular signaling, caused by the other two non functional forms i.e. membrane bound monomer and non-lytic pre-pore of α-HL are still not clear. In order to understand the changes in cellular signaling caused by these forms of α-HL we have employed a mutant form of α-HL viz., H35N (Histidine 35 mutated to Aspargine), that cannot assemble beyond membrane bound monomer, because Histidine-35 of α-HL is essential for inter monomer interactions during oligomerization. In the present study, we demonstrate that the H35N blocks the fission and fusion cycles of caveolae with the cell membrane. This is a novel observation because blockade in the caveolae dynamics by a pathogenic molecule results in apoptosis of target cells.

Aims and objectives

Delineating the Apoptotic pathway during the assembly of a-HL and role of caveolae vesicles.

Work Achieved

Considering the nature of action of pore forming toxins on mammalian cell membranes, it is anticipated that mere binding, i.e. without pore formation, cannot result in either cell death or substantial changes in the signaling pathways of target cells, except initiating steps for repairing membrane damage, if any. Earlier studies have shown that low doses of α-HL treatment of Jurkat T cells resulted in activation of caspases via mitochondrial pathway and oligonucleosomal DNA fragmentation. However, the caspase activation is not inhibited by zVADfmk or overexpression of Bcl-2 and the process is independent of death receptor pathway. However, recent observations are surprising that the low doses of α-HL still induced necrotic form of cell death despite caspase activation. Due to these contrasting observations, how α-HL manages to initiate the intrinsic pathway of apoptosis is unclear to date. The present study was aimed to resolve these contrasting observations with the help of a select mutant of α-HL *viz.* H35N.

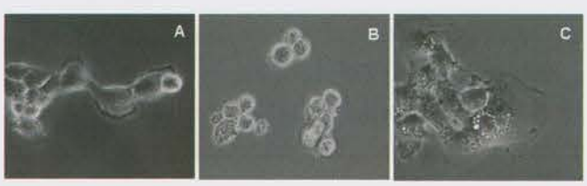

Fig. 1: H35N induced morphological changes of A431 cells: Cells were left untreated (A) or treated with H35N for 10 hr (B) or α-HL for 10 hr (C). After 10 hours, the cells were viewed using phase contrast microscope.

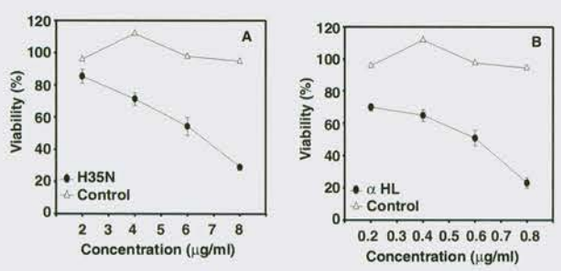

Fig. 2: H35N mediated cell death: Cells treated with H35N (A) or with α-HL (B) for indicated concentration of proteins for 30 hr as mentioned in materials and methods. The cells were then assayed by addition of MTT. The error bars represent the average of two sets of independent experiments.

In the present study, the morphology of A431 cells after H35N treatment (addition of recombinant H35N mutant α-HL protein) showed extensive rounding and cell shrinkage (Fig 1B) when compared to untreated cells (Fig. 1A). Both α-HL and H35N induced A431 cell death in a dose dependent manner. The percentage of cell death caused by H35N was around 65% and that caused by α-HL was around 75% in 30 hr (Fig. 2). Moreover, treatment of A431 cells with H35N has resulted in accumulation of hypo-diploid DNA and time dependent increase in the sub-G1 population of cells, while α-HL treatment did not exhibit a significant sub-G1 peak, demonstrating that cell death caused by H35N is mechanistically different from that of the death caused by α-HL.

In view of this unanticipated cell death we have investigated the nature of cell death in detail. Cleaved forms of caspase-3 (17 and 20 kD) appeared after 12 hr of H35N treatment (Fig. 3A), which are consistent with the bands seen in case of Cisplatin treatment (500 M), a potent apoptosis inducer. In contrast, α-HL showed no caspase-3 cleavage. Presence of active caspase-3 was also confirmed by FITC labeled active anti-caspase-3 monoclonal antibody staining. In comparison to untreated cells, a seven fold increase in the active caspases-3 in H35N treated cells in 24 hr (Fig. 4A) while α-HL showed negligible increase in the active caspase-3. In order to elucidate whether or not the mitochondrial pathway is responsible for H35N induced caspase-3 activation, untreated, α-HL or H35N treated A431 cells were examined for the presence of active caspase-9. Similar to caspase-3 activation, H35N induced time dependent increase (about 70% in 24 hours) in FITC-LEHD-FMK staining in A431 cells (Fig. 4B). In contrast, α-HL treated cells showed just about 25 % caspase-9 activity in 24 hr. A431 cells pretreated with zVAD-fmk, a broad spectrum caspase inhibitor, inhibited the caspases-9 activation induced by H35N treatment.

In summary, these observations suggest that the induction of apoptosis by α-HL is due to presence of monomeric form of α-HL or the form that did not

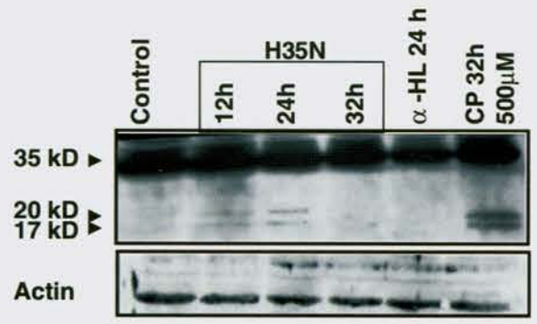

Fig 3. H35N induced Caspase-3 acitivity: Immunoblot analysis demonstrating caspase-3 processing in A431 cells that were either left untreated or treated with α-HL(800ng/ml) or H35N (8μg/ml) for the indicated time. The last lane represents treatment with 500μM cisplatin (CP) which was used as positive control. The lower panel represents total actin as loading control.

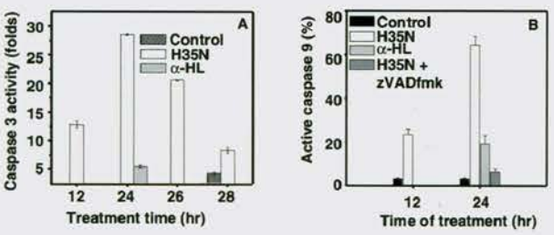

Fig. 4: (A) H35N induced Caspase-3 activation: A431 cells were incubated with H35N, α-HL for the mentioned time and active caspase-3 level was measured by using FITC labelled anti active caspase-3 antibody. The graph represents one of the two independent experiments. **(B) H35N induced Caspase-9 activation:** Following treatment of A431 cells with H35N or α-HL or H35N + zVADfmk for the indicated time points, the cells washed with PBS and incubated with FITC-LEHD-FMK which binds irreversibly to activated caspase-9 and cells analyzed using flow cytometry. The graph is a representation of average of two independent experiments.

undergo conformational changes after binding to target membranes as we could clearly observe all the important features of apoptotic cell death. Based on the present study and the published data, the reason for caspase activation might be as follows: When α-HL binds to target cells, only a fraction of the protein converts to lytic pore, while a substantial amount of the protein remains as membrane bound monomer and as non-lytic, pre-pore. In the absence of both pre-pore and functional pore, our data clearly highlights the activation of the intrinsic apoptotic pathway. Hence, it is possible that the membrane bound monomer will activate the intrinsic apoptotic pathway while a successful pre-pore/pore assembly will result in classical necrotic form of death. However, the causes for the induction of apoptotic pathway are unclear as the H35N can neither undergo large scale conformational changes nor is efficient in inducing membrane damage.

In our earlier work, we provided several evidences by biochemical analysis and molecular modeling, which suggested that the α-HL can interact with the scaffolding domain of Caveolin-1 (Cav-1; amino acids 81-101) through its 9 amino acid motif 'W^{179}GPYDRDSW^{187}'. Firstly, α-HL targets itself to lipid rafts of mammalian cells after binding as it was detected in Cav-1 enriched membrane fractions and it co-precipitates with Cav-1. Secondly, purified Cav-1 blocks the hemolysis of RBCs caused by α-HL. Furthermore, treatment of A431 with α-HL results in clustering of Cav-1 at cell-cell contacts and depletion of cholesterol in A431 cell membranes completely arrests the assembly of α-HL. Theoretically, the Cav-1 matches the dimensions and stiochiometry of α-HL for the facile assembly of its β-barrel. Thus, Cav-1 can act as an anchor for the α-HL beneath the cell surface through protein-protein interactions. There are also reports which suggest that cell shrinkage is required for tyrosine phosphorylation of Cav-1 and p38 MAP kinase activation.

The caveolae of mammalian cells exist as static platforms as well as undergo a continuous cycle of 'kiss and run' dynamics with the plasma membrane. The kiss and run dynamics of Cav-1-GFP was also observed by us in HeLa cells by Total Internal Reflection Fluorescence Microscopy. While a few caveolae remain static (permanently docked with the plasma membrane), several caveolae undergo fusion with and detachment from the plasma membrane within a small volume beneath it (Fig. 5). It has been reported that the phosphorylation of Cav-1 (during stimulation with EGF and also during stress) leads to internalization of caveolae. In view of the phosphorylation of Cav-1 due to H35N treatment, we examined the dynamics and internalization of caveolae by TIRFM upon H35N and -HL treatment. Interestingly, upon α-HL treatment, the kiss and run dynamics was completely arrested within 30 to 45 min while there was no detectable membrane damage. Similar results were also observed with the H35N treatment while there was no detectable membrane damage during this period. The caveolae

Research Reports

Diabetes

Evaluation of differentiation potential of tissue resident progenitors for regenerative therapy in diabetes and its complications

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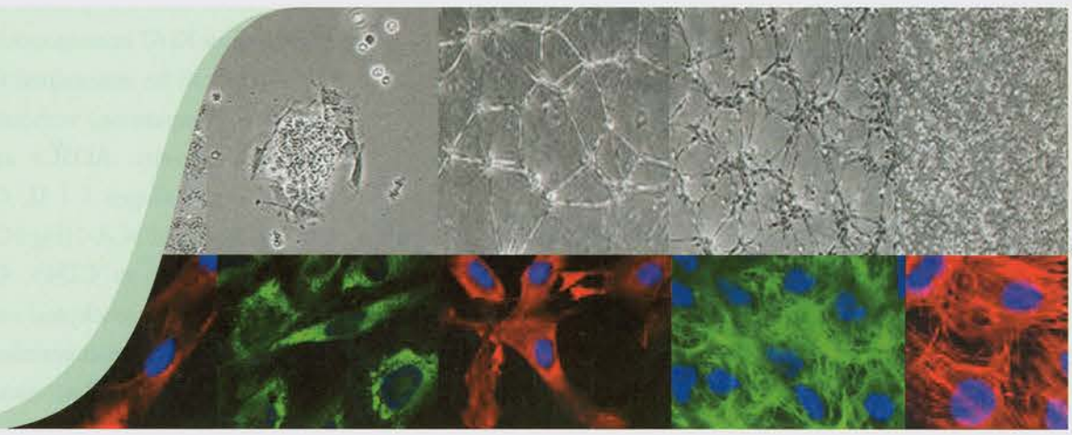

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Background

Resident somatic stem cell population plays a pivotal role in the tissue homeostasis of adult organs. Stem cell therapy aims at harnessing the potential of resident stem cells to achieve endogenous regeneration for repairing the damaged organs. Diabetes is a multi-factorial metabolic disorder caused due to partial/total damage of insulin producing beta cells of the pancreas leading to hyperglycemia. Chronic, uncontrolled, hyperglycemia would also lead to many life threatening secondary complications such as vasculopathy, nephropathy, retinopathy etc. Current therapies offer better management of diabetes by aiming at tight glycemic control. However none of the existing therapies offer a permanent remedy. Hence there is a need to look for alternative measures. Stem cells display robust proliferation along with the required plasticity to differentiate into various cell lineages. Since adult beta cells have limited potential to replicate, there is a need to replenish the lost beta cell mass by new cells. Adult stem cells offer an attractive source for cell replacement therapy. The present report summarizes the isolation, expansion, characterization and differentiation potential of adipose tissue derived stem cells into islet like aggregates and their role in diabetic reversal.

Also, the present study investigates the role of kidney resident progenitors in renal repair and regeneration after diabetic nephropathy, which is one of the most common secondary complications faced by diabetic patients.

Aims and Objectives

1. To test the potential of adult stem cells derived from adipose tissue for differentiation into insulin secreting islet like aggregates
2. To identify progenitors from postnatal organs, with special reference to kidney for assessing their regenerative potential in diseased mice models

Work Achieved

Adipose Tissue-derived Stromal Cells for Cell Replacement Therapy in Diabetes

One of the major limitations in cell replacement therapy for diabetes has been the availability of an unlimited supply of non-malignant progenitor cells. Though embryonic stem (ES) cells have tremendous capacity for proliferation, several limitations restrict their use for replacement therapy. Adult somatic progenitor cells are thought to be lineage committed but generally do not proliferate well. We describe here a highly proliferative population of mouse adipose tissue-derived stromal cells (ADSCs) having ability to differentiate into pancreatic islet-like cell aggregates (ICAs) in addition to their multilineage differentiation potential. White adipose tissue from epididymal fat pads of Swiss albino mice (n=6) is enzymatically digested to obtain stromal cell populations. These fibroblast-like cells demonstrate immunopositivity for two mesenchymal proteins (smooth muscle actin and vimentin) as well as intermediate filament protein Nestin(Fig 1B). Such ADSCs show Ki 67 immunopositive cells (54.69% in S-phase) and can be maintained for at least 80 passages (trillion fold expansion) without any significant loss in telomerase activity. ADSCs are found to produce Fibronectin, Collagen I / II, CD29, CD44, CD55, CD73, Thy-1 and SCA-1(Fig 1C)) but do not show any immunopositivity to CD45, CD31, CD11b, c-kit, suggesting their mesenchymal nature. When exposed to a serum free medium containing a cocktail of growth factors and ITS (insulin, transferrin and selenium) ADSCs migrate to form ICAs (Fig 1A). Such cell aggregates express several genes involved in pancreatic endocrine development as well as mature islet (pro)-hormones including insulin, glucagon, somatostatin (Fig 1E), Pdx-1, Glut-2, Nkx2.2, Ngn3 and Pax-6 etc (Fig I D and F). These ICAs demonstrate glucose-stimulated insulin release in vitro and can also maintain normal glucose concentrations when transplanted in streptozotocin (STZ)-induced diabetic

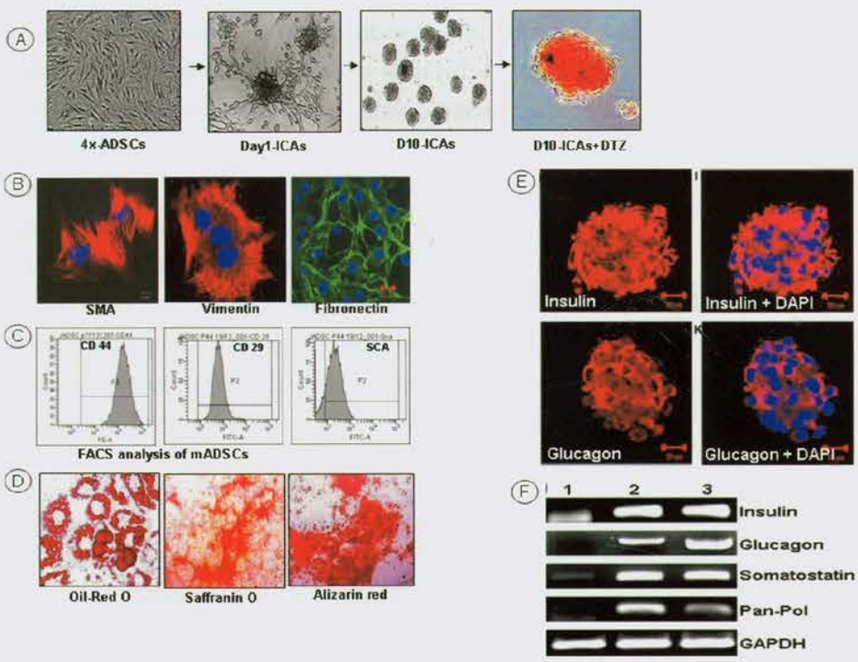

Fig. 1: Isolation, characterization and multilineage differentiation potential (adipogenic, chondrogenic and osteogenic) of murine adipose tissue derived stromal cells and in-vitro differentiation into insulin producing islet like Cell addregates (ICAs)

immunocompetent mice. On induction with specific differentiation cocktails, ADSCs differentiate into adipogenic, chondrogenic, and osteogenic as well as neuronal lineages. ADSCs obtained from human peritoneal fat mass (n=5) also demonstrate similar growth and differentiation potentials. Further studies that are presently being carried out in our laboratory demonstrate the therapeutic potential of ADSCs as autologous ICA-grafts for treatment of type 1 diabetes.

Evaluation of primitive renal progenitors present in adult mouse kidney and their role in renal regeneration

Resident somatic stem cell population plays a pivotal role in the tissue homeostasis of adult organs. Stem cell therapy aims at endogenous stimulation or exogenous transplantation of these cells into the damaged organs. Kidney is an organ with limited turn over of cells but show remarkable regenerative potential as evidenced by compensatory renal growth after unilateral nephrectomy (Fig 2E). Here we report the isolation and extensive characterization of mesenchymal progenitor population from the adult (8 wk) murine kidney. The cells were obtained as glomerular outgrowths (Fig 2A) and exhibited high proliferation potential and were telomerase positive. Transcript analysis revealed that the cells abundantly express genes which are characteristic to primitive renal progenitors, viz. Pax2, Gdnf, odd1, six1, Eya1, lim1, hoxb7, K-Cadherin. The cells showed high immunopositivity for CD 24, CD 29, CD 44 and Sca-1, weakly positive for CD73, CD 54 and negative for CD 34, CD 45, CD11b and CD 31. Immunocytochemistry of the cells showed the expression of nestin, vimentin, alpha smooth muscle actin, collagen 1, fibronectin (Fig 2B), thus reiterating their mesenchymal nature. On induction, the cells showed early events of differentiation into osteogenic, chondrogenic, endothelial (Fig 2D) and neuronal lineages. When transplanted under kidney capsule in unilateral nephrectomised mice, the cells differentiated into renal glomeruli (Fig 2F) and tubules, and improved renal functioning. This is the first report demonstrating the presence of primitive renal progenitor pool resident in the adult murine kidney and their active participation in renal regeneration in unilateral nephrectomy model.

Future Work

1. To understand the underlying mechanism of insulin resistance using adipocyte as a model.
2. To compare the adipose tissue stromal cells derived from different sources of human origin such as subcutaneous, retroperitoneal, visceral and mesenteric.
3. To evaluate the role of RRMCs in regeneration of kidney in diabetic nephropathy models.

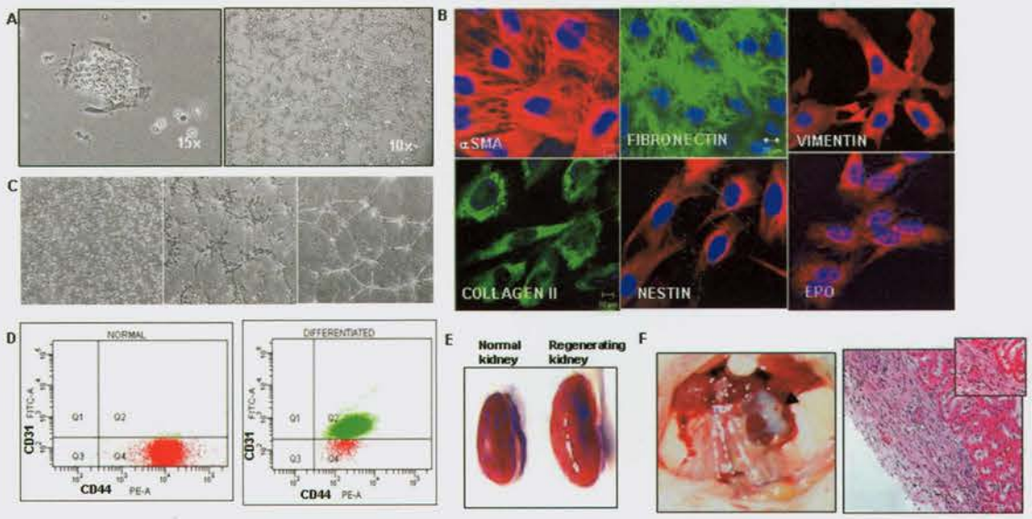

Fig. 2: Isolation and characterization of Renal Resident Mesenchymal Cells (RPMCs) from adult murine kidney (A & B), RRMCs exhibit in vitro tube formation potential when grown on matrigel (C).The differentiated cells exhibit immunopositivity for CD31 (D). Compensatory renal growth after unilateral nephrectomy in mice. (E), RRMCs exhibit renal commitment when introduced into a regenerating niche of compensatory growth (F)

Investigation on mitochondrial dysfunction, oxidative damage and apoptotic cell death stress mechanisms in diabetic cardiomyopathy

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Background

Diabetes causes various cardiovascular complications, which have become the major cause of morbidity and mortality in the diabetic population. Several studies have shown that hyperglycemia as an independent risk factor causing cardiac damage, leading to diabetic cardiomyopathy. However, mechanisms for the pathogenesis remain unclear.

Diabetic cardiomyopathy is characterized by systolic and diastolic dysfunctions. Impaired diastolic function is the most prominent mechanical abnormality manifested as prolonged relaxation and decreased compliance. Several mechanisms have been proposed for the pathogenesis of impaired cardiac excitation-contraction (E-C) coupling, however, oxidative stress, induced by reactive oxygen and nitrogen species derived from hyperglycemia and alteration of Ca^{2+} signaling has been a hallmark of cardiomyopathy and heart failure. Because cardiac contraction is highly dependent on intracellular Ca^{2+} levels ($[Ca^{2+}]_i$), its regulation through redox modification of Ca^{2+} channels and transporters has a profound effect on cardiac function.

Changes in critical processes that regulate intracellular Ca^{2+} concentration have been shown to occur in streptozotocin (STZ)-induced Type I diabetic rats. These include expression of sarcolemmal L-type Ca^{2+} channel that triggers Ca^{2+} release from the sarcoplasmic reticulum (SR), Ca^{2+}-ATPase pump (SERCA2), dephosphorylation of phospholamban (PLB) which decreases the affinity of SERCA2 for

Ca^{2+}, ryandine receptor (RyR) and sarcolemmal Na^{+}/Ca^{2+} exchanger (NCX) which mediates Ca^{2+} efflux from the cell.

Whether these cellular effects progress as a result of hyperglycemia or indirectly via glucose toxicity-induced secondary cellular alterations has not been understood completely. A direct correlation between hyperglycemia and oxidative stress in diabetic injuries, in particular mitochondrial damage, has been shown. A reduction in the number of calcium channels, alterations in heart sarcolemmal Ca^{2+}-ATPase and pump activity, and Na^{+}-Ca^{2+}-exchange activity has been shown to be associated with ROS in ischemia/reperfusion (I/R) injury. However, there has not been a critical examination of the link between hyperglycemia, oxidative and nitrosative stress and defective intracellular Ca^{2+} signaling in diabetic cardiomyopathy.

The purpose of the present study is to determine at the cellular and molecular levels whether oxidative stress and defects in intracellular Ca^{2+} signaling contribute to cardiomyopathy. Abnormal E-C coupling is apparent in ventricular myocytes isolated from diabetic rats even after only a few days of diabetes and these abnormalities are reproduced in normal myocytes cultured in a "diabetic-like" medium containing high glucose (HG). Therefore, we are using isolated adult cardiac ventricular myocytes in the present study.

Aims & Objectives

1. To study the redox regulation of cardiac calcium channels and transporters in diabetic cardiomyopathy
2. To study high glucose induced changes in the mechanical properties, calcium signaling, oxidative and nitrosative stress in isolated adult cardiac ventricular myocytes
3. To trace cardiac contractile dysfunction from live diabetic rats to isolated individual myocytes, then determine whether contractile dysfunction in $[Ca^{2+}]_i$ in individual myocytes occur in parallel with oxidative and nitrosative stress
4. To determine whether the oxidative stress and defects in $[Ca^{2+}]_i$ are consistent with alterations of expression and function ofproteins that are involved in intracellular Ca^{2+} signaling

Work achieved

During the last year, we had observed that high glucose-induced damage in the H9c2 cells is mediated by mitochondrial apoptotic pathway, suppression of ROS and RNS pathway and activation of p38 and PI3K/AKT pathways. Interestingly, this damage could be protected by insulin.

Further, we studied the redox regulation of cardiac calcium channels and transporters in diabetic cardiomyopathy. Adult rat ventricular myocytes maintained in a high glucose (HG) culture medium exhibit abnormalities in excitation-contraction coupling similar to myocytes from diabetic rats. Therefore, we used this model and since Reactive oxygen species (ROS) are shown to regulate cell function through redox modification of target proteins and one of them is the L-type Ca(2+) channel we used Nicardipine, a Ca^{2+} entry blocker - which inhibits Ca^{2+} entry via L-type Ca^{2+} channels. Nicardipine is a potent coronary and systemic vasodilator without evidence of depression of ventricular function also has the myocardial protective effect, and reduces the severity of myocardial ischemia, but its direct impact on cardiac function is not fully understood. To examine the role of nicardipine on HG-induced cardiac dysfunction at the cellular level, normal adult ventricular myocytes were cultured for 1 day in a serum-free insulin-containing medium with either normal glucose (5.5 mmol/l glucose) or HG (33 mmol/l glucose) in the presence or absence of nicardipine. Mechanical properties were evaluated using a high-speed video-edge detection system, and intracellular Ca^{2+} transients were recorded in fura-2-loaded myocytes. Oxidative and nitrosative stress was evaluated using specific fluorescent probes by confocal microscopy and changes in protein expression by western blotting. As previously reported, culturing myocytes in HG depresses peak shortening, prolongs time to 90% relengthening (Fig. 1 A-D), and slows Ca^{2+} transient decay. Culturing cells with nicardipine (2 micromol/l)

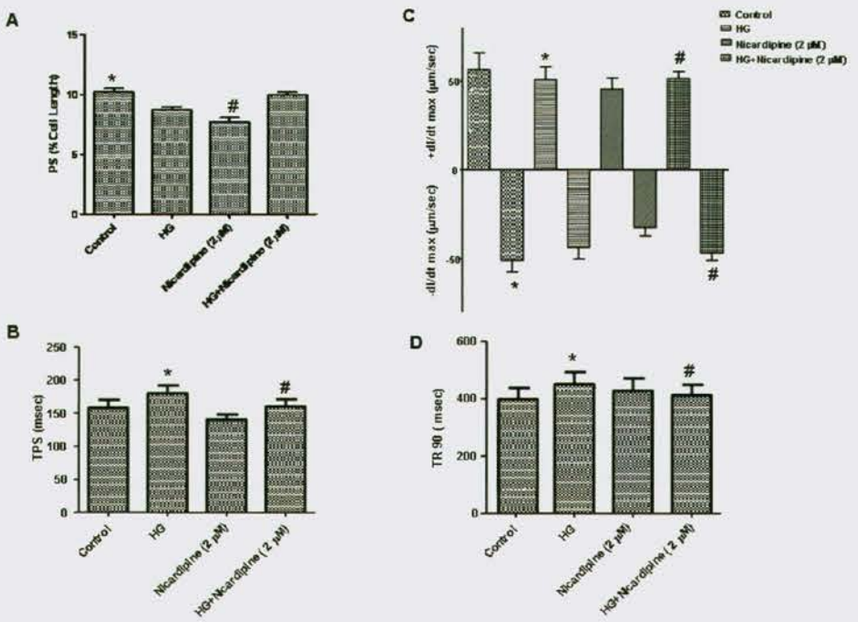

Fig. 1 A-D. Contractile properties of myocytes cultured for 24 h in normal or high glucose medium with or without the Ca^{2+} entry blocker Nicardipine (2 µmol/l) Mechanical indices are peak shortening (PS, panel **A**), maximal velocities of shortening/ relengthening ± dL/dt, Panel **B**), time-to-peak shortening (TPS, panel **C**) and time-to-90% relengthening (TR90, panel **D**). Means ± SEM, *n*=40-57 cells/group. **p*<0.05 vs respective normal group, #*p*<0.05 vs nicardipine supplemented high glucose group

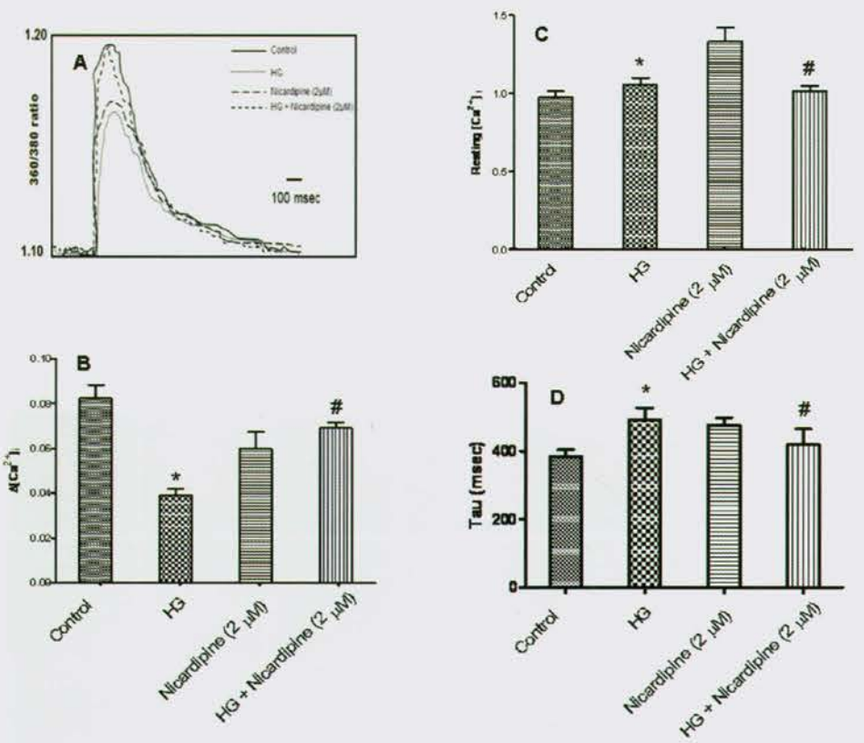

Fig. 2 A-D. Intracellular Ca^{2+} transient properties in fura-2- loaded ventricular myocytes cultured for 24 h in normal or high glucose medium, with or without nicardipine (2 µmol/l). **A** Representative traces of fura-2 fluorescence ratio in myocytes from normal, high glucose and high glucose+nicardipine group; **B** Baseline intracellular Ca^{2+} concentrations; **C** Increase in intracellular Ca^{2+} transient in response to electrical stimulus; **D** rate of cytosolic free Ca^{2+} decrease (tau). Means ± SEM. *n*=48-55 cells/group. **p*<0.05 vs respective normal group, #*p*<0.05 vs nicardipine supplemented high glucose group

prevented the HG-induced abnormalities in relaxation and depressed peak-shortening amplitudes (Fig. 2 A-D). Incubation of the cells with nicardipine also prevented slower intracellular Ca^{2+} clearing induced by HG. Incubation of the cells in high glucose for 24 h resulted in decrease in the cardiac Ca^{2+} regulatory protein SERCA2a, increase in RyR and no change in phospholamban (Fig. 3A). Interestingly, nicardipine also reduced HG-induced increase in superoxide and increased NO (Fig. 3B).

These data demonstrate that, nicardipine provides cardioprotection against HG-induced abnormalities in myocyte relaxation, perhaps through their antioxidant effect as well.

In conclusion, the results suggested that oxidative and nitrosative stress play a role in glucose-induced cardiomyocyte contractile dysfunction and in the pathogenesis of diabetic cardiomyopathy and nicardipine provides cardioprotection against HG-induced abnormalities in myocyte relaxation, perhaps through their antioxidant effect as well.

Future work

1) Characterization of high glucose induced mitochondrial ROS, RNS, Ca^{2+} influx and efflux mechanisms in cardiac muscle cells and determine how these mechanisms regulate excitation-contraction coupling ultimately leading to new insights for therapy using various specific calcium anion channel blockers.

2) Study signaling pathways involved both in vitro and in vivo using specific inhibitors.

3) Study the effect of good glycemic control with insulin-mimetic molecules and anti-diabetic pharmaceutical preparations and therapeutic potential of multiple anti-oxidants on oxidative and nitrosative stress in diabetic rat.

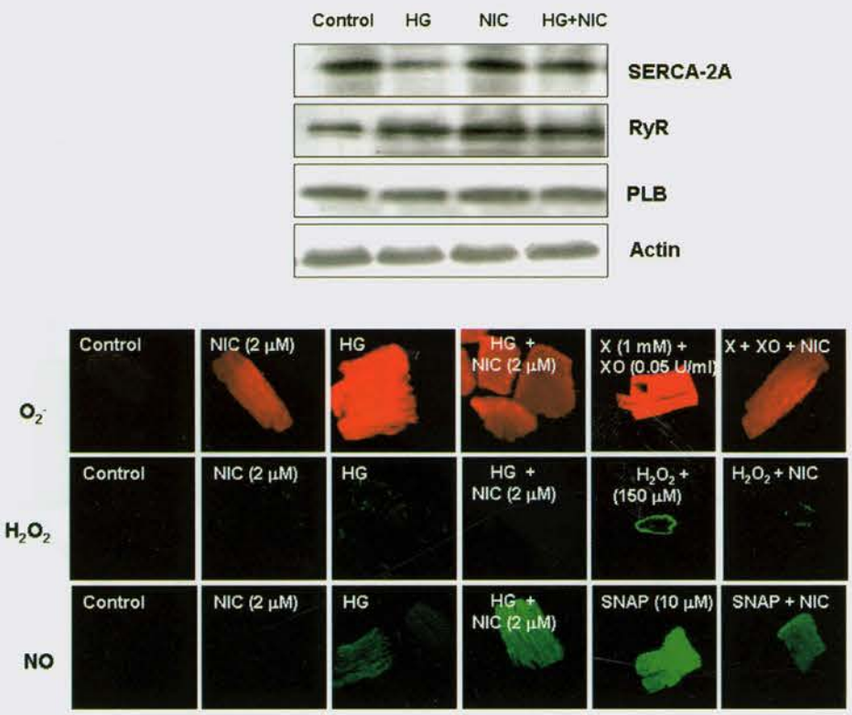

Fig. 3AB. Effect of nicardipine (NIC, 2 μmol/l) on the cardiac Ca^{2+} regulatory proteins including SERCA2a, RyR and phospholamban in myocytes cultured for 24 h in normal or high glucose medium. **A** Representative gels depicting immunostaining using anti- SERCA2a, anti-RyR and anti-PLB antibodies; **B** Oxidative and nitrosative stress in normal and high glucose myocytes incubated with or without nicardipine

Understanding the role of microRNAs in endocrine pancreas development and function

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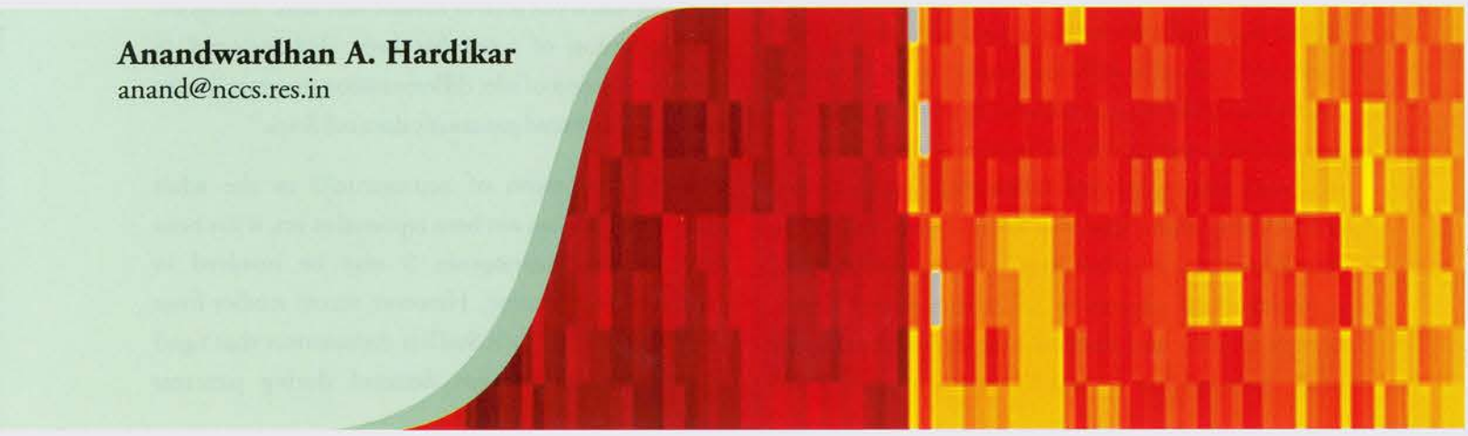

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Background

Failure in maintenance of pancreatic β-cell mass is recognized to be a major player in pathogenesis of type 1 and type 2 diabetes mellitus. Insulin replacement therapy, achieved by transplantation of cadaveric pancreatic insulin-producing cells has been demonstrated with some success. However, considering the limited number of available pancreatic islets, several researchers are trying to understand ways to generate insulin-producing cells from pancreatic progenitors and / or stem cells. To achieve efficient differentiation of pancreatic progenitors, we need to understand the process of endocrine pancreas development. The major focus of our laboratory is to understand mechanisms that are involved in generation of endocrine pancreas. We intend to use this information to achieve differentiation of pancreatic progenitor cells *in vitro*.

Aims and Objectives

1. To understand the developmental biology of endocrine pancreas.
2. To identify microRNAs involved in regulating endocrine pancreas development and function.
3. To study proliferation of insulin-producing beta cells in vitro and their contribution to generate human fetal islet-derived progenitor cells (FIPCs).
4. To understand the mechanism involved in generation of FIPCs.

Work Achieved

Pancreas development has been well studied in mice. It is well known that the rodent pancreas develops from dorsal and ventral endodermal buds near midgut region. As the gut tube rotates during development, dorsal and ventral buds fuse with each other to form the definitive pancreas. Endocrine cells are known to be generated close to duct-like structures in the developing pancreas. At embryonic day (e) 14, the termini of these duct-like structures form acini and differentiate into exocrine cells. During mouse pancreas development, cell fate is determined by a very finely regulated and synchronized spatio-temporal expression of transcription factors. Studies in mice and humans have demonstrated that pdx1 (pancreas and duodenal homeobox gene 1) and neurogenin-3 (Ngn3, a bHLH transcription factor), mark pancreatic endocrine progenitor cells. Loss of pdx1 leads to pancreas agenesis (no pancreas) while that of ngn3 leads to absence of endocrine cells within the pancreas. Ngn3 protein is detected largely during the 2nd trimester and is not seen in mature islet cells. Transgenic over-expression of ngn3 has been demonstrated to induce activation of islet differentiation program *in vivo* as well as in cultured pancreatic duct cell lines.

Although expression of neurogenin-3 in the adult mouse pancreas has not been reported as yet, it has been suggested that neurogenin 3 may be involved in pancreatic regeneration. However, recent studies from the laboratory of Doris Stoffers demonstrate that ngn3 immunopositivity is not detected during pancreas

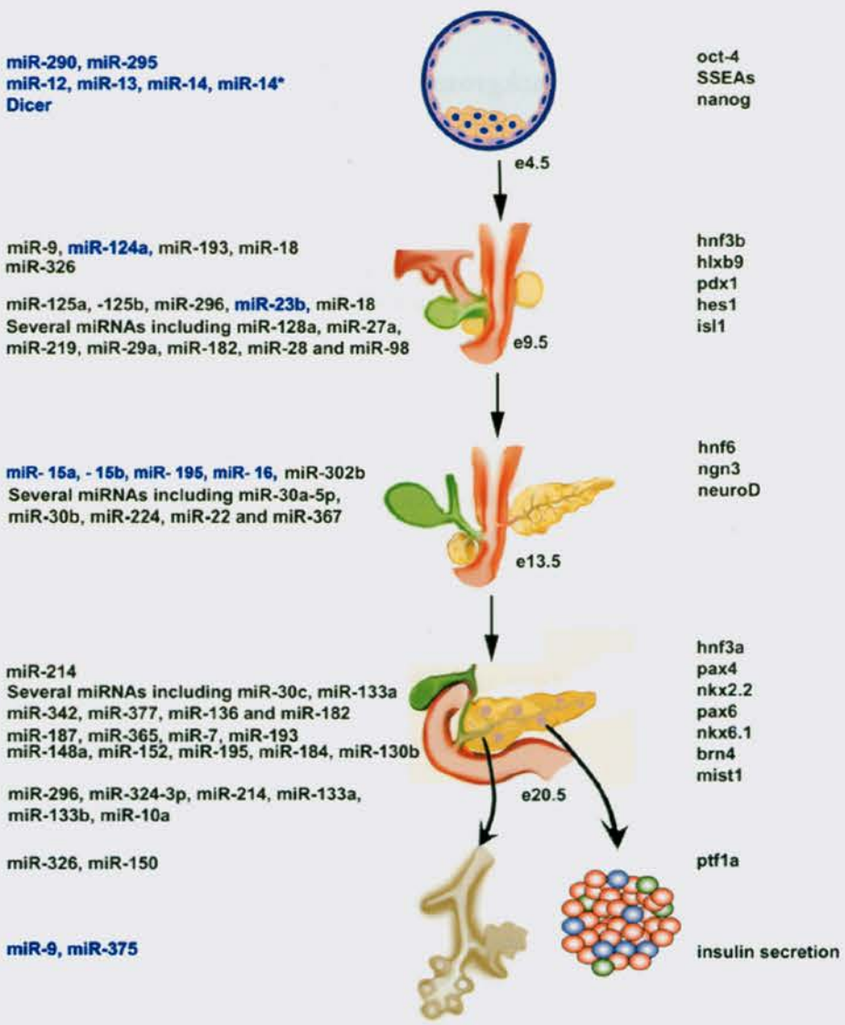


Fig. 1: MicroRNAs involved in pancreas development. Pictoral representation of pancreas development is presented in the central panel. Various transcription factors that are known to be involved in pancreas development are shown in the right panel. Bioinformatically predicted microRNAs that can bind to each transcription factor are shown in left panel in front of respective protein. The miRNAs shown in blue have been verified for their function.

regeneration. These investigators did not report transcript data for neurogenin3 during pancreas regeneration. We observe that ngn3 transcripts are detectable during development, post-natal life as well as during pancreatic regeneration following partial pancreatectomy. However, no immunopositive cells were visualized during regeneration, consistent with previous reports. We reasoned that ngn3 transcripts may be post-transcriptionally regulated. We looked at expression of microRNAs, a class of non-coding RNAs that are known to be important regulators of post-transcriptional gene expression. MicroRNAs (miRNAs) are approximately 22-nucleotides long, evolutionary conserved class of non-protein-coding RNA molecules. These are known to act by negatively regulating gene expression at the post-transcriptional level either by blocking translation through incomplete binding to the 3'UTR of their target mRNA or by directing degradation of the target mRNA. We carried out miRNA profiling of developing and regenerating pancreas to gain insights into mechanisms that regulate islet-cell regeneration. We find significant high expression of miRNAs targeting ngn3 (miR-15a, miR-15b, miR-16 and miR-195) during pancreas regeneration suggesting possible mechanism of post-transcriptional regulation. Further studies using anti-miRs and LNAs for miR-195 family miRNAs have contributed to our understanding of microRNAs that regulate pancreas development in mice (summarized in Fig. 1). These studies have helped us in identifying microRNAs that are specifically important in generation and function of the endocrine pancreas. Our studies indicate that though ngn3 protein is not produced during pancreas regeneration, there are no differences in expression of mature islet hormones or transcription factors. It appears that since insulin-producing cells in regenerating pancreas arise mainly from pre-existing β-cells, ngn3 mediated pathway of islet regeneration is inhibited by miRNA-mediated post-transcriptional regulation. These studies are now taken over to human fetal pancreas so as to understand if inhibition of these miRNAs may help in generation of ngn3-producing precursor cells.

On similar lines, we have been working on understanding the differentiation potential of human pancreatic progenitor cells. We observe that human fetal pancreatic islet cells can proliferate *in vitro* to produce mesenchymal-like cell populations. We believe that such pancreatic progenitor cells that are derived from hormone-producing islet cells are better precursors / candidates for differentiation since they have a promoter conformation that is necessary to achieve efficient gene transcription. Initial studies involved screening insulin promoter region in several human cell types (ES cells, umbilical cord blood MNCs, bone marrow mesenchymal cells, adipocytes, endothelial cells, hepatocytes, biliary epithelial cells, pancreatic islet cells) to understand their chromatin conformation. Of the several cell types studied in our laboratory, we found that human fetal islet-derived progenitor cells showed very high levels of H3K4-me2/3 as compared to H3K9-me2/3 or H3K27-me3. Since these histone modification are seen to be retained during *in vitro* expansion, we believe that human pancreatic islet-derived mesenchymal cells are better precursors. In the past few years there has been significant debate over understanding the proliferative potential of pancreatic beta cells. In order to confirm that insulin-producing

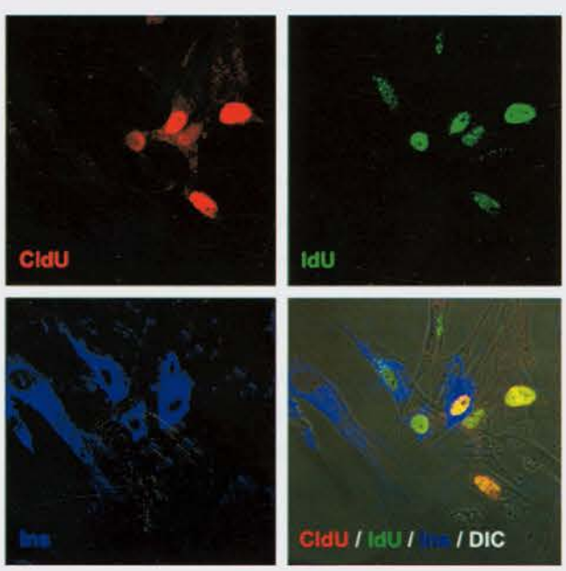


Fig 2: Lineage tracing of human fetal pancreatic beta cells using thymidine analogs. Freshly isolated human fetal pancreatic islets were labeled with CldU for 2 days in serum containing medium. This pulse phase is followed by a day of wash out, where no analog is present in the medium. The next pulse was given using IdU for 2 days. After this 5-day experiment, insulin producing cells were assessed for the incorporation of either or both of the analogs. Around 40% insulin positive cells co-expressed both CldU and IdU, demonstrating the in vitro proliferation ability of human fetal pancreatic beta cells.

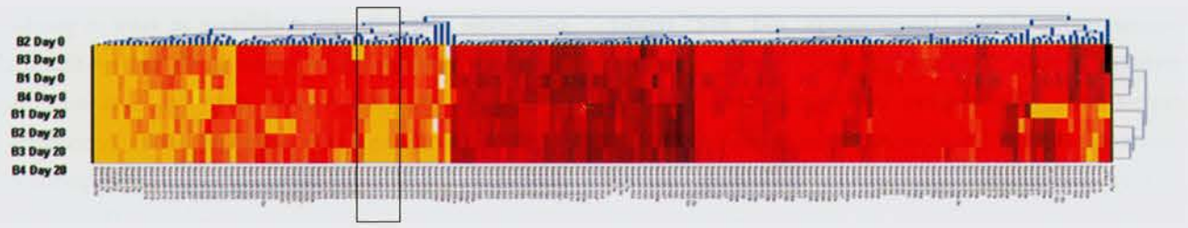
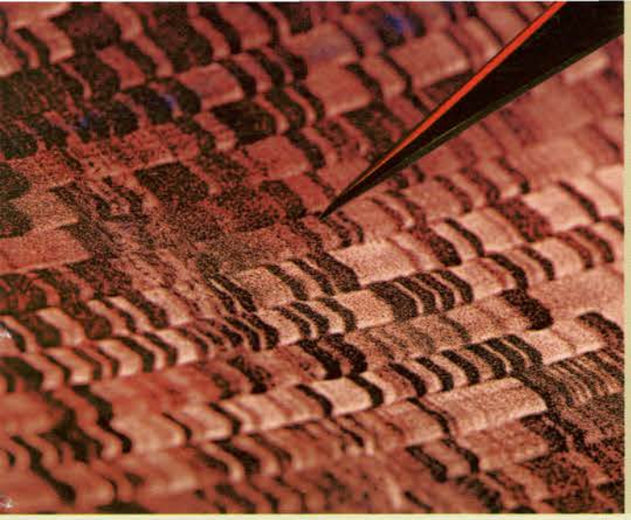


Fig. 3: Bidirectional cluster analysis of miRNAs during epithelial to mesenchymal transition. Profiling of microRNAs was carried out by TaqMan based real-time PCR for all miRNAs in version 10.1 (Sanger center). Normalized cycle threshold (Ct) values were taken into Matlab™ for bidirectional cluster analysis. Four different fetal islet preparations (B1, B2, B3 and B4) were taken for RNA isolation on day 0 or 20 days after in vitro expansion. Differentially expressed group of miRNAs involved in epithelial-to-mesenchymal transition are outlined. These are presently being tested and validated for their ability to induce mesenchymal to epithelial transition.

cells in human pancreatic islets proliferate *in vitro*, we took several approaches including immuno-staining, combined ICC-FISH, gene expression analysis in single cells and populations, clonal expansion analysis and lineage tracing using the thymidine analogs CldU and IdU. The use of such thymidine analogs is novel, well established and unbiased method of assessing progeny of cells during expansion. The method involves the use of 2 different thymidine analogs that can be detected independently using specific antibodies. Using this thymidine analog based labeling we observe around 40% of insulin-producing cells incorporate CldU and IdU in initial 5 days of *in vitro* expansion (Fig. 2). These and other studies demonstrate that insulin-producing cells within islets proliferate *in vitro*. Apart from this, we are also beginning to understand the role of microRNAs in epithelial to mesenchymal transition of fetal pancreatic islet cells. We compared miRNA profiles of pancreatic islets and mesenchymal cells generated from them (Fig. 3). Our studies demonstrate that miRNAs that show specificity to a unique sequence in 3'UTR of mesenchymal genes regulate epithelial phenotype in islet hormone producing cells.

Future Work

1. Understanding the growth or differentiation factors involved in differentiation of FIPCs.
2. Understanding insulin promoter conformation in various islet progenitor cells.
3. Assessing the functionality of differentiated FIPCs after transplantation in diabetic animals.



Research Reports

Biodiversity

Insect and Microbial Genomics

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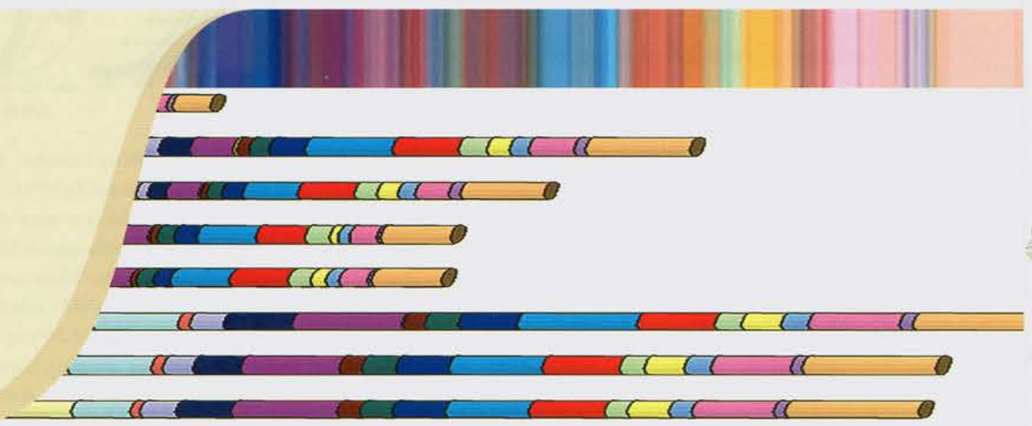

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Background

Microbial Genomics:

Prokaryotes outdo eukaryotes by several orders of magnitudes in terms of numbers as well as biomass. They are also reservoirs for the discovery of new drugs and metabolic processes. Despite this, we have very little information about their diversity due to inherent limitations of conventional methods to study them.

Use of 16S rRNA gene sequences as molecular chronometers, which was established by Carl Woese, enabled the studies of microbial phylogeny, taxonomy and ecology by overcoming the limitations of conventional methods. More recently whole genome sequencing and metagenomic analysis are becoming powerful and popular tools for such studies.

Our laboratory uses these methodologies to understand community structure and function of some unique ecological niches.

Insect Genomics:

Mosquitoes are vectors for diseases like malaria, filaria and many arboviruses. After completion of the human genome project, researchers worldwide have turned their attention to sequencing of the mosquito genome. The genome sequencing project of *Anopheles gambiae,* which is the vector for malaria in Africa, is already completed and *Aedes aegypti* is already completed. To complement this effort we have performed extensive EST (Expressed Sequence Tag) analysis of *Anopheles stephensi*, which is the vector of malaria in India.

Aims and Objectives:

1. To understand the "uncultured" microbial diversity with the long term aim of utilizing it for biotechnological purposes
2. To study the diversity and evolutionary relationships in different organisms
3. To generate Expressed Sequence Tags from uninfected and *Plasmodium*-infected mid-gut of female *Anopheles stephensi* mosquito

Work Achieved

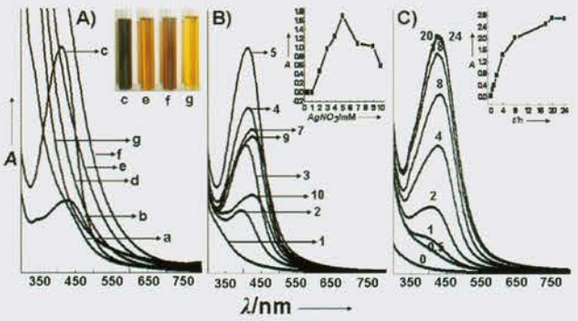
Microbial Genomics

Insect Mid-gut:

Invertebrates provide a rich habitat for micro-organisms. The interactions between invertebrate hosts and the microbes they harbor are numerous, and they play a key role both in host physiology and the ecosystem. Dipteran flies are one of the most abundant and important groups of insects which act as mechanical and / or biological vectors for various pathogenic agents. House flies (*Musca domestica* L.) are cosmopolitan, ubiquitous and synanthropic, and are known to be intimately associated with vertebrate pathogens. We have investigated bacterial communities associated with the gut of house flies using a culture-dependent and culture-independent approach. This was followed by a phylogenetic analysis to compare the communities.

Microbes isolated from the gut of insects from Western Ghat were screened to explore the possibility of extracting bioactive compounds. We have shown a bacterium, originally isolated from an insect gut, is capable of synthesizing extracellular silver nanoparticles. We have proposed a mechanism for the synthesis of nanoparticles by studying its resistance to silver cations (Fig 1a and b).

Microorganisms associated with different vectors could have several effects on the host including direct pathogencity, interference with reproduction or reduction of vector competence. Earlier, we had studied microbial flora associated with gut of *Culex quinquifasciatus*, we have now extended these studies to other species of mosquitoes including *Anopheles*



1a: A) UV/Vis spectra recorded from the culture supernatant that shows the production of AgNPs from *Morganella* sp. Curve a, b and c shows AgNPs production after the addition of aqueous AgNO3 solution to the overnight-grown *Morganella* sp. after 0 h, 1 h and 20 h of reaction, respectively. While curves d and e were recorded after the addition of aqueous AgNO3 solution only to the LB broth (without added NaCl) after 1 h and 20 h of reaction, respectively. Curve f was recorded (without diluting supernatant) after the addition of aqueous AgNO3 solution to the overnight-grown *Escherichia coli*. Curve g was recorded from the culture supernatant of *Morganella* sp. without added aqueous AgNO3 solution after 24 h of growth. Inset Figure shows images of color of solution of curves c, e, f and g. B) UV/Vis spectra that were recorded from culture supernatant from different concentrations of AgNO3 that were added to overnight-grown *Morganella* sp. The numbers given to the respective curves represent the concentration (mM) of the added aqueous AgNO3 solution. Inset graph shows the absorbance of AgNPs that were produced against respective concentrations of added aqueous AgNO3 solution. C) UV/Vis spectra showed the time-dependent kinetics of AgNPs production. The numbers over respective peaks indicate the time at which the spectrum was recorded. Inset graph shows the saturation curve of AgNPs production over a period of 24 h.

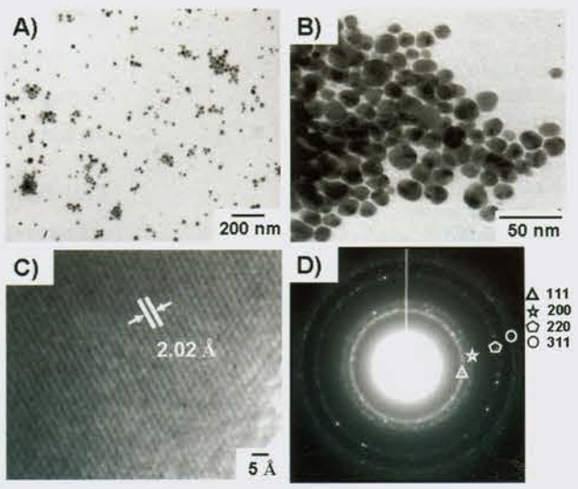


Fig. 1b: A) and B) Transmission electron microscopy images of AgNPs from *Morganella* sp. at different magnifications. C) HRTEM image showing characteristic d spacing for the [200] plane. D) Selected area electron diffraction showing the characteristic crystal planes of elemental silver.

stephensi and *Aedes aegypti*. Bacteria present in the midgut of *Aedes* mosquito could have important roles as determinants of survival and development of virus in mosquito, and therefore, can contribute to the modulation of vector competence. Libraries of PCR amplified 16S rDNA genes were constructed from the midgut of three different *Aedes aegypti* strains having different ability to transmit viruses, MOYO-R (wild refractory strain), MOYO-S (wild sensitive strain) and MOYO-D laboratory reared refractory strains.

Human Gut:

Microbes of human digestive tract are being investigated for their possible association with the development of obesity. Human gut mainly harbors two prominent bacterial phyla, bacteroidetes and firmicutes. Recent studies involving human subjects and mice have shown a changed proportion of the above bacterial phyla in guts from obese individuals helping them to harvest more energy from the food. Indian population differs from the western in terms of food habits and genetic make-up. Therefore, the focus of the study is; (1) to identify the gut microbial community in normal, obese and treated-obese individuals (patients undergoing gastric intervention treatments which limit patient's hunger by reducing effective gastric volume), (2) to investigate any possible significant difference in community composition at phyla to species level and to see whether there is a correlation between decreasing Body Mass Indices (BMI) (treated-obese individuals) and gut microbe dynamics, and (3) to compare the genomes of prominent species (if possible) in order to identify the diverse metabolic capabilities of prominent bacteria and correlate them with their differential presence (Fig 2).

Lonar Lake:

Lonar Crater Lake, Lonar (19°58_N, 76°31_E), is situated in the Buldhana district (Maharashtra, India) in the formerly volcanic, Deccan-Trap geological region. It is almost circular, with its longest and shortest diameters being 1875 m and 1787 m, respectively, with a raised rim of about 30 m and a depth of 135 m. Based on geological studies, it is postulated that the lake originated as a meteorite impact crater around 50 thousand years ago. It is the third largest crater in the world and the only known crater formed by meteoritic impact in basaltic rock. We had undertaken a study for the complete understanding of archeal and eubacterial diversity of this unique ecosystem. We have completed the assessment of diversity and the work has been published, we have currently undertaken a metagenomic analysis of this ecosystem.

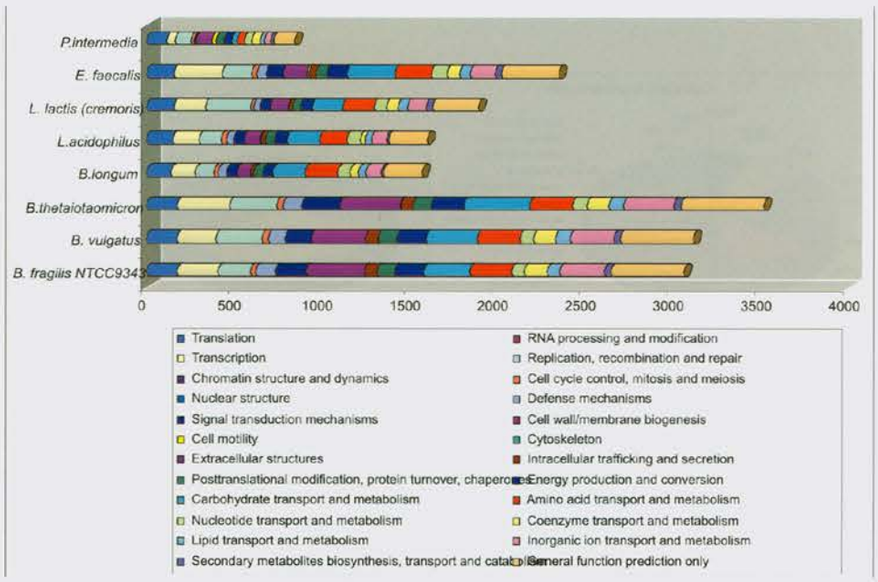


Fig. 2 : Classification of Proteomes in COG functional categories of bacteria present in human gut. The comparison shows bacteria verses number of proteins in give in given COG category (Y axis is number of proteins). Large number of proteins are involved in defence mechanism, lipid metabolism, carbohydrate metabolism and inorganic nutrient metabolism for genus Bacteroides

In order to construct metagenomic library samples were collected from Lonar Lake. Metagenomic library was prepared with expression vector (ZAP express vector) and Bacterial Artificial Chromosome vector using high quality DNA.

Insect Genomics

Malaria is one of the deadliest tropical diseases, affecting 200-300 million people worldwide with a mortality rate of ~1 million people every year. Controlling the spread of the disease demands an in-depth analysis of vector-parasite interaction at various levels. Availability of genomic sequence information from *Anopheles gambiae*, the African malarial vector, assisted researchers in designing novel anti-malarial strategies along with a better understanding of the interaction with the parasite. The spread of malaria in a particular area is determined by the presence of dominant anopheline mosquito. *Anopheles stephensi* is a major malarial vector in urban settings in the Indian subcontinent, but sparse genomic sequence information is available in various public databases. Thus, we carried out generation, analysis and annotation of expressed sequence tags (ESTs) from two cDNA libraries prepared from sugar-fed and *Plasmodium yeolii*-infected, blood-fed (post 24 h) adult female *Anopheles stephensi* midgut tissue.

We obtained 8,306 ESTs from the blood-fed infected library and 7,061 ESTs from the sugar-fed library. Clustering and assembly of ESTs from blood-fed infected library resulted in 591 contigs and 1355 singlets, thus giving 1946 unique transcripts. In the case of ESTs from sugar-fed library 822 contigs and 1818 singlets 2640 unique transcripts could be identified. Putative functions were assigned to the transcripts using BLASTX at NCBI. Transcripts showing no significant homology in the Blast search were scanned using ESTscan in order to predict UTRs. With Blast2GO analysis, GO terms were assigned to 56% and 32% unique transcripts obtained from blood-fed infected and sugar-fed *Anopheles stephensi*, respectively (Fig 3). All the transcripts were mapped on the *Anopheles gambiae* genome in order to find out the chromosomal location, numbers of exons and splice sites. We were successfully able to map approximately 1723 transcripts on *Anopheles gambiae* genome. We also identified 297 novel *A. stephensi* transcripts which were mapped to the unannotated regions in the *A. gambiae* genome. We also identified many novel *A. stephensi* ESTs that were not mapped on *A. gambiae* genome, indicating significant differences among these closely related malaria vectors.

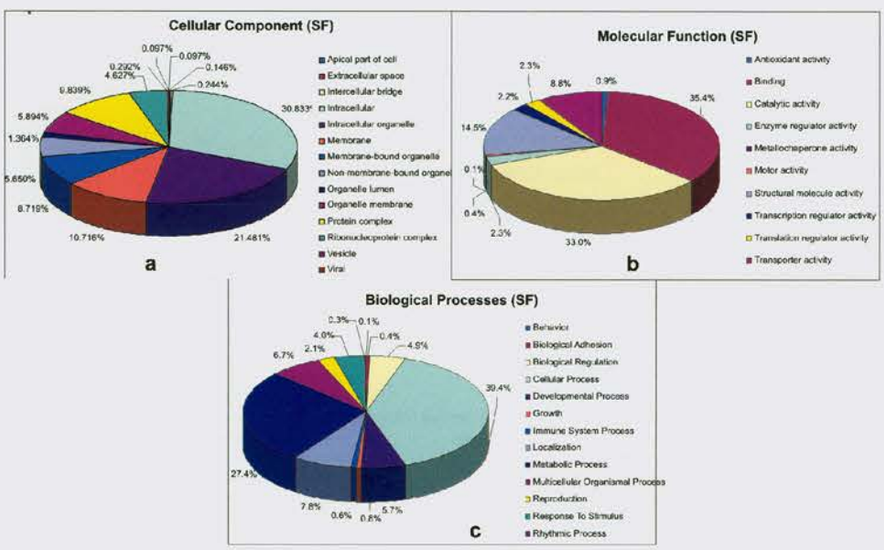


Figure1. Gene Ontology (GO) assignment (level 2 GO terms) of SF library ESTs based upon Cellular Component (a), Molecular function (b).and Biological processes (c)

Fig. 3: Gene Ontology (GO) assignment (level 2GO terms) of SF library ESTs based upon Cellular component (a), Molecular function (b), and biological process ©).

Future Work

1. Exploration of biotechnological potential of microbes present in insect gut using various approaches.
2. Studies on geographical and developmental variation in the gut flora of mosquitoes.
3. Studies on role of gut flora on disease carrying capacity of the mosquitoes.
4. Understanding of role of human gut microbes in health and disease.
5. Metagenomic analysis of Lonar lake and marine sediment / water for exploration of biotechnological potential in order to dissect the ecological structure.
6. Analysis and annotation of EST sequences from *Anopheles stephensi*.

Research Reports

Infection & Immunity

Studies on Cell Cycle & Schizogony in Plasmodium falciparum erythrocytic stages

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Background

The malarial parasite *Plasmodium falciparum* multiplies within human erythrocytes. The life-cycle of the parasite involves several discrete steps whose molecular controls are poorly understood. The process of cell division in Apicomplexa, a group of protests to which *Plasmodium falciparum* belongs, is very different from binary fission observed in most other eukaryotes. Daughter cells are assembled within mother cells, using so-called 'inner membrane complex". Cell cycle control elements are presumably involved not only in cell multiplication processes themselves, e.g. during schizogony, but also in transitions between developmental stages in their proliferation status. A clear correlation between the G1, S, G2 and M phases of the typical eukaryotic cell cycle and those of the Plasmodium parasite is yet to be established. The eukaryotic cell cycle is regulated by a group of highly conserved cyclin dependent protein kinases (CDKs). Though several CDKs have been identified in malaria parasites, their regulatory mechanisms as well as their role in parasite growth and differentiation are not understood fully. The biology of parasites has many unusual facets that might be exploited for drug design. The cell cycle of the parasites shows important structural and functional differences with mammalian cell cycle control machineries and signal transduction pathways which might be utilized for rational drug design. Potential targets include protein kinases from cyclin-dependent kinase, cAMP-dependent kinase and mitogen activated kinase families.

This project aims at understanding the role and effect of different cell cycle regulators and schizogony (merozoites formation) in *P.falciparum* erythrocytic stages.

Aims and Objectives

1. Stage specific effect of various cyclin inhibitors on transformation (regulation of growth and development) of erythrocytic stages (ring, trophozoite and schizont) of *P.falciparum* malaria parasite. CDKs-regulatory mechanisms as well as their role in parasite growth and differentiation.
2. Studies on Schizogony - merozoites formation
3. Gametocytes formation

Work Achieved

Studies on Schizogony: - The studies on schizogony (merozoite formation) has revealed that the merozoites infectivity to the erythrocytes (invasion assay) is less than 30% and the remaining 70% merozoites do not infect (Table.1). This may be due to defective merozoites and this is not dependent on RBC blood group. The preliminary results indicates that the infectivity of merozoites decline with age of erythrocytes. We have observed merozoites formation in single and multiple (2, 3 & 4) parasite infected erythrocytes. The number of merozoites formed by the parasites varies with multiple parasite infection (Fig.1). The multiple infection of RBC by malaria parasite increased with increased parasitemia (Table 2). However the multiple infections decreased with increasing hematocrit level.

Future Work

1. Infectivity of merozoites formed in the multiple parasites infected RBCs.
2. Effect of different CDKs on transformation as well as development of erythrocytic stages of the malaria parasite.

Table 1. Efficacy of Merozoites : Invasion into erythrocytes.

Parasitemia (schizonts)	2.3 %
Merozoites/schizonts (n=10)	16.30 %
Parasitemia (rings) after invasion	8.70 %
Percent Merozoites successful invasion	23.51 %
Expected Parasitemia (rings) after invasion	37.49 %

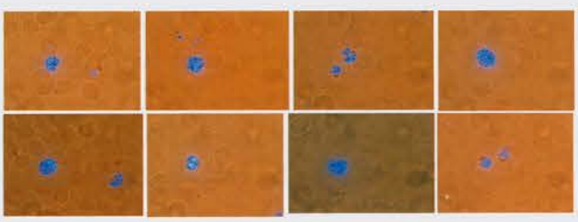

Fig. 1: DAPI stained malaria parasites. Different number of merozoites formation in schizonts in multiple parasite infected erythrocytes

Table 2. *Parasitemia v/s multiple infections in P.falciparum (3D7) in vitro cultures*

	% parasites	% multiple parasites in a RBC			
	parasites /rbc :	I	II	III	IV
1.	5.68	5.05	0.63	0.00	0.00
2	10.12	6.57	1.77	1.24	0.53
3	18.48	12.26	2.68	2.18	1.34

Signaling requirements for differentiation of CD8$^+$ T-cells and memory generation

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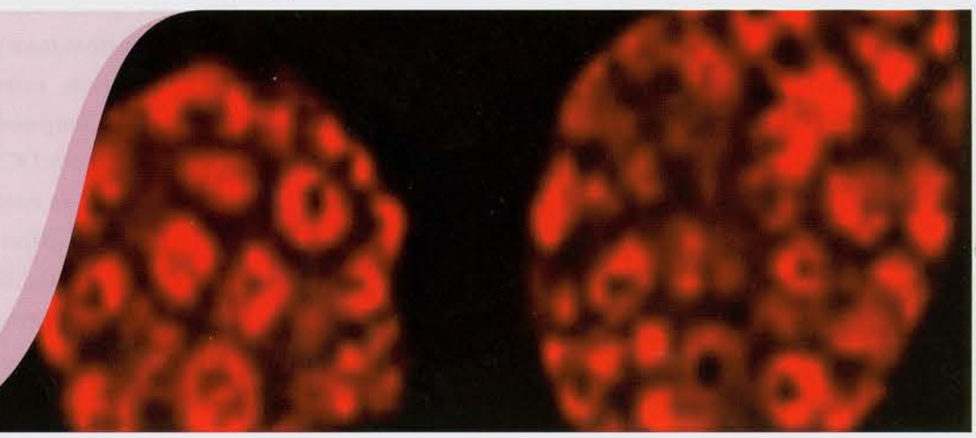

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Background

Dengue disease is a serious health problem all over the world. The hallmark of the clinical features associated with dengue infection is manifestation of dengue fever or life threatening syndromes like hemorrhagic fever and shock syndrome. These symptoms are consequence of the immune response to the virus rather than the virus induced cytopathology. Dengue virus (DV) primarily targets dendritic cell via DC-SIGN receptor to establish the infection.

In the previous study, we have shown that DV targets both monocyte-derived dendritic cells 1 (DC1) and plasmacytoid-derived dendritic cells 2 (DC2) to set up the infection. DV infected DC1 could enhance the activation of CD4$^+$ T cells; while DV infected DC2 could suppress CD4$^+$ T cells. However, infected DCs did not have any effect on CD8$^+$ T cells. Thus, the stimulatory ability of infected DC1 and suppressive property of infected DC2 was restricted to CD4$^+$ T cells.

To further validate the differential property of infected DCs, we performed *in vivo* experiments, in which, C57BL/6 mice were injected i.v. with mock or infected DC1 or DC2. After 25 days, splenocytes from immunized mice were harvested and re-challenged *in vitro* with DV2 or DV3 for proliferation assay. The role of DV-DC1 and DV-DC2 priming on Tcell activation and differentiation may provide important information in understanding the pathogenesis of DV infection.

Aims and Objectives

1. To understand the molecular mechanism of $CD4^+$ and $CD8^+$ T-cell activation by dengue virus infected DC1 and DC2
2. Differentiation *in vitro* and *in vivo* of $CD4^+$ and $CD8^+$ T-cells by dengue virus infected DC1 and DC2.

Work Achieved

The *in vivo* results showed that priming with infected DC1 induced a strong response in the spleen as seen by increased proliferation of splenocytes *ex vivo* when cultured in the absence of a second stimulus (Figs. 1A and B). Interestingly, re-challenge with DV2 resulted in a drastic reduction in splenocyte proliferation. To ascertain whether the observed phenomenon was also seen with heterologous serotype, we re-challenged splenocytes with DV3 and observed a similar inhibitory effect on proliferation of splenocytes. Importantly, splenocytes from mice primed by infected DC2 showed no proliferation, instead the cultures showed lower counts when compared to cultures from mice that were primed with mock DC2. There was no effect when cells were stimulated *in vitro* with both DV2 and DV3. (Fig. 1B). Thus, the effect of priming mice with infected DC1 and DC2 was different and re-challenge with virus *ex vivo* had a suppressive effect on the DC1 induced splenocytes proliferation.

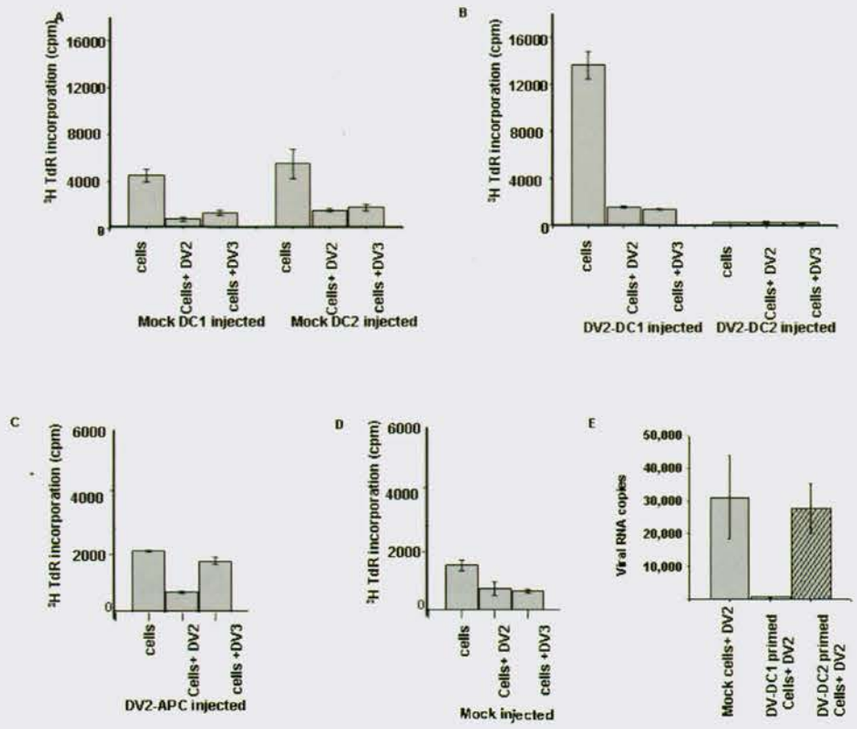

Fig. 1: *In vivo* priming with infected DC1 induces strong primary response, while priming with infected DC2 shows no sensitization. C57BL/6 mice were injected i.v. with mock (A) or infected DC1 or DC2 (3 x 10^5 cells/mice, B). After 25 days, splenocytes from immunized mice were harvested and pooled together, and 10^6 cells were re-challenged in vitro with DV2 or DV3. Cells were cultured for further 72 h and cell proliferation was assayed by ^{3}[H] TdR incorporation (n = 5 mice per group). (C) C57BL/6 mice were injected i.v. with mock or infected APCs (3 x 10^5). After 25 days, splenocytes from immunized mice were harvested and pooled together, and 10^6 cells were re-challenged *in vitro* with DV2 or DV3. Cells were cultured for further 72 h and cell proliferation was assayed by ^{3}[H] TdR incorporation. (D) C57BL/6 mice were mock injected (inactivated DV) and after 25 days splenocytes were re-challenged with DV2 or DV3. Bar represents variability within the wells. E) Viral RNA copies in the re-challenged splenocytes from mock and infected DC1 and DC2 primed mice. Results are representative of four independent experiments.

In order to distinguish the effect on immune response by DV infected DC1 or DC2 from MHC-II positive spleen APCs, we performed the same experiment with DV2 infected APCs from splenocytes. Our results demonstrated that there was 7-fold decrease in proliferation of splenocytes primed with infected APCs as compared to priming with infected DC1. However, there was a 10 fold increase in proliferation of splenocytes when compared with priming with infected DC2. Re-challenge with DV2 decreased the proliferation of splenocytes in APC primed mice (Fig. 1C). DV3 re-challenge showed less suppressive effect on splenocytes as compared to DV2. To check the effect of inactivated virus itself on the proliferation of splenocytes we injected mice with inactivated DV2 and re-challenged with DV2 or DV3. It was found that inactivated DV2 did not induce proliferation of splenocytes (Fig. 1D). These results suggest the differential properties of infected DC1 and DC2 in regulating the primary immune response. To ascertain the effect of enhanced proliferation of splenocytes by infected DC1 and the suppression of splenocytes by infected DC2 on dengue virus replication, we estimated viral RNA copies in splenocytes with or without virus addition. It was found that re-challenge of splenocytes of DV-DC1 primed mice drastically decreased the viral RNA copies as compared to splenocytes from mock DCs. However, there was no difference in the viral RNA copies in re-challenged splenocytes of DV-DC2 primed mice as compared to mock DCs (Fig. 1E). The splenocytes from primed mice without virus addition did not show viral RNA copies (data not shown).

Since infected DC1 showed strong primary immune response we analyzed splenocytes cultures for the Th1 and Th2 cytokine profile in the supernatant of infected DC1 and DC2 primed cultures. The infected DC1 priming induced high IFN-γ, TNF-α and IL-2 production which play important role in the generation of effector cells. We found that there was a decrease in IFN-γ, TNF-α and IL-2 levels by DV2 re-challenge (Table I). Although IL-4 production was not high in the culture supernatant, DV2 re-challenge further decreased its level. Splenocytes of infected DC2 primed mice did not show any cytokine production.

	DV2-DC1 primed		DV2-DC2 primed	
	Cells	Cells + DV2	Cells	Cells + DV2
IL-2 (pg/ml)	259.5 ± 78	192.5 ± 38.5	U. D.	U. D.
IFN-γ (pg/ml)	849.7 ± 142.6	601.4 ± 24	U. D.	U. D.
TNF-α (pg/ml)	290. 5 ± 94.3	147. 4 ± 26.9	U. D.	U. D.
IL-4 (pg/ml)	54.3 ± 14.6	20.3 ± 3.6	U. D.	U. D.

Table 1. C57BL/6 mice were injected i.v. with infected DC1 or DC2 (3×10^5 cells/mice). After 25 days, splenocytes were harvested and 10^6 cells were re-challenged in vitro with DV2 at 1 MOI. Cells were cultured for further 72 h and analyzed for Th1 and Th2 cytokines estimation by FACS using CBA kit. n = 5 mice per group. U.D.= undetected (below 10 pg/ml).

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), which is defined by a reduction in the number of $CD4^+$ T cells (less than 200 cells/ µl) and the onset of opportunistic infections. The incidence of HIV infection has reached alarmingly high levels worldwide including India. The therapeutic strategies 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, related to viral pathogenesis, 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 us to new antiviral strategies.

Aims and Objectives

1. Role of viral regulatory proteins Tat and Nef in HIV pathogenesis, and differential gene expression studies in HIV-1 infected cells.
2. CD40 CD40L signaling in HIV infection.
3. Identification of novel molecules with anti-HIV activity from plant source.

Work Achieved

Role of viral regulatory proteins Tat and Nef in HIV pathogenesis, and differential gene expression studies in HIV-1 infected cells

The Human Immunodeficiency Virus Type 1 encodes a 27 kDa protein, Nef, which has come a long way from being termed as a negative factor to being one of the most important proteins of HIV-1. However, its role in HIV-1 replication and gene expression remains to be clearly understood. Although involvement of heat shock proteins in viral pathogenesis has been reported earlier, a clear understanding of their role remains to be elucidated. We have shown earlier that Nef not only interacts with the heat shock protein 40 (Hsp40) but also induces the expression of Hsp40 in HIV-1 infected cells. The interaction between Nef and Hsp40 is important for increased Hsp40 translocation into the nucleus of infected cells, which seems to facilitate viral gene expression by becoming a part of the cyclin dependent kinase 9 (cdk9) associated transcription complex regulating long terminal repeat (LTR) mediated gene expression. As Hsp40 is normally associated with Hsp70, we have also studied the role of Hsp70 in HIV pathogenesis. Our results now show that Hsp70 is also present in the Nef-Hsp40 complex reported earlier. Furthermore, Hsp70 over expression inhibits viral gene expression whereas its down regulation leads to increase in viral gene expression and replication. Taken together, Hsp40 and Hsp70 proteins, which are closely associated with each other in their chaperone function, seem to act contrary to each other in regulating viral gene expression. It seems that Hsp70 acts as an antiviral factor whereas Hsp40 works as a pro-viral factor. In addition, we have initiated studies on the regulation of HSP40 gene expression during HIV infection and our results indicate that Heat shock factor -1 plays an important role in this regulation.

We have earlier shown a direct interaction of Tat with nuclear factor kappa B (NFκB) enhancer, a global regulatory sequence for many cellular genes both *in vitro* and *in vivo*. As Tat is known to modulate expression of many cellular genes in the infected cells and this interaction could be the molecular basis for such modulation. Thus we have now initiated studies on the global recruitment of the Tat protein in the infected cell chromatin and are also trying to identify the molecular mechanism of Tat mediated modulation of selected genes. Using differential gene expression studies we have identified several Tat modulated genes a few of which are being studied to identify the molecular mechanism of transcriptional regulation by Tat.

Studies done till date to elucidate the pathways involved in HIV-1 induced T cell depletion have 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. We have previously shown specific down regulation of the complex I subunit NDUFA6 with simultaneous impairment of mitochondrial complex I activity in HIV infection. We have been looking in to the role of other complexes of the oxidative phosphorylation system in HIV induced T cell apoptosis and our recent results show modulation of several components of the system during HIV induced T cell apoptosis.

CD40-CD40L signaling in HIV infection

The nature of interaction between HIV-1 and the immune system is complex and the mechanism of T cell response and cytokine induction to restrict the infection is not well understood. In early stage of infection HIV infected individuals have high frequency of HIV-1 specific CTLs, which play an effective role in reducing the viral load, however, in later stage of infection or as the infection progresses these CTLs are unable to clear the infection. Dysregulation of cytokines that are induced due to interaction of HIV-1 specific T cells with APC is one of the possible causes of CTL dysfunction. IL-12 is secreted from APCs and acts on T cells and NKT cells to induce IFNγ from these cells. Peripheral blood mononuclear cells (PBMC) from HIV infected patients, asymptomatic or with acquired immunodeficiency disease produced less IL-12 than PBMC from uninfected healthy donor. We have now studied the role of IL-12 in CTL dysfunction by using DNA immunization with HIV-1 gp120 antigen in murine system. Immunization of wild type (WT) and IL-12 deficient mice with gp120 resulted in impaired

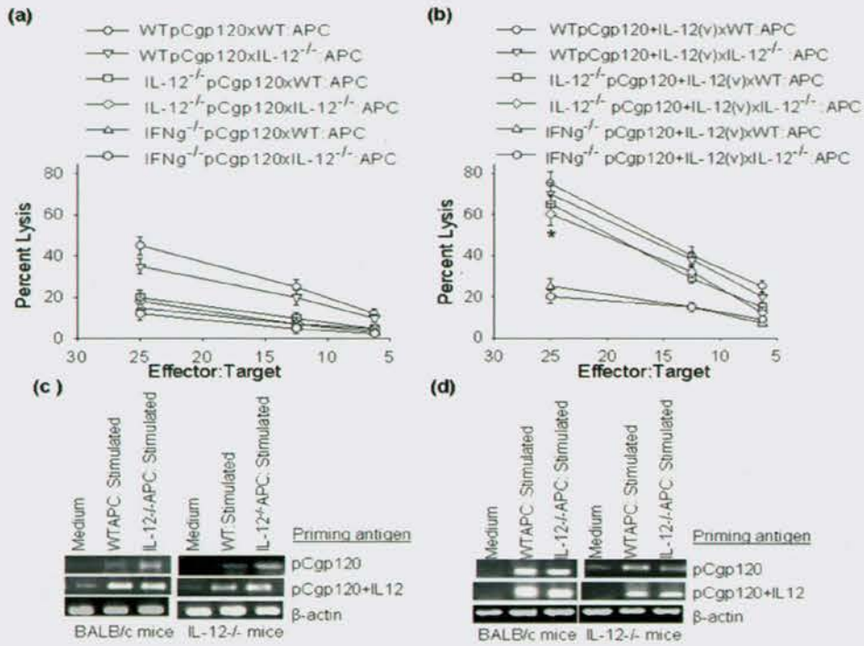

Fig. 1: Antigen presenting cell derived IL-12 is not required for restimulaion but IFNγ is necessary for CTL response. BALB/c, IL-12 and IFNγ deficient mice were immunized with pCgp120 and pCgp120+ Il-12 vector. Splenocytes from pCgp120 and pCgp120+ IL-12 injected WT, IL-12, IFN mice were plated $2x10^6$ per well in 24 well plate with gp120 peptide pulsed $1x10^6$ irradiated naïve WT or IL-12-/- splenocytes. After 5 days of culture, cells were harvested and plated against ^{3}(H) thymidine-incorporated gp120 pulsed p815 cells and tested for their cytolytic activity in standard $3_{1/2}$ hours JAM test. The Effector:Target ratio used are shown in the figure. Each data point is the mean of triplicate samples. The results represent three individual experiments and error bars represent the mean ±SD of a given group. The results obtained using WT and IL12-/- APC are not statistically significant as indicated by $p>0.05$ where as the data obtained in co-immunization with IL-12 is statistically significant as indicated by * $p<0.05$. (a) gp120 specific CTL response in pCgp120 immunized WT, IL-12 and IFNγ deficient mice stimulated with WT or IL-12$^{-/-}$ APCs. (b) gp120 specific CTL response in pCgp120 or pCgp120 + IL-12 immunized WT, IL-12 and IFN deficient mice stimulated with WT or IL-12-/- mice. (c) RT-PCR for IFNγ was performed with RNA isolated from the stimulated cells using gene specific primers. (d) RT-PCR for perforin was performed with RNA isolated from the stimulated cells using gene specific primers.

CTL response in IL-12 deficient mice as compared to WT mice. Our results further demonstrate that co-immunization with IL-12 vector restores the impaired CTL response in IL-12 deficient mice immunized with gp120. However, immunization with IL-12 vector fails to rescue the CTL response in IFNγ deficient mice (Fig-1). This finding suggests a phase specific role of IL-12 in CTL response, specifically in priming of $CD4^+$ T cells that provide help to $CD8^+$ T cells. Our results also suggest that IL-12 is vital for priming of antigen specific T cells and plays an essential role in induction of IFNγ from T cells. We have also initiated studies on CD40 and CD40 ligand expression in HIV-1 infected population and signaling mediated by CD40 during HIV 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 from natural resources. Our earlier studies have resulted in identification, characterization and synthesis of a novel reverse transcriptase inhibitor from Black Clam (Fig-2). We are also working along with NIPER (Mohali, India) for screening of compounds isolated from medicinal plant extracts and new synthetic compounds for identification of potential microbicides. More than two hundred extracts, fractions, isolated and synthetic compounds have been screened till date and a number of new molecules showing anti-HIV activity have been identified. Further characterization of the activity is in progress.

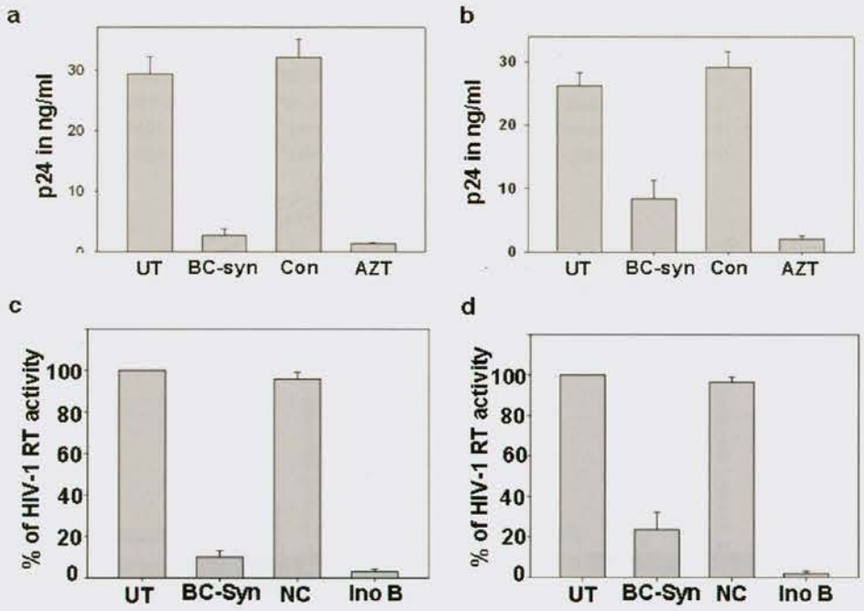

Fig. 2: Anti-HIV activity and reverse transcriptase inhibitory activity of the novel molecule BC-syn. CEM-GFP, a human CD4+ T cell line was infected with 0.1 MOI of NL4.3 and IIIB virus isolates and the cells were incubated with BC-syn for 7 days in CO_2 incubator. After incubation, the supernatants were analyzed for virus production using HIV-1 p24 antigen capture ELISA. Azt was used as a positive control and a structural analog of BC-syn was used as a negative control. (a) CEM-GFP cells infected with HIV-1 NL4.3 virus isolate. (b) CEM-GFP cells infected with HIV-1 IIIB isolate. Reverse transcriptase assay was performed with HIV-1 NL4.3 (subtype-B) and HIV-1 Indie-C (subtype-C) Reverse transcriptase in vitro in presence off BC-syn using the colorimetric kit of Roche, Germany. Inophyllum-B was used as a positive control. (c) NL4.3 reverse transcriptase; (d) Indie-C reverse transcriptase.

Future Work

Our results indicate that heat shock proteins, specifically HSP40 and HSP70 play an important role during HIV-1 infection. We are now trying to elucidate the role of all the other heat shock proteins in HIV pathogenesis, which will provide us a comprehensive knowledge about the role of HSPs in HIV infection and pathogenesis. We are also continuing characterization of some of the other Nef interacting clones identified by yeast two hybrid systems and identifying their functional relevance in HIV lifecycle. Furthermore, we are studying the recruitment of Tat protein on the chromatin during HIV infection, both in acute and latently infected cells, that might lead to the elucidation of the mechanism of Tat mediated regulation of both vital 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 in infected cells. We have also initiated studies on the role of CD40 and CD40L in HIV infection with emphasis on the study of CD40 mediated signaling in HIV infection. Finally, studies are in progress to identify novel anti-HIV molecules, both from natural resources and synthetic chemistry, with the objective to identify novel lead molecules with potential for use as anti-HIV microbicides.

Host-Pathogen Interactions

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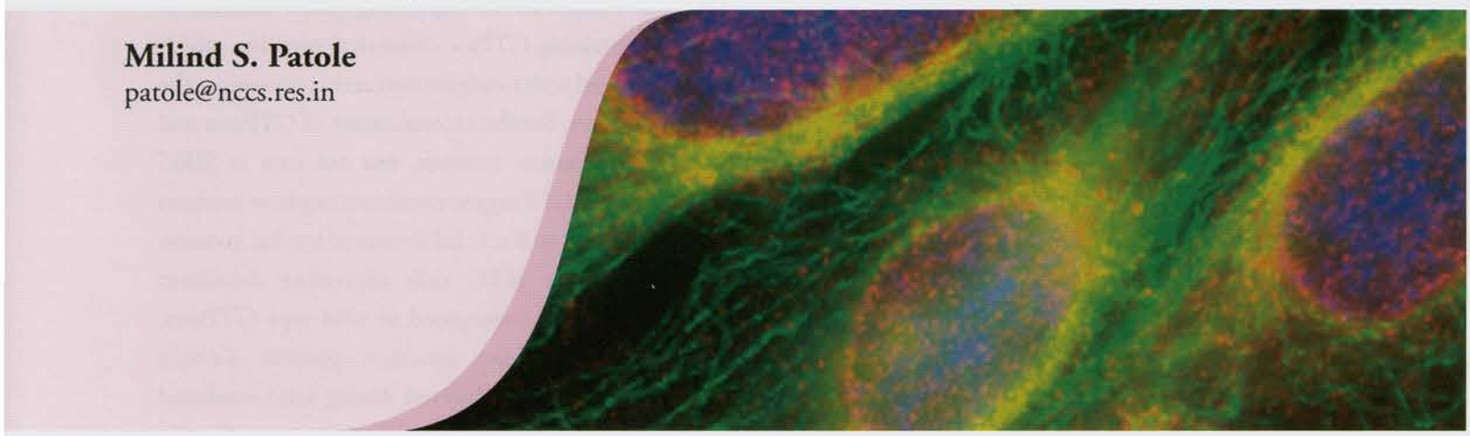

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Background

The kinetoplastid protozoa cause many diseases of medical and economic importance in hosts ranging from invertebrates to plants and mammals. They are the earliest-branching organisms in eukaryotic evolution to have either mitochondria or peroxisome-like microbodies. Protein trafficking in kinetoplastids is in many respects similar to that in higher eukaryotes, including mammals and yeasts. Search of different peroxisomal targeting signal-2 (PTS2) is an ongoing process and with bioinformatics tool new topogenic signals have been discovered from genome sequences of plants, yeast and human. Although kinetoplastids are important human parasites, the information related to PTS2 sequences is inadequate. Therefore in this study, attempt has been made to verify different PTS2 sequences from known glycosomal enzymes and their ability to target reporter proteins to glycosomes and mammalian peroxisomes.

Another pathogen which we are interested in is *Candida albicans*, a diploid fungus that causes opportunistic oral and genital infections in humans. *C. albicans* adhesins were shown to bind specifically to N- and E-cadherin present on membranes of endothelial and oral epithelial cells, respectively, which is believed to be responsible for actin-mediated endocytosis of the organism. It would be interesting to find out the sequence of events that leads to cytoskeletal organization after binding of *C. albicans* to its receptors like E-cadherin. It would be interesting to study the site of actin polymerization during invasion of epithelial cells by *C. albicans* and involvement of small GTPase family members and other cytoskeletal interacting proteins in the invasion process.

Aims and Objectives

1. To elucidate functionality of the kinetoplastid PTS2 in mammalian cells

2. To study the role of actin remodeling and involvement of small GTPases during the invasion of *Candida albicans*

Work Achieved

Functionality of kinetoplastid PTS2 in mammalian cells

The glycolytic enzyme hexokinase in the *Leishmania donovani* promastigote and amastigote stages co-localizes exclusively to the glycosome. Trafficking of hexokinase to this subcellular organelle is dependent on an N-terminal topogenic signal designated as peroxisomal targeting signal-2 [PTS2]. Fusion of GFP to C-terminus of hexokinase [Hex-GFP] or addition of the first 12 amino acids of *Leishmania* hexokinase, which contain a PTS2 domain, [PTS2-GFP] was sufficient to target this reporter protein to glycosome in promastigote and amastigote life cycle stages. Deletion of the PTS2 sequence resulted in targeting of the GFP reporter to the cytoplasmic compartment. Transfection of the Chinese Hamster Ovary [CHO] cell line with an EGFP construct containing the *Leishmania* hexokinase PTS2 failed to target this reporter protein to the peroxisome. In contrast, EGFP tagged with the human thiolase PTS2 lead to complete trafficking of the reporter to the peroxisome. Also other kinetoplastid PTS2 sequences and EGFP fusion constructs were mislocalized to cytoplasm in CHO cell line. These results suggest that *Leishmania* and human PTS2 motifs and possibly their receptors may not be identical. The studies in this line will help to strengthen the notion of developing drug discovery programme for glycosome biogenesis in kinetoplastids including *Leishmania*.

Association of small Rho GTPases during the invasion by *Candida albicans*

Actin remodeling was assessed in corneal epithelial cell line SIRC after *C. albicans* invasion and it was seen that newly formed actin filaments created a ring like rigid structure at the site of hyphal invasion. During the invasion, a marked increase in the mRNA content of Cdc42, Rac1 and RhoA members of small Rho GTPase family was observed in SIRC cells. Immunochemical staining showed that all these three GTPases colocalize at the site of actin polymerization and hyphal invasion. This was confirmed by expressing green fluorescent protein containing GTPase chimerical proteins, which also colocalized with newly formed actin polymers at the hyphal invasion. Similar colocalization of GTPases and actin polymerization however, was not seen in SIRC cells expressing GFP tagged dominant negative mutants of RhoA, Cdc42 or Rac1. Inhibition of hyphal invasion was observed in SIRC cells expressing dominant negative mutants as compared to wild type GTPases. Involvement of tight junction protein Zonula Occludens-1 was also observed during actin mediated endocytosis of *C. albicans*. Colocalization of actin filaments, GTPases and ZO-1 was also observed in the epithelial cells during the uptake of polymethylmethacrylate beads coated with spent medium from *C. albicans* culture. The results indicate that in host cells, actin remodeling and recruitment of small GTPases occurs at the site of *C. albicans* invasion and molecule(s) that is secreted or released by *C. albicans* is probably responsible for such cytoskeletal reorganization.

Future Work

1. To study interaction of PTS2 sequences from Leishmania with its receptors from human and Leishmanial cells.

2. To study different peroxins from *Leishmania* in glycosome biogenesis.

3. To characterize the secretory molecules responsible for host cytoskeleton reorganization during invasion of *C. albicans* using bead uptake studies

CD40 signaling in the regulation of immune response

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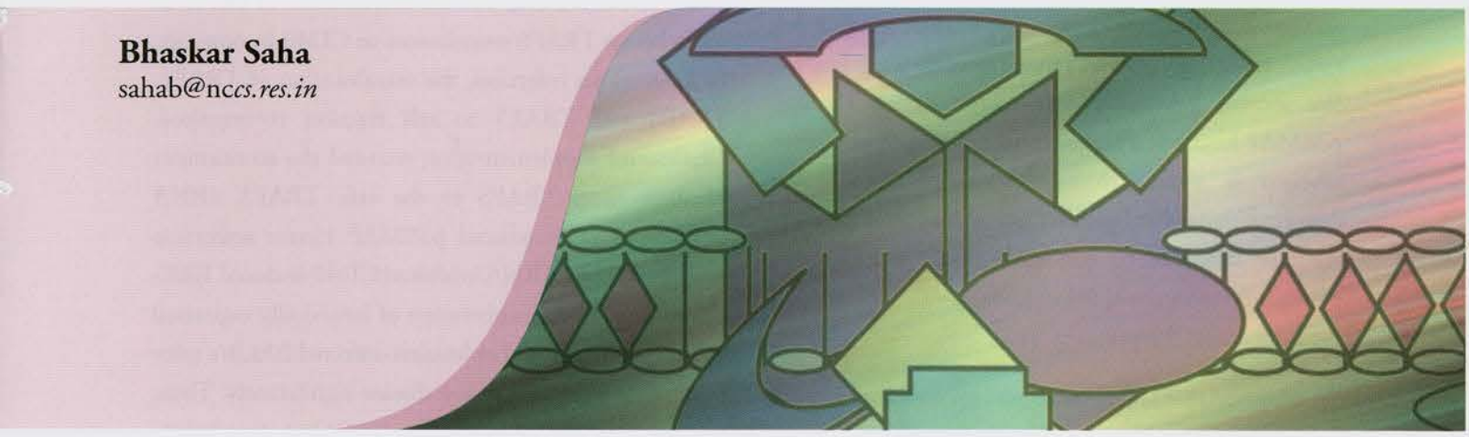

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Background

Immune responses to auto-antigens, allo-antigens, pathogens and tumor antigens are regulated by activation of T-cells. It has been shown that the interaction between T-cell-expressed CD40-ligand (CD40-L) and antigen presenting cell-expressed CD40 plays a crucial role in T-cell activation. CD40-L- or CD40-deficient mice are susceptible to tumor growth and infection by pathogens. CD40-based therapies are also shown to control tumor growth and infection significantly. On the other hand, blockade of CD40-CD40-L interaction prevents allograft rejection and autoimmune reactions. We have shown earlier that CD40 signaling in macrophages triggers counteractive effector functions. However, how a single receptor triggers such contrasting reactions is not known. Because effector functions are the results of signaling, we studied whether CD40 initiates reciprocal signaling and the mechanism of initiation of such signaling. In addition, we aimed at deciphering how *Leishmania* may switch the CD40 signaling from p38MAP kinase to ERK-1/2 pathway.

Work Achieved

We demonstrate that CD40 remains in the detergent-soluble membrane domain at low dose of CD40 stimulation whereas with increasing CD40 ligation, the receptor moves to the detergent-resistant membrane domain called lipid raft. When CD40 signals from raft, it activates predominantly p38MAP kinase whereas signaling from the non-raft domain results in ERK-1/2 activation. As shown previously, ERK-1/2 activation leads to primarily IL-10 production and exaggerated *Leishmania* infection whereas p38MAP kinase activation

results in IL-12-dependent protection from the infection.

Macrophages express CD40 and in response to CD40-ligand binding produce both IL-10 and IL-12. While the former aggravates *Leishmania* infection, the latter ameliorates the infection. How such contrasting responses are regulated by a single receptor is not known. We showed previously that CD40 signals through p38MAP kinase at a higher concentration of its ligands whereas it signals through ERK-1/2 at a lower concentrations of its ligands. How activation of the same receptor can lead to p38MAP kinase or ERK-1/2 phosphorylation remained unknown. We have studied the mechanism this year.

We observed that CD40 molecules on mouse peritoneal macrophages are distributed in the non-raft fractions. Upon increasing concentrations of the ligand- either an agonistic antibody or recombinant ligand- CD40 moves into the raft fractions (Figure 1). Depletion of cholesterol by β-methyl cyclodextrin but not by its inactive isomer -cyclodextrin- and *Leishmania* infection impeded the CD40 relocation. Cholesterol supplementation to the -methyl cyclodextrin-treated or *Leishmania*-infected macrophages restored CD40 relocation to raft. The *Leishmania*-infected macrophages had reduced cholesterol. The reduction of cholesterol is at least partially due to reduced HMGCoA reductase expression in these macrophages.

Because CD40 cytoplasmic domain does not have any kinase activity, it signals via recruitment of TRAFs (Tumor necrosis factor alpha receptor-associated factors). Analysis of the distribution of the six TRAFs in the raft and non-raft membrane fractions shows TRAF2, TRAF3 and TRAF5 recruitment to CD40 in raft whereas TRAF6 recruitment to CD40 in non-raft. In Leishmania infection, the translocation of TRAF2, TRAF3 and TRAF5 to raft fraction is impaired. Cholesterol supplementation restored the recruitment of these three TRAFS to the raft. TRAF3 siRNA inhibited CD40-induced p38MAP kinase activation whereas TRAF6 siRNA inhibited CD40-induced ERK-1/2 activation. Administration of lentivirally expressed TRAF6 shRNA into Leishmania-infected BALB/c mice resulted in reduction of the disease significantly. Thus, we concluded that at lower doses of CD40 stimulation, the receptor remains primarily in non-raft fractions but at higher doses, it moves to raft fractions. In these two domains, CD40 assembles constitutionally and functionally different signalosomes that trigger the reciprocal activation of p38MAP kinase or ERK-1/2, as shown in the model here.

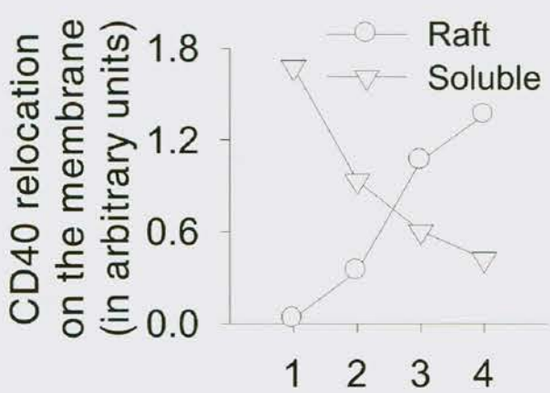

Fig.1: With increasing concentration of CD40 ligation, the receptor moves into the raft.

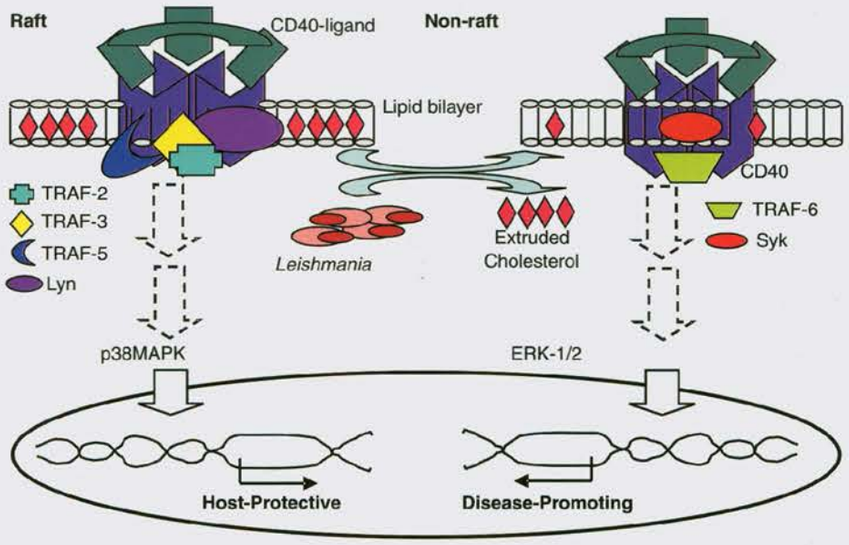

Fig. 2: CD40 assembles two constitutionally and functionally different signalosomes in raft and non-raft membrane domains.

Role of viral complement control proteins in immune evasion

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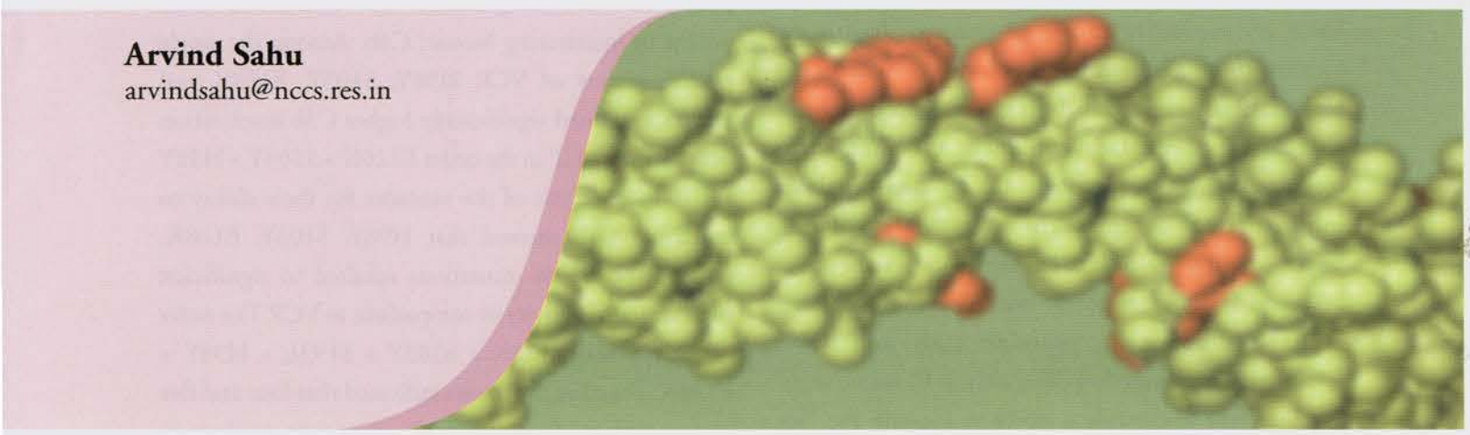

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Background

The first barriers that micro-organisms including viruses must overcome for being a successful pathogen are imposed by the innate immune system, of which the complement system constitutes a major arm. Studies on the primary structure of genomes of viruses have now made it clear that large DNA-viruses entrust a significant part of their genome to encode for immuno-modulatory genes, which include genes for complement regulatory proteins. In particular, it has been shown that members of Poxviridae and Herpesviridae encode for homologs of human complement regulators. We, therefore, started the present study with a hypothesis that viruses encode complement regulators (vCCP) to escape from the complement system surveillance and play a critical role in viral pathogenesis. Currently, we are focusing on unraveling the molecular basis for complement inhibition by complement control protein homologs of variola virus (SPICE), vaccinia virus (VCP), Herpesvirus saimiri (sCCPH) and Kaposi's sarcoma-associated herpesvirus (Kaposica). In addition, we are also looking into the role of these proteins in viral pathogenesis.

Aims and Objectives

1. How vCCPs inactivate complement?
2. Which are the determinants of vCCPs important in complement inactivation?
3. What role vCCPs play in viral pathogenesis?

Work Achieved

Vaccinia virus and variola virus are two important members of the family Poxviridae: vaccinia virus was used as a vaccinating agent against variola virus, the causative agent of the dreadful disease smallpox, that still holds an imperative situate of concern due to the looming threat of its reemergence owing to its usage as a biological weapon. Both these viruses encode soluble complement regulators named vaccinia virus complement control protein (VCP) and smallpox inhibitor of complement enzymes (SPICE), respectively. Earlier, SPICE has been shown to be about 100- and 6-fold more potent than VCP in inactivating human complement proteins C3b and C4b, respectively, although they differ only in 11 amino acids. We therefore undertook a study to identify the 'hot spots' in SPICE that impart functional advantage to SPICE over VCP.

Identification of 'hot spots' in SPICE for complement regulation

To identify the amino acids that provide functional advantage to SPICE over VCP we generated single point mutants of VCP by substituting each of the eleven VCP residues that vary between VCP and SPICE with the corresponding residue of SPICE (Fig. 1). These mutants along with VCP and SPICE were then expressed in E. coli and assayed for complement regulatory activities (inactivation of complement proteins C3b and C4b, and decay of C3 convertase) and binding to the target proteins (C3b and C4b). We observed that in comparison to VCP, SPICE is about 90-fold more potent in inactivating human C3b and 5-fold more potent in inactivating human C4b. Among the single point mutants of VCP, H98Y, S103Y, E108K and E120K exhibited significantly higher C3b inactivation activity than VCP in the order E120K > S103Y > H98Y > E108K. Analysis of the mutants for their ability to inactivate C4b showed that H98Y, S103Y, E108K, E120K and S193L mutations resulted in significant increase in the activity in comparison to VCP. The order of activity was E120K > S103Y > S193L > H98Y > E108K. Together the results indicated that four and five amino acid residues of the eleven variant residues in SPICE are responsible for its increased ability to inactivate C3b and C4b, respectively.

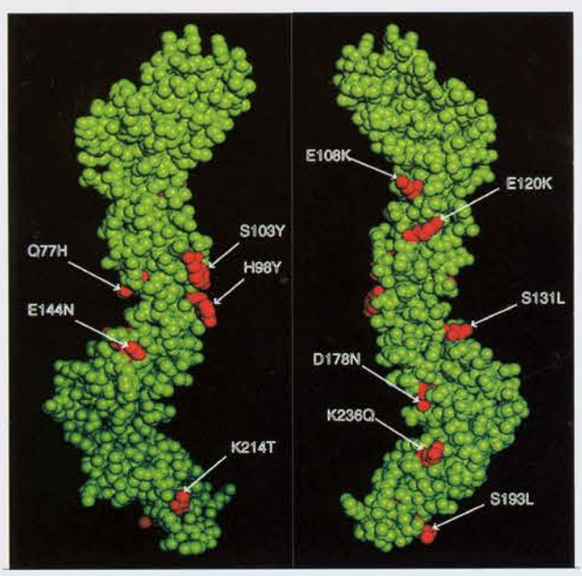


Fig. 1: Space-filling model of SPICE depicting the eleven mutations in SPICE compared to VCP. The model was built by utilizing crystal structure of VCP as the template using program SWISS-MODEL.

Another activity through which VCP and SPICE inhibit the complement system is the decay of the classical pathway C3 convertase. We therefore compared the C3 convertase decay activity of SPICE, VCP and the single point mutants of VCP. The data showed that SPICE was only two-fold more active than VCP and none of the single point mutants significantly increased the C3 convertase decay activity in comparison to VCP. Our data therefore suggested that increased cofactor activity of SPICE was mainly responsible for its enhanced function against human complement.

Inactivation of C3b and C4b by complement regulators involve two steps, i) binding of complement regulator to C3b or C4b and ii) interaction of the protease factor I with C3b or C4b and the regulator. We therefore designed a surface plasmon resonance (SPR) assay for binding and asked if increased cofactor activity of SPICE was due to its enhanced binding to C3b/C4b. Single point mutations those enhanced the C3b cofactor activity (H98Y, S103Y, E108K, E120K) showed moderate increase in C3b binding, while mutations that resulted in gain in C4b cofactor activity (H98Y, S103Y, E108K, E120K and S193L) did not correlate with C4b binding. We therefore suggest that increased C3b/C4b inactivation ability of SPICE is likely due to its better interaction with factor I.

Identification of minimum number of residues responsible for imparting functional advantage to SPICE

Although the above exercise allowed us to identify the hot spots in SPICE for complement regulation it did not reveal how many residues need to be changed to achieve activity as that of SPICE as none of single point mutants showed full activity. Because four point mutants showed increased C3b inactivation and five mutants showed increased C4b inactivation we generated the tetra-mutant (H98Y/S103Y/E108K/E120K) and the penta-mutant (H98Y/ S103Y/E108K/E120K/S193L) and assessed their activity with respect to C3b and C4b inactivation. We observed that both tetra- as well as penta-mutants were as potent as SPICE in inactivating C3b and C4b. Thus, mutations of four residues (H98Y/S103Y/E108K/E120K) are enough to formulate VCP as potent as SPICE.

Poxviral complement control proteins display species specificity

Variola virus shows strict human tropism, but the exact reason for this is not known. If SPICE serves as an immune evasion protein then it is likely that it is evolved to be more human specific. We therefore asked if SPICE displays increased specificity towards human complement compared to VCP. Interestingly, we observed that in a hemolytic assay SPICE was 25-fold more potent towards human complement than VCP and conversely VCP was 12-fold more potent against dog complement and the complement from other domestic animals (Fig. 2). Encouraged by this data we then looked into the influence of the amino acid variations in SPICE on species specificity. Analysis of inhibitory activity of tetra- and penta-mutants towards human and dog complement showed that they behaved like SPICE i.e., their inhibitory activity against human complement was comparable to that of SPICE (Fig. 2). Our data therefore indicate that amino acid variations in SPICE increase its specificity towards human complement.

Future Work

1. Structural basis for species specificity in poxviral complement regulators.
2. Fine mapping of functional sites in VCP and Kaposica.
3. Effect of modulation of electrostatic potential on functional activities of vCCPs.

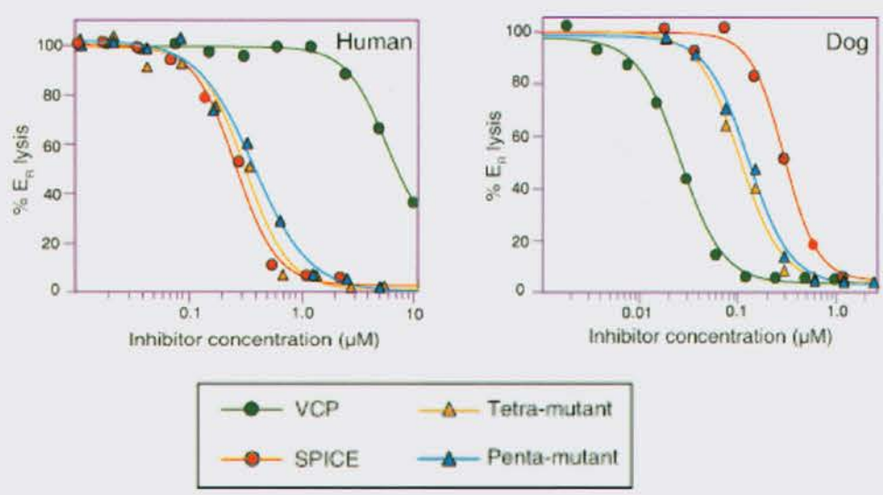
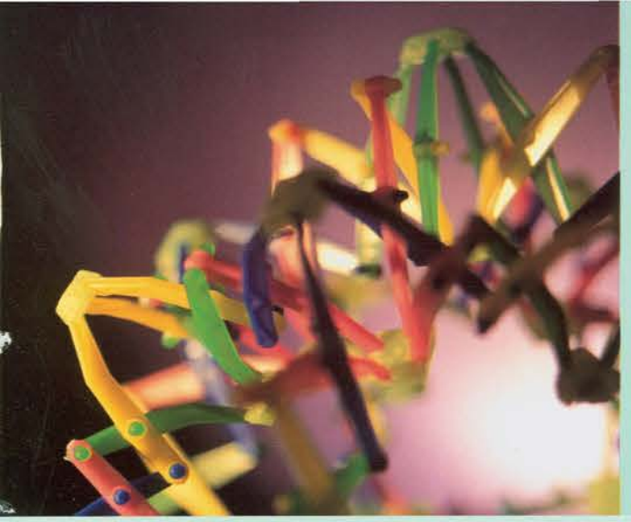


Fig. 2: Inhibition of human and dog complement by SPICE, VCP and the tetra- and penta-mutants of VCP. Complement inhibition was assessed by measuring the effect of these inhibitors on the alternative pathway-mediated lysis of rabbit erythrocytes (E_R).



Research Reports

Chromatin Architecture & Gene Regulation

Gene regulation by tumor suppressor MAR binding protein SMAR1

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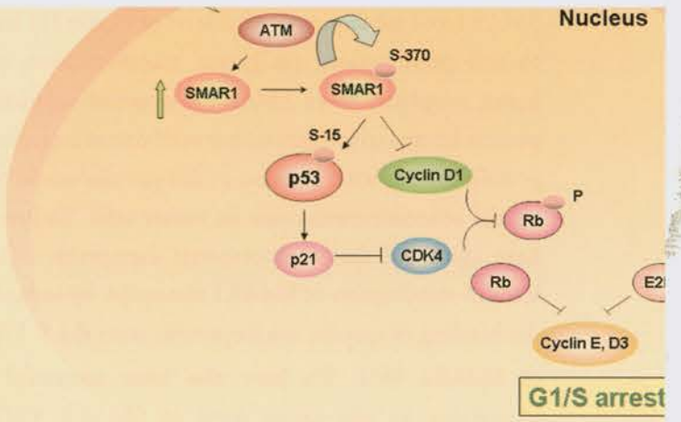

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Background

The tumour suppressor functions of MAR binding protein SMAR1 have been attributed to its role as a transcriptional regulator and modulator of p53. In this context, we have identified the minimal domain of SMAR1 essential for interaction with p53 and the transcriptional repression mediated by its recruitment on Cyclin D1 promoter. We have also identified the cross talk between SMAR1 and NF-κB molecule that governs several cellular pathways important for maintenance of cellular homeostasis. Further studies have been carried out to delineate the specific signaling pathways underlying the stress responsiveness of SMAR1 and its effect on transcriptional regulatory functions.

Aims and Objectives

1. Identification of factors regulating SMAR1 at mRNA and protein level
2. Regulation of cell growth and differentiation by SMAR1 in p53 dependent and independent mechanisms
3. Structure function studies of different domains of SMAR1
4. Regulation of Th1-Th2 lineage commitment

Work Achieved

Identification of factors regulating SMAR1 at mRNA and protein level

In breast cancer derived cells, low level of endogenous SMAR1 and subsequently high level of Cyclin D1 leads to cell proliferation, malignant transformation and hence neoplasia. Since SMAR1 is a tumor suppressor protein known to interact with several tumor suppressor proteins, the down regulation of this protein would be a crucial selection mechanism in tumor cells. Earlier we have identified that the hormonal therapeutic PGA2 leads to stabilization of SMAR1 transcript, by inducing the binding of specific nucleoprotein onto the 5' UTR of SMAR1 (ϕ1). We have also been successful in identifying an alternative form of SMAR1 5'UTR (ϕ17), whose stability is poor in breast cancer derived cell lines. The ϕ1 UTR of SMAR1 is a thermodynamically stable structure and differs from its variant form in hosting a minor stemloop structure, the one formed by deletion of 18 bases. Further investigations indicated that this sequence is critical for SMAR1 stabilization brought about by nucleoprotein complex formation on the stem loop structure. The competition studies using different mutants showed that the secondary structure of the loop was important. It is thus possible that upon PGA2 treatment certain proteins that recognize this fold, bind to the stem and loop structure and regulate mRNA stability, resulting in increased SMAR1. This could be an alternate selection mechanism in tumor cells, whereby a variant UTR containing a transcript, encoding a tumor suppressor protein is retained for yet other unknown functions. Interestingly, we also find that the growth inhibitory response of cells to PGA2 is in part due to the stabilization of SMAR1 mRNA by 5' UTR, as the knock down of SMAR1 leads to compensation in the growth-arrest function of PGA2. We have now identified HSP-70 as one of the factors binding to ϕ1 UTR that regulates mRNA stability and growth inhibitory potential of SMAR1. Since PGA2 is shown to induce HSP-70 levels (at ~ 6h), it is quite conceivable that this induced HSP-70 binds to SMAR1 and stabilizes the SMAR1 mRNA (Pavithra et al 2007).

Regulation of cell growth and differentiation by SMAR1 in p53 dependent and independent mechanisms

a. Regulation of Cytokeratin 8 expression in breast cancer

The loss of cellular differentiation is a characteristic feature of malignant transformation that leads to a whole series of structural changes, which enables the tumor cells to detach from the epithelial layer and metastasize. Since the invasiveness of a tumor is often determined by the profile of expressed genes, we studied the possible regulation of cytokeratin genes by tumor suppressor protein SMAR1. Microarray analysis upon SMAR1 overexpression in MCF-7 cells reflected a drastic downregulation of Cytokeratin genes, of which CK8 was prominent. Our recent findings have pointed out at the complex regulation of CK8 by cross talk between SMAR1 and p53. Tumor suppressor SMAR1 downregulates Cytokeratin 8 gene expression by modulating p53 mediated transactivation of this gene. The recruitment of SMAR1 on CK8 promoter following genotoxic stress results in deacetylation and displacement of p53, leading to chromatin changes associated with the inactive state of chromatin. Additionally, expression of CK8 and SMAR1 are negatively correlated as evaluated by normal- metastatic tissue microarray. Therefore, our studies identify the role of a candidate tumor suppressor protein SMAR1 that regulates the expression of intermediary filament protein CK8, thereby ensuring the normal phenotype of the cells (Fig. 1).

b. Regulatory function of SMAR1 during IR induced DNA damage

Preliminary studies from lab suggest that SMAR1 is responsive to various stress stimuli. Therefore identification of stress responsive nature of this protein and delineating the signaling pathway that specifically stimulates the functions of this protein is of paramount importance. Utility of specific post-translational modifiers allows temporal and spatial control over protein relocalization and interactions, and may represent a means for trans-regulatory activation of protein activities. The ability to recognize these specific modifiers also underscores the capacity for signal

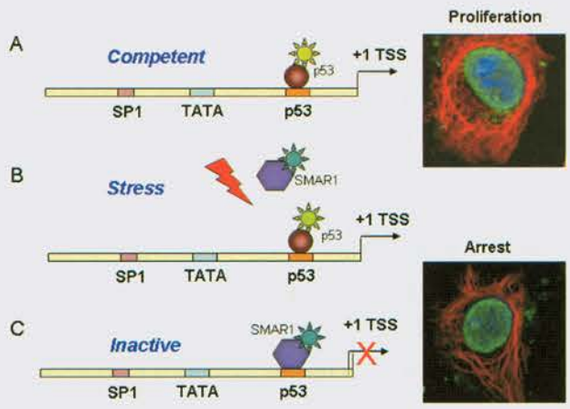

Fig. 1. Schematic representation of CK8 repression by interplay between SMAR1 and p53. (A) Under endogenous conditions, CK8 gene is competent for transcription, following recruitment of different factors and p53, allowing cellular proliferation. (B) Upon stress, DNA damage triggers SMAR1 expression that results in the displacement of p53 from its cognate site in CK8 gene. Further, SMAR1-HDAC1 corepressor complex deacetylates the local histones, causing a transcriptional pause, silencing the CK8 gene expression.

amplification, a crucial step for the maintenance of genomic stability and tumor prevention. In context with the stress response, we identified a novel ATM phosphorylation site at serine 370 of SMAR1 that mediates an increased association of SMAR1 with Cyclin D1 promoter. The dual effect of SMAR1 i.e. induction of p21 through p53 activation and downregulation of Cyclin D1 by recruitment of corepressor complex causes cell cycle arrest. The phosphorylation mutant form of SMAR1 is partially defective in arresting cells at G1 phase, highlighting the role of DNA binding activity of SMAR1 in regulating G1/S phase of cell cycle. Thus our studies demonstrate that SMAR1, a novel ATM substrate is a critical determinant of cell cycle, linking p53 and Rb signaling pathways in an ATM dependent manner. This is for the first time we show a direct connection of ATM mediated phosphorylation of p53 ser 15 modulated by SMAR1 (Fig. 2).

Structure function studies of different domains of SMAR1

The intra-cellular level of tumor suppressor protein p53 is tightly controlled by an autoregulatory feedback loop between the protein and its negative regulator, MDM2. The role of MDM2 in down regulating p53 response in unstressed cells and the post stress recovery phase is well documented. The interplay between the N-terminal phosphorylation and C-terminal acetylation of p53 in

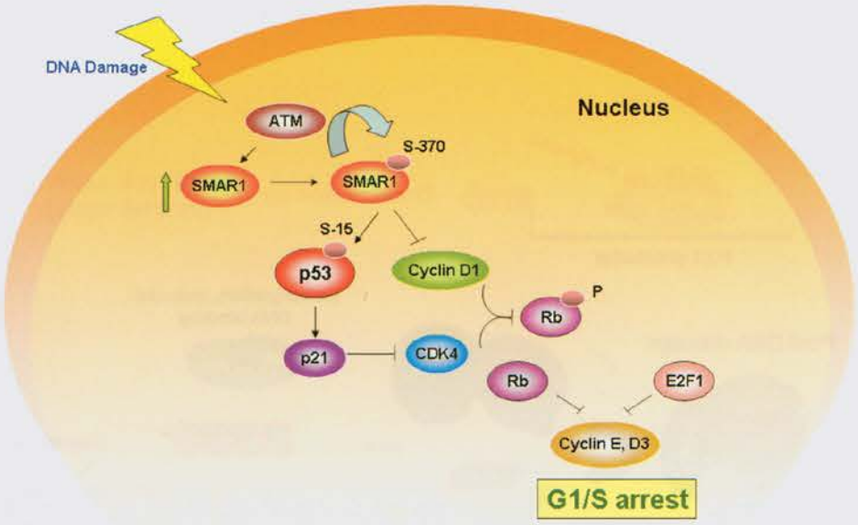

Fig. 2. Schematic representation of SMAR1 induction and the cascade of events leading to growth arrest following p53 and Rb activation. Upon DNA damage, ATM induces and phosphorylates nuclear SMAR1 that leads to stabilization of p53 and repression of Cyclin D1 gene. This results in hypophosphorylation of Rb by combinatorial effect of p21 induction and CDK4-Cyclin D1 complex disruption. This is followed by E2F1 sequestration and halt in the synthesis of Cyclin E and D3. This results in the G1/S phase arrest upon IR.

the context of MDM2 mediated degradation of p53 however remains unclear. We have now identified that SMAR1, a MAR binding protein, interacts with MDM2 and serine-15 phosphorylated form of p53, forming a ternary complex in the post stress-recovery phase. In this context, we are now aiming to understand the structural modules of SMAR1-p53-Mdm2 complex through various bioinformatics and mutation studies. Deciphering of these structural motifs and domains will aid in the design of artificial molecules that can be used for targeted therapeutic intervention (Fig. 3).

Role of SMAR1 in T helper (Th) cell differentiation

The development and function of the T lymphocyte lineage are regulated tightly by signaling pathways that involve lineage-restricted cell surface receptors, intracellular signaling molecules and nuclear transcription factors. Naïve T helper cells differentiate into two subsets, TH1 and TH2, each with distinct functions and their respective cytokine profiles. We have earlier shown that SMAR1 downregulates Th1 specific transcription factor T-bet thereby effecting Th1 lineage commitment of T cells. Most our observations were made in SMAR1 transgenic mice. Now we are in process of generating SMAR1 knock out mice that will validate our earlier hypothesis. Moreover we find that SMAR1 expression is induced drastically during Th2 polarization. The increase in the levels of SMAR1 is because SMAR1 promoter has putative GATA binding elements. During polarization of T cell to Th2 lineage, GATA3 the master regulator of Th2 type response, is induced which in turn binds to SMAR1 promoter thereby transcriptionally inducing SMAR1. SMAR1 is then recruited to T bet promoter along with a corepressor complex. Thus we have tried to understand how SMAR1 act as switch in regulating these kinds of responses. Further, we are now trying to address the implication of this in disease models.

Future Work

1. Identification of post translational modifications that act as switch in regulation of SMAR1
2. Regulation of p53 transcriptional activity by SMAR1 that act as switch in cell cycle arrest and apoptosis
3. Elucidating the structure of various domains of SMAR1 and their role in the protein function.
4. Role of SMAR1 in regulation of HIV1- LTR mediated transcription.

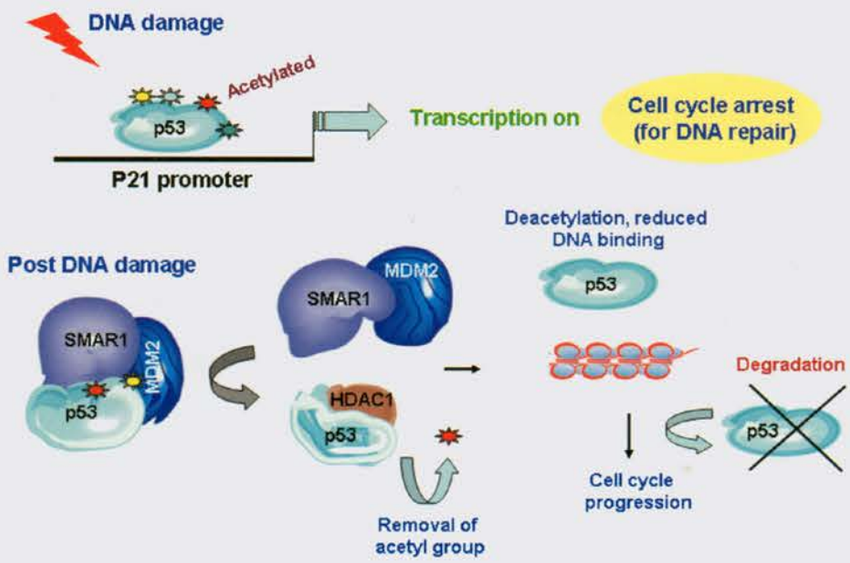

Fig. 3. Schematic representation of the formation of SMAR1 mediated ternary complex and its role post DNA damage. When the cells are subjected to stress, p53 is activated and binds to its consensus site in target genes that elicit cell cycle arrest or transcriptional arrest, aiding in the cellular repair machinery for damage rescue. Post damage repair, p53 response is dampened by MDM2 and SMAR1. SMAR1 masks the p53 serine 15 phosphorylation, aiding in association of MDM2 with p53. As a consequence of this interaction, p53 is deacetylated by HDAC1 and there is a reduction in DNA binding of p53. This leads to switching off the transcriptional activation of antiproliferative genes like p21 and cells progress to S phase.

Study of the mechanism(s) involved in the regulation of the MAR-binding activity of SATB1

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Background

Special AT-rich sequence binding protein 1 (SATB1) participates in the maintenance of chromatin architecture by organizing it into distinct loops via periodic tethering of matrix attachment regions (MARs) to the nuclear matrix. SATB1 acts as a 'docking site' for several chromatin modifiers including ACF, ISWI and HDAC1 and these chromatin modifiers were suggested to affect gene expression through histone modifications and nucleosome remodeling at SATB1-bound MARs. SATB1 also regulates gene expression by recruiting corepressors (HDACs) and coactivators (HATs) directly to promoters. Post-translational modifications of its N-terminal PDZ-like domain act as molecular switches regulating the transcriptional activity of SATB1 via modulating its association with other proteins. The PDZ-like domain is also important for DNA- and chromatin-binding ability of SATB1 through homodimerization. In the C-terminal half, amino acids (aa) 346-495 harbor two Cut-like repeats and hence can be referred to as the Cut Domain (CD). This region is also referred as the MAR-binding domain (MD) due to its probable role in highly specific recognition of MARs. Additionally, SATB1 harbors a homeodomain (HD) spanning aa 641-702 that is believed to act in concert with the MD and direct SATB1 to bind to the core-unwinding element within a MAR with high affinity. Gene profiling studies using RNA from cells overexpressing point mutants of SATB1 defective in phosphorylation or acetylation revealed that SATB1 regulates more than 10% of genes demonstrating the importance of these modifications towards the ability of SATB1 to act as a global regulator of gene expression. However, only a limited number of SATB1 binding sites (SBSs) have

been characterized so far, most of which were isolated based on their ability to serve as base unpairing regions (BURs) that are hallmark of MARs. Comparison of these SBSs and various other sequences reported to be bound by SATB1 in vivo did not reveal any specific consensus element, giving rise to the notion that SATB1 binds DNA in a sequence-independent but context-dependent manner. However, such analyses identified an ATC context that has been proposed to be involved in targeting SATB1. Due to lack of consensus binding element the precise mechanism of how SATB1 binds to MARs or non-MAR DNA sequences with high affinity and specificity remains poorly understood.

In this study, we set out to understand how SATB1 binds to its target sequences specifically by characterizing its binding targets. We used the approach of systematic evolution of ligands by exponential enrichment (SELEX) to isolate a pool of synthetic DNA sequences that were bound with high affinity by SATB1. We found a conserved pattern of 10-12 nucleotides in all enriched sequences consisting of two inverted AT-rich (4 to 6 bases) repeats resembling the homeodomain binding site separated by 1-2 non-AT nucleotides. Substitution by cytosine (C) at any position in the conserved homeodomain binding region "TATTAG" abolishes the DNA binding activity of SATB1 indicating that it is mediated primarily by the homeodomain. The minor groove binding agent Distamycin has been shown to abolish the binding of SATB1 to the IgH MAR, indicating that SATB1 binds via the minor groove of the DNA. Results of our *in vitro* binding studies in conjunction with the recently solved structure of the N-terminal Cut repeat 1 (CUTr1) led us to propose that SATB1 binds to the inverted consensus palindromic repeats via homeodomain in dimerization-dependent manner via the minor groove, whereas the Cut repeats enhance the binding via hydrogen bonding interactions in the major groove. As a functional consequence, we demonstrate for the first time that the strength of repression mediated by SATB1 is proportional to its affinity to the target sequence. Collectively, our results provide evidence for sequence specific binding of SATB1 to target DNA and not only to the ATC context as thought before. Furthermore, we show that high affinity binding by SATB1 is dimerization-dependent and its specific high affinity binding is mediated by its homeodomain (HD) in collaboration with the Cut repeat containing domain (CD).

Aims and Objectives

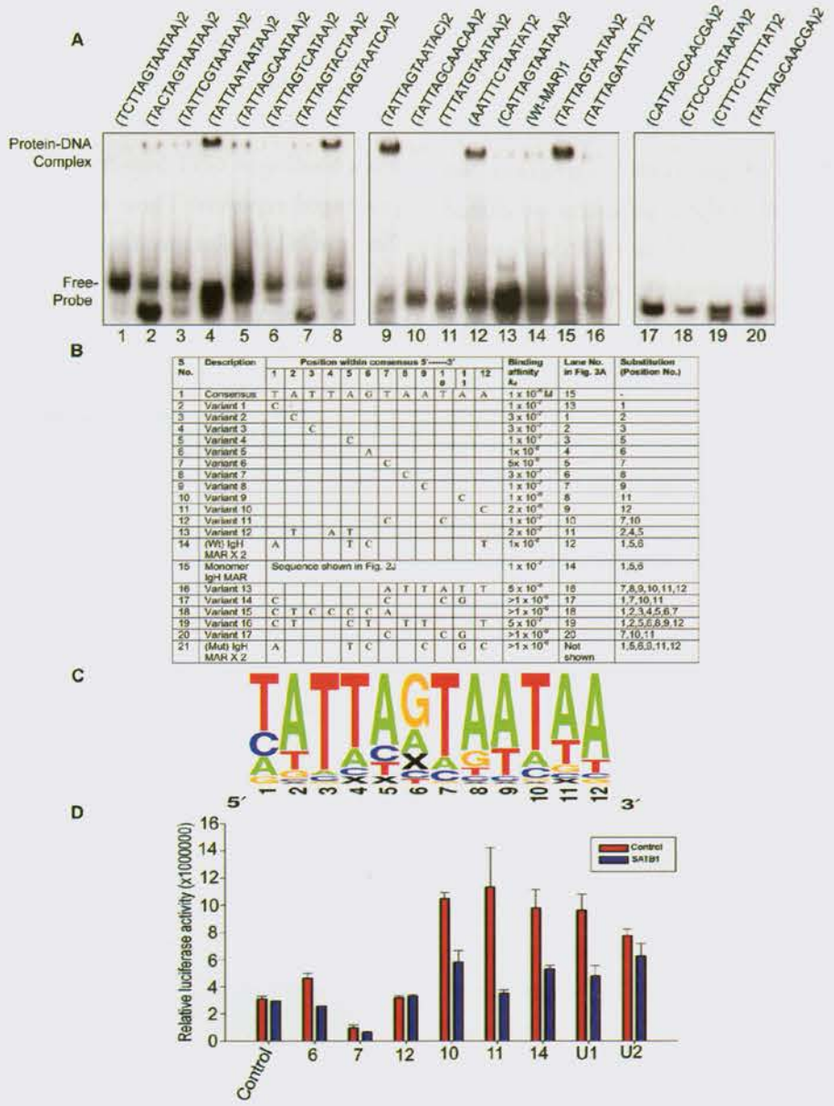
1. To identify proteins interacting with SATB1 through its PDZ-like domain.
2. To study the mechanism(s) by which SATB1 regulates transcription by interacting with other cellular proteins through its PDZ-like domain.
3. To monitor the effect of post-translational modifications of SATB1 on its MAR-binding activity and on global gene regulation.

Work Achieved

Consensus SATB1 binding sequence (CSBS) harbors a conserved homeodomain binding element that is essential for transcription regulation by SATB1

We analyzed the AT-rich regions in 40 unique sequences obtained by SELEX procedure. We aligned these sequences manually and also using the online software tool MEME and found that the motif TATTAGTAATAA occurs in most of the sequences whereas few sequences harbor its variants. We synthesized a dimer of TATTAGTAATAA and also of its variant sequences in which nucleotides were varied at different positions within the 12 mer consensus sequence. We then performed EMSA and determined the SATB1 binding affinity to individual sequences in terms of dissociation constant (K_d). Forty nmoles of SATB1 was sufficient to bind various enriched sequences and therefore used for the EMSA using synthetic dimeric sequences (Figure 1A). Inclusion of Cytosine (C) at positions 1 (lane 13), 2 (lane 1), 3 (lane 2), 5 (lane 3), 8 (lane 6), 9 (lane 7) or at two positions 7 and 10 (lane 10) instead of Adenine (A) or Thymidine (T) reduced SATB1 binding drastically. Substitution of central Guanosine (G) (6^{th} position) with A (lane 4) or substitution of A or T with C at position 11 (lane 8) or 12 (lane 9) had no significant effect on SATB1 binding. The monomeric wild type IgH MAR has lower binding activity than the consensus sequence identified here (sequence 15 in Figure 1B). The alignment of the core of the IgH MAR sequence with the CSBS revealed that it

varies from CSBS at 4 places out of 12. At position 1, T is substituted by A and T by A at position 5, central (6th) position G is replaced by C and at the last position A is replaced by T (Figure 1B, sequence 14). Next, we analyzed SATB1 binding affinity for a synthetic oligonucleotide containing dimer of this sequence and found it to be comparable with the affinity of the CSBS (Figure 1A, lane 12 versus 15), indicating that these substitutions do not have much effect on the binding affinity of SATB1. The relative binding affinities of several variants of CSBS are summarized in Figure 3B. These results indicate that SATB1 binds strongly with the 12 mer CSBS or its variants and a similar sequence is also present in the IgH MAR. Strikingly, the CSBS is divided into two AT-rich regions flanking a central region (one or two nucleotides) that could be either A, C or G. The substitution of A or T residues with a single nucleotide (C) at several positions abrogated the binding



S No.	Description	Position within consensus 5'------3'												Binding affinity K_d	Lane No. in Fig. 3A	Substitution (Position No.)
		1	2	3	4	5	6	7	8	9	10	11	12			
1	Consensus	T	A	T	T	A	G	T	A	A	T	A	A	1 x 10^{-10} M	15	-
2	Variant 1	C												1 x 10^{-7}	13	1
3	Variant 2		C											3 x 10^{-7}	1	2
4	Variant 3			C										3 x 10^{-7}	2	3
5	Variant 4					C								1 x 10^{-7}	3	5
6	Variant 5						A							1x 10^{-8}	4	6
7	Variant 6							C						5x 10^{-8}	5	7
8	Variant 7								C					3 x 10^{-7}	6	8
9	Variant 8									C				1 x 10^{-7}	7	9
10	Variant 9											C		1 x 10^{-8}	8	11
11	Variant 10												C	2 x 10^{-8}	9	12
12	Variant 11							C				C		1 x 10^{-7}	10	7,10
13	Variant 12		T		A	T								2 x 10^{-7}	11	2,4,5
14	(Wt) IgH MAR X 2	A				T	C						T	1x 10^{-8}	12	1,5,6
15	Monomer IgH MAR	Sequence shown in Fig. 2J												1 x 10^{-7}	14	1,5,6
16	Variant 13							A	T	T	A	T	T	5 x 10^{-8}	16	7,8,9,10,11,12
17	Variant 14	C						C			C	G		>1 x 10^{-6}	17	1,7,10,11
18	Variant 15	C	T	C	C	C	C	A						>1 x 10^{-6}	18	1,2,3,4,5,6,7
19	Variant 16	C	T			C	T		T	T			T	5 x 10^{-7}	19	1,2,5,6,8,9,12
20	Variant 17							C			C	G		>1 x 10^{-6}	20	7,10,11
21	(Mut) IgH MAR X 2	A				T	C			C		G	C	>1 x 10^{-6}	Not shown	1,5,6,8,11,12

Fig. 1: Identification of consensus SATB1-binding sites. (**A**) EMSA using radiolabeled synthetic duplex oligonucleotides. The sequences of various dimeric oligonucleotides are depicted on top of individual lanes. Positions of bound and unbound probes as indicated on left. (**B**) Table summarizing the various positional variants and their relative binding affinities. (**C**) Consensus SATB1 binding site (CSBS) derived from the SELEX-enriched sequences is represented in LOGO (http://weblogo.berkeley.edu/logo.cgi) format. The positions of each nucleotide within the 12 mer consensus are indicated below using numbers 1-12. The percentage frequency of occurrence of a specific nucleotide at a position is also shown in the supplementary table 1. (**D**) In vivo reporter assay was performed to monitor the transcriptional activity of dimeric CSBS and its variants. These sequences were separately cloned in the pGL3 promoter vector and their activity towards the modulation of the SV40 promoter derived luciferase reporter and effect of SATB1 towards the regulation of activity of these sequences in a transient transfection experiment are depicted in the histogram. The numbers on X-axis correspond with the serial numbers in the table in (B). Error bars represent standard deviation calculated from triplicates.

affinity drastically, suggesting that SATB1 may exhibit more stringent sequence specificity for binding than the presumed ATC context. The positional mutation analysis along with analysis of the naturally in vitro selected sequences led us to propose that a combination of 12 mer palindromic sequence possessing two AT-rich repeats in inverse orientation is essential and sufficient for specific binding by SATB1. The palindromic consensus SATB1 binding sequence (CSBS) is represented in LOGO format in Figure 1C. Analysis of the consensus sequence revealed that it harbors two copies of a conserved homeodomain binding sequence (TAATA) (Figure 1C) and mutational analysis in this region reduces affinity of SATB1 very significantly. To monitor the effect of positional variations on transcriptional potential of these sequences we cloned several point mutations in the 12 mer SBS as listed in Figure 1B as well as the two unique sequences in pGL3P vector. These reporter DNAs were used to transfect 293 cells, either along with 3X FLAG vector or with 3XFLAG-SATB1 construct. Most of these sequences enhanced SV40 promoter-driven transcription except sequence 7, which acted as a silencer of the SV40 promoter-driven transcription (compare red bar for sequence 7 with that of control sequence in Figure 1D) and SATB1 coexpression led to repression of enhancer activity of these sequences (Figure 1D, blue bars corresponding to sequences 6, 7, 10, 11 and 14) whereas there is no effect of SATB1 on sequence 12 which displays weaker affinity to SATB1. The two SELEX-enriched unique sequences enhanced the activity of luciferase reporter driven by SV40 promoter (U1 and U2 in Figure 1D). The stronger SATB1 binding unique sequence (U1) enhanced the luciferase activity to a relatively higher extent compared to the weaker SATB1 binding unique sequence (U2). Furthermore, overexpression of SATB1 resulted in stronger repression with the U1 (>2-fold, Figure 1D) compared to the U2 (~ 1.2-fold, Figure 1D). Thus, SATB1 binding sequences generally seem to possess a cis acting enhancer function, whereas binding of SATB1 to these sequences leads to the repression of enhancer activity which is directly proportional with the binding affinity of SATB1. Taken together, these results suggest that the relative binding affinity of SATB1 to the palindromic homeodomain consensus sequences determines its transcriptional activity.

Dimeric HD is the determinant for the higher affinity and specificity to CSBS variants and IgH MAR, but not the dimeric CD

To directly evaluate and compare the binding affinities of the various proteins we performed Southwestern analysis using GST:346-763 (CD+HD), GST:346-763 •HD (lacking 641-702 aa), GST alone, GST:CD, and GST:HD. We observed binding of CSBS (Figure 2B) and wild-type heptamer IgH MAR (Figure 2C) with GST:346-763 (lanes 2-5) and with GST:HD (lanes 15-17). Strikingly, no significant binding observed with GST:CD (lanes 12-14), GST:346-763 •HD lanes 5-7), and GST alone (lanes 8-10). Very faint signal indicating weak binding of GST:346-763 could be detected upon prolonged exposure. These results clearly confirm that dimer of HD makes specific contacts with the DNA at a concentration when there is no binding by GST:CD or GST:346-763 •HD. Furthermore, neither HD (lanes 8-

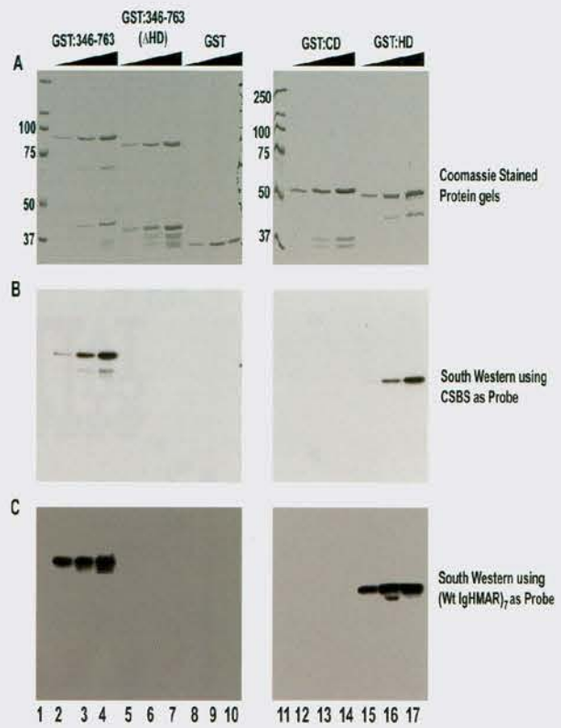


Fig. 2: Dimerization-dependent specific and higher affinity binding is a property of HD. (A) Coomassie brilliant blue stained protein gel indicating quality of various proteins used for Southwestern blotting. Lane 1, Molecular weight standards; lanes 2-4, GST:346-763; lanes 5-7, GST:346-763 (ΔHD); lanes 8-10, GST; lane 11, Molecular weight standards; lanes 12-14, GST:CD and lanes 15-17, GST:HD. For each of the proteins resolved, the three lanes contain 0.25 μg, 0.5 μg, and 1 μg of protein respectively. (B) Southwestern blot using CSBS as probe. (C) Southwestern blot using heptameric IgH MAR as probe.

10) nor CD (data not shown for monomer CD) exhibited binding with the probe in their monomeric (GST-free) form. Taken together these results confirm that dimeric status of HD and not CD is essential for the specific binding of SATB1 with the DNA.

Role of PDZ domain-mediated dimerization in the high affinity binding by SATB1

Based on missing nucleoside experiments it was suggested that HD and CD contact the same site simultaneously, possibly from opposite sides of the DNA helix. However, this model does not incorporate the fact that SATB1 exists as a homodimer in vivo. It is noteworthy that crystal structure of the even-skipped (eve) homeodomain showed that two homeodomains are bound by one 10-bp consensus sequence on both faces of the DNA in a tandem fashion. This unusual binding mode involving simultaneous occupation of one binding site from both sides of the DNA helix could stabilize the protein-DNA complex. In the transcription factor Oct-1, the bipartite DNA-binding domain is composed of a POU-specific domain (POUs/ one cut domain) and a POU-homeodomain (POUhd) connected by a flexible linker. Solution structure revealed that the left half of the optimal POU binding site, the octamer ATGCAAAT, is recognized by POUs and the right half by POUhd. Interestingly, another Pou homeodomain protein LFB1/HNF1 binds as a homodimer to an inverted palindromic consensus binding element. HNF1 • crystal structure indicates that a monomer can occupy more than half site of the DNA when bound to a 21 bp oligonucleotide sequence harboring 13 bp palindrome sequence. In light of the findings that SATB1 is a homodimer and that its binding consensus is an inverted palindrome, we propose that the PDZ-like dimerization domain bridges DNA binding regions of two SATB1 monomeric subunits such that they bind in an anti-parallel fashion to the inverse palindromic consensus binding element (Figure 3). In this model, all three domains have unique contributions towards the high affinity DNA binding by SATB1. The CD binds DNA through major groove without much specificity and with low affinity whereas the HD binds target DNA specifically through the minor groove and with high affinity. The affinity is increased many folds when both domains are held together in dimeric form by the PDZ domains (or GST). Thus, the dimer of SATB1 may form a clamp-like structure that wraps around the helix via occupying both major and minor grooves (Figure 3). This mode of binding is similar to that of LFB1/HNF1, wherein the DNA-independent dimerization domain is required to increase the DNA-binding affinity, but does not influence the dimer geometry. It is not surprising therefore, that the replacement of SATB1's N-terminal PDZ with any other dimerization imparting polypeptide including glutathione-S-transferase can restore DNA binding to the wild-type levels. Thus, the functional MAR-binding domain of SATB1 is constituted by the CD and HD together and not by the

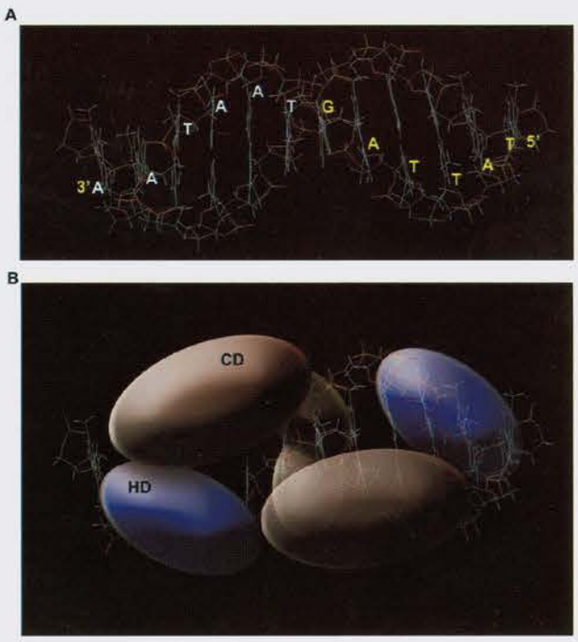
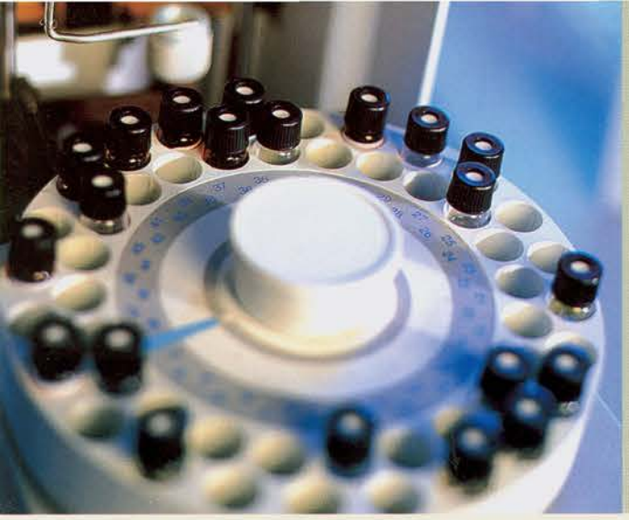


Fig. 3: Model depicting binding of the consensus element by SATB1. (**A**) The consensus DNA sequence was created using the 'B' software (http://www.scripps.edu/mb/case/Biomer/). A pdb file was created from the sequence and rendered in wireframe model assuming B-form of DNA, and then the structure was minimized. This was imported in Photoshop (Adobe) and labeled with alphabets representing the nucleotides. (**B**) Three-dimensional representation of SATB1-CSBS complex. The B form of CSBS DNA as obtained using the "B" software was imported to the Photoshop CS2 (Adobe) and the cartoon of SATB1-DNA complex was created by adding the colored blobs representing each of the domains of SATB1. The figure represents that one monomer of the dimer occupies one face of DNA and the other monomer occupies the other side where HD recognizes the minor groove whereas CD recognizes major groove and the N-terminal PDZ provides the dimerization function by bridging two monomers together; however, the PDZ domain does not contact with the DNA. Transparent blobs are on the face of the helix away from the reader. For details see text. The sizes and shapes of the blobs depicting the three domains of SATB1 are strictly for schematic presentation purpose only.

CD alone. The CD may principally occupy the major groove whereas the N-terminal arm of the HD may occupy the minor groove. The recognition helix of the HD may also occupy the major groove but without any significant contribution towards binding, since mutation in the 3rd helix of HD does not affect DNA binding of SATB1 significantly. This is yet another unique mode of DNA binding that may provide exceptional stability to the complex, and may therefore explain the remarkable increase in binding specificity and affinity with dimeric CD+HD. Therefore the final proposed model is quite similar with the structure of the dimeric HNF1 α where dimerization domain resides independently and one monomer of the dimer occupies one face of DNA and the other monomer occupies the other side. Additionally, one monomer occupies more than half of the palindrome sequence which could be also true for SATB1 since the distance between the half-sites of CSBS is critical for binding.

Future Work

1. To investigate the role of the PDZ-mediated interactions on regulation of transcription of multiple genes by SATB1.
2. To study SATB1-mediated dynamic organization of chromatin at its genomic binding sites.



Support Units

Experimental Animal Facility

Dr. B. Ramanamurthy

The Team:
Dr. R.M. Bankar
Mr. Md. Shaikh
Mr. A. Inamdar
Mr. P.T. Shelke
Ms. Vaishali Bajare
Mr. Sanjay Gade
Mr. Dilip Thorat

The Experimental Animal Facility is an infrastructural service department of the Institute facilitating animal experimentation. It is a barrier-maintained facility for the breeding, maintenance and supply of high quality and standardized 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
129/SvJ
FVB/NJ
NOD/LtJ
SWISS#
BALB/c*
NMRI $^{nu/nu}$
Genetically engineered mutant mice (knock-out, transgenic & mutant mice-**34 lines**)

RATS:

WISTAR
LEWIS

RABBITS:

NEWZEALAND WHITE

Defined barrier practices are followed scrupulously without any exception or allowance, with access to select personnel, to minimize the risk of microbial infection to the animals housed in the facility.

The breeding program for the propagation of the two different inbred lines viz. BALB/c and C57BL/6 is structured in a three-tier format, i.e. the Foundation colonies (FC), Pedigreed expansion colonies (PEC) and the Production colonies (PC). The remainder inbred and mutant (gene knock-out) mice are maintained as foundation colonies. Strict full-sib pairing only propagates the animals in the FC. These colonies are the nuclear colonies for the long-term propagation of an inbred strain.

During the reporting period, the facility has added a further 2 different gene knock-out / transgenic mice lines to the existing ones, taking the total now to 34 different mutant mice lines. With this, the total number of mice strains, both inbred and mutant, being maintained at the Experimental Animal Facility stands at *45 (forty five)*. These mice are housed in Individually Ventilated Caging systems. An aseptic/sterile routine standardized in-house is in practice for the housing, breeding and handling of these mice. These mice have been supplied in reasonable numbers for use in ongoing research projects.

Complete technical 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: collection of blood and other samples, immunizations, surgical procedures, injection of tumor cells in 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. The details of the laboratory animals supplied for various R & D activities are given below.

1-4-2007---31-03-2008

SR.NO.	STRAINS/SPECIES	ANIMALS SUPPLIED
1.	**MICE**	
	BALB/c	5801
	C57BL/6	980
	SWISS#	309
	DBA/2	32
	DBA/1	221
	Nude (nu/nu)	310
	BALB/c*	65
	FVB/NJ	514
	NOD-SCID	401
	Mutant Mice	604
2.	**RATS**	
	WISTAR	436
	LEWIS	50
3.	**RABBIT(NZW)**	4

*** BALB/c with cataract mutation.**
# Outbred

Library

The Team:

Mr. Rameshwar Nema,
Sr. Lib. and Info. Assistant

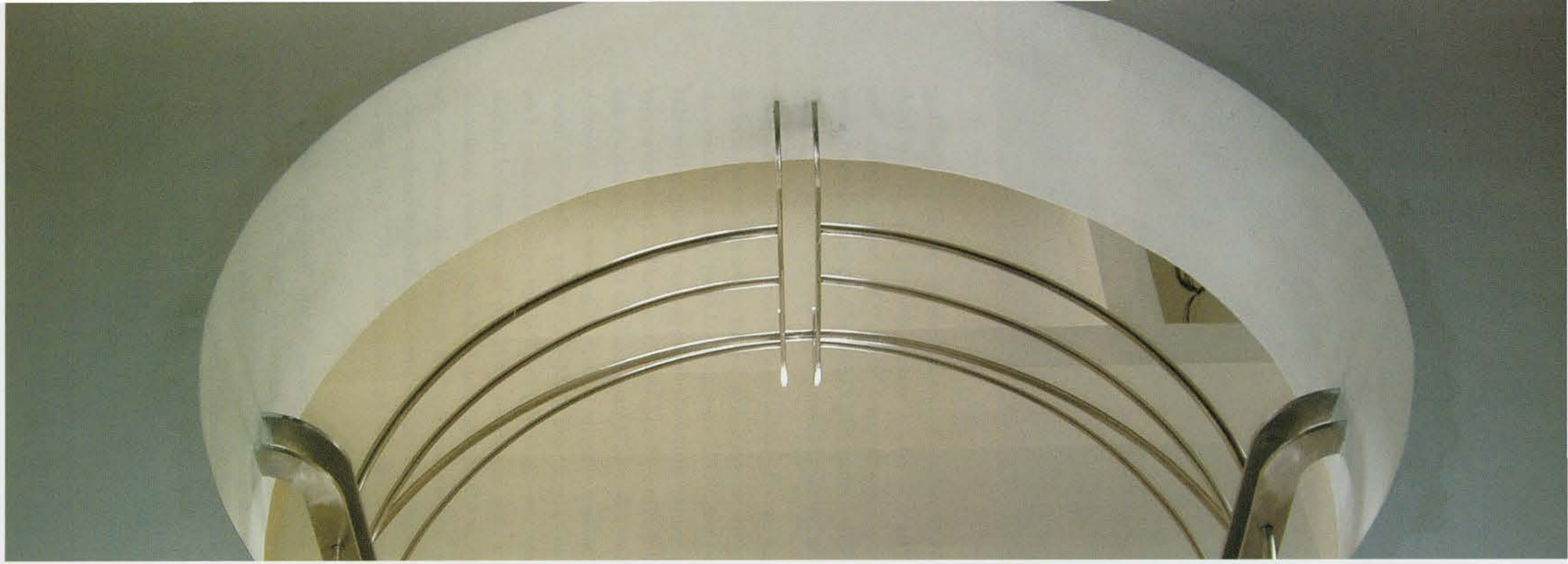
Mrs. Aparna Panse,
Office Assistant B

Mr. Madhukar Randive,
Helper A

The NCCS Library has collection in the frontier areas of biotechnology having relevance to NCCS research activities. The Library holds approximately Eight thousand Seventy One bound journals, Two thousands One hundred Fifty Eight books, and subscribe to Seventy five Scientific Journals and Twenty Eight other periodicals.

In the development of its collections, the Library's priority is to support NCCS research activities. The Library collection is expanded in consultation with NCCS scientists. The Library's print collections are growing by approximately 900 volumes per year. During the period of 2007-2008, the Library has added 87 books and 822 bound volumes of journals to its collection. A limited full text Online Journals are also provided for faster access to research information. The Library is equipped with Linux based SLIM++ LX library software for library house keeping operation and Web-OPAC for online searching of the library documents. In addition, the Library has also installed Barcode Technology for Circulation (Issue & Return) of library documents. The Library information (in Hindi & English) is available on the library Webpage which includes free Online Medical database links, NCCS research publication list, library form, NCCS in News and Ph.D. thesis collection.

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



Computer Section

The Team:

Mr. R. J. Solanki,
Mr. S. S. Jadhav
Ms. R. C. Patwardhan
Ms. K. S. Jadhav

The Computer Centre has currently enhanced its infrastructure as follows -

- **Local Area Network Upgradation**

  Computer Section has recently completed the upgradation of the Network Backbone with the use of OFC and manageable 96 Gigabit chasis switch. This has facilitated Centralized Network Management, Monitoring and Access Control. The endnodes are now connected with Cat 6 cables replacing the Cat 5 cables. This has resulted in a remarkable increase in the data transfer speed.

- **Rack Mounted Servers Installed and Functional**

  The installation and configuration of the four high End Rack Mounted Servers completed and the servers are functional thereby providing an additional storage space for the Mails, Domain based User Interface.

- **Procurement of an upgraded Network Antivirus Software Package**

  An up gradation of the current antivirus software is performed there by providing security to the invaluable data on all the computers of the Institute and for a safe Internet connectivity.

- **Installation of Wi-Fi Networks in all the Labs in progress**

  The Centre is in process of Installing Wireless Connections in all the labs. It will soon be functional thereby providing an uninterrupted wi-fi connection.

- **Internet Leased Lines Upgradation in progress**

  NCCS is currently connected to the internet via two leased lines namely one from Reliance(1 Mbps) and the other from VSNL(256 Kbps). The process of upgradation of these leased lines to 2 Mbps is in progress thereby enhancing the internet bandwidth remarkably.

The Computer Section at NCCS is involved in the following main activities :

a) Providing technical support to more than 120 computers and more than 80 printers.

   Computer centre provides support such as installations of Operating Systems, Other Softwares and Drivers and troubleshooting other hardware problems.

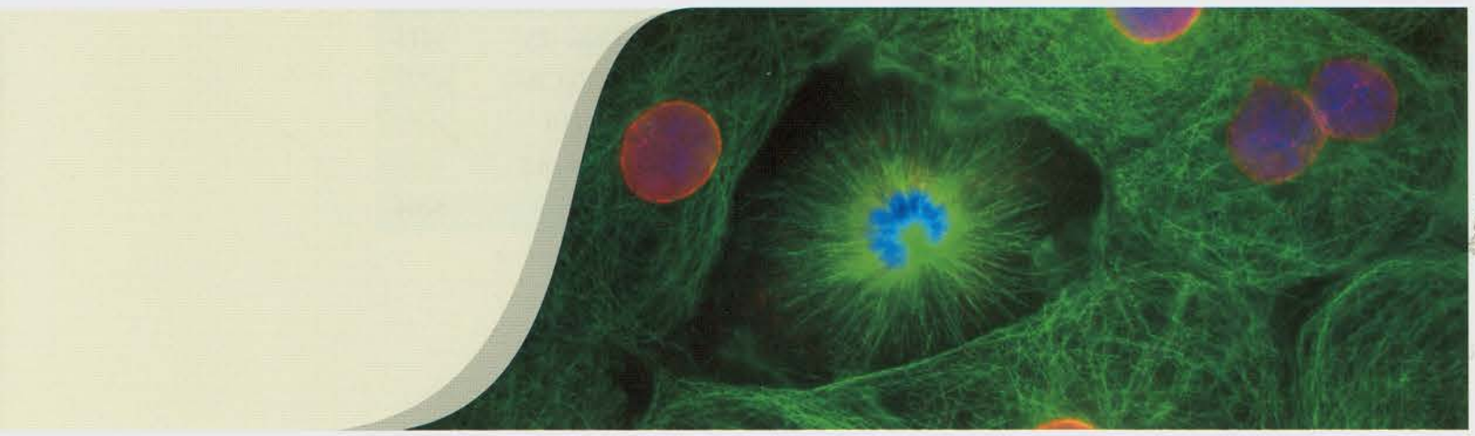
b) Management of the invaluable information in the Institute.

c) Providing Network Support to all the sections and facilities thereby contributing to the smooth functioning of the routine administrative and research work in the Institute.

d) Providing Support to the students and scientist for their scientific presentations required when attending National and International conferences / seminars. Computer centre helps in the DTP work, CD writing, scanning of images and transperancy printing on color laserjet Printer.

e) Regular updation of the NCCS website for any changes in the information. Publishing of Tender Notices and advertisements for available posts such as Project Posts, Project Training, Summer Training and other Administrative / supportive staff. Updating individual scientists' webpages.

NCCS Facilities



Confocal Laser Microscopy

The Confocal facility has analyzed about 6140 during the year 2007-08 and about 145 samples belonging to outside organizations. Another Confocal microscope LSM 510 on inverted platform is being installed at the facility and ready for service to NCCS research community.

FACS

There are four FACS equipments under the Central FACS facility of the Institute viz. FACS Vantage, Calibur, Aria and Canto II. Out of these four equipments, FACS Calibur and Canto II were made functional in June 2007. A training session was organized for the research students for operating Calibur. The fluidics of FACS Vantage was upgraded in July 2007.

The three technicians who are operating the four equipments on rotation basis are:

1. Hemangini Shikhare
2. Swapnil Walke
3. Pratibha Khot

The number of samples processed during the period April 2007 to March 2008 on three equipments is summarized below.

Type of equipment	No. of samples acquired		Outside samples	Total
	Immunophenotyping	DNAanalysis		
FACS Vantage	5173	617	Pune Univ. -25	5815
FACS Calibur	3931	404	NIV - 290(CBA) AFMC - 110 Pune Univ - 42	4777
FACS Canto II	3202	62	Nil	3264

A total of 252 samples were acquired for analysis on FACS Aria in the initial days. Later on from July 2007, it was used as a dedicated sterile sorter. During the period under consideration, 101 samples were sorted on UV laser and 183 samples were sorted on Blue or Red lasers. A variety of cells like SP cells, GFP transfected cells and surface stained samples were successfully sorted. A total of 536 samples were processed on Aria.

Moflow FACS Facility

A Moflo cytometer (3 lasers, 9 colours, 11 parameters) is being used for high-speed sorting and analysis of samples.

Total Internal Reflection Fluorescence (TIRF) Microscopy

TIRF is being used for studying surface protein dynamics at single molecule resolution.

Time-lapse Microscopy Live cell imaging

This is being used for studying in vivo dynamics of biological molecules.

DNA Sequencer

During the year 2007-2008 more than 1, 00, 000 samples were processed for sequencing on Automated DNA sequencer.

Publications and Awards

Publications and Patents

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43. Hasan S, Singh S, Parikh RY, Dharne MS, Patole MS, Prasad BLV and Shouche YS. Bacterial Synthesis of Copper/Copper Oxide Nanoparticles. *Journal of Nanoscience and Nanotechnology* (In press).

44. Satlewal A, Soni R, Zaidi M, Shouche Y and Goel R. Comparative Biodegradation of HDPE and LDPE Using an Indigenously Developed Microbial Consortium. *J. Microbiol Biotechnol.* 2008; 18: 477-482.

45. Selvakumar G, Kundu S, Gupta AD, Shouche YS and Gupta HS. Isolation and Characterization of Nonrhizobial Plant Growth Promoting Bacteria from Nodules of Kudzu (Pueraria thunbergiana) and Their Effect on Wheat Seedling Growth. *Curr Microbiol.* 2008; 56: 134-139.

46. Shukla R and Bhonde RR. Adipogenic action of vanadium: a new dimension in treating diabetes. *Biometals.* 2008; 21(2): 205-10.

47. Bavikar SN, Salunke DB, Hazra BG, Pore VS, Dodd RH, Thierry J, Shirazi F, Deshpande MV, Kadreppa S and Chattopadhyay S. Synthesis of Chimeric Tetrapeptide Linked Cholic Acid Derivatives: Impending Synergistic Agents. *Bioorganic & Medicinal Chemistry Letters* (In press).

48. Vaidya AA, Sharma MB and Kale VP. Suppression of p38-stress kinase sensitizes quiescent leukemic cells to anti-mitotic drugs by inducing proliferative responses in them. *Cancer Biol Ther.* 2008; 7: 1-9.

49. Vijayakumar MV and Bhat MK. Hypoglycemic effect of dialyzed fenugreek seeds extract is sustainable and is mediated, in part, by the activation of hepatic enzymes. *Phytotherapy Research* 2008; 22: 500-525.

50. Wani AA, Rangrez AY, Bapat SA, Kumar H, Suresh CG, Barnabas S and Shouche YS. Analysis of reactive oxygen species and antioxidant defenses in complex I deficient patients reveals a specific increase in superoxide dismutase activity. *Free Radical Research.* 2008; 42: 415-427.

51. Yadav VN, Pyaram K, Mullick J and Sahu A. Identification of hot spots in the variola virus complement inhibitor (SPICE) for human complement regulation. *J Virol.* 2008; 82(7): 3283-3294.

Patents

Dr. Manoj Kumar Bhat

Protein based product from Fenugreek seeds that regulates dyslipidemia and obesity, and a process for the preparation thereof.

Manoj Kumar Bhat, Vimal Pandey and Maleppillil Vavachan Vijayakumar Indian, EPO as well as PCT application is being processed by DBT through NRDC.

Patenting is done by NRDC, New Delhi.

Ref. # IPR/4.13.16/07092/2008 Dated April 1st 2008.

Patent application: 1621/DEL/2008 dated 26th June 2008.

Dr. Debashis Mitra

A pharmaceutical composition having virucidal and spermicidal activity.

Syed N. Kabir, Heramba N. Ray, Bikash C. Pal and Debashis Mitra.

U. S. Patent application No. 12/154847 filed on 27th May 2008.

Dr. Samit Chattopadhyay

"Tumor suppressor Activation polypeptide and uses thereof"

Indian patent file number: IPR/4.19.20/06083/2006.

International patent is filed. (PCT#NO7 / 000402).

Memberships / Awards / Fellowships

Memberships & Awards

Dr. Vaijanti P Kale

- Member of International Society for Experimental Hematologists
- Member of International Society for Stem Cell Research

Dr. Lalita S Limaye

- Life member,
  - Indian Society for Cell Biologists
  - Indian Society for Biotechnologists
  - Indian Women Scientists Association
  - Indian Association of Microbiologists
- Fellowships,
  - Awarded DBT short term overseas fellowship for the year 2007

Dr. Nibedita Lenka

- Indian Academy of Neuroscience: Life Member
- International Society for Stem Cell Research (2005 continuing).
- Visiting Scientist (Invited), JSPS-DST Exploratory Exchange under the Indo-Japan Co-operative Program between Japan Society for the Promotion of Science (JSPS), Japan and DST, India (2007).
- International Society for Stem Cell Research (ISSCR) travel award (2008).

Dr. Mohan Wani

- Member of the Executive Committee of Indian Society of Cell Biology for the term April 2007-March 2009.
- Member of CPCSEA for NIV and Raj Biotech, Pune.
- Member of the American Society for Bone and Mineral Research, USA, 2005 onwards.
- Member of International Chinese Hard Tissue Society, 2005 onwards.
- Member of Molecular Immunology Forum, India 2004 onwards.
- Life Member of Indian Society of Cell Biology, 2002 onwards.

Dr. Sharmila A Bapat

- Active Member of American Association of Cancer Research (AACR)
- Active Member of International Society of Stem Cell Research (ISSCR)
- Member - International Epigenetics Society (earlier DNA Methylation Society)
- Member - Indian Association of Cancer Research (AACR)
- Member - Indian Women Scientists Association

Dr. Anjali Shiras

- Member of the Editorial Board for International Journal: Journal of Clinical Rehabitative Tissue Engineering Research 2007-10.
- Member of the International Society of Stem Cell Research (ISSCR), USA

Dr. Ramesh R Bhonde

- Member of Research and Recognition Committee of Mumbai University, Mumbai.
- Interview committee for Scientist 'C' selection at SCTIMST, Trivandrum.
- Examiner for M.Sc. Biotech Devi Ahilya University, Indore.
- Member of Ethical Committee of H. V. Desai Eye Hospital, Hadapsar.
- Member of NCLAS NIN, Hyderabad.

Dr. Debashis Mitra

- Fellow, The National Academy of Science, India
- Executive Member, Microbicide Society of India

Dr. Arvind Sahu

- Member of the International Complement Society (since 1999)
- Member of the Molecular Immunology Forum (since 2005)
- Member of the American Society for Microbiology (since 2005)

Dr. Sanjeev Galande

- Member of American Society for Microbiology (ASM)
- Received the Swarnajayanti Fellowship from the Department of Science and Technology.

Dr. Samit Chattopadhyay

- Fellow, The National Academy of Science, Allahabad, India**Dr. Bhaskar Saha**
- Received the National Bioscience Career Development Award 2007, DBT, India

Dr. Anandwardhan Hardikar

- Received Vidya Vyaas Award, Vidya Bank Association, Pune

Dr. Gopal C Kundu

- Received the "Outstanding achievement award in Oncology" from International Journal of Oncology, Oncology Reports and International Journal of Molecular Medicine

Extramural Funding

Dr. Jomon Joseph

- Molecular characterization of the interaction between the tumour suppressor Adenomatous Polyposis Coli (APC) and the nucleoporin Nup358. **DBT** (2008-2011).

Dr. Vaijanti P Kale

- Harnessing the potential of multi-potent adult stem cells: Development of three dimensional cultures with mesenchymal cells to examine their effects on development of hematopoietic cells. **DBT**
- Identification of molecular mechanisms involved in the adipogenic conversion of hematopoietic cells by mannose specific dietary lectins namely, banana lectin and garlic lectin, and exploration of differentiation inducing capacity of lectins having defined binding characteristics. **DBT**
- Identification of the molecular mechanisms involved in the induction of proliferative responses in primitive hematopoietic cells. **DBT**

Dr. Lalita S Limaye

- *Ex vivo* expansion and cryopreservation of haematopoietic cells: Prevention of apoptosis to improve the outcome. **DRDO-LSRB** (2004-2007).
  Co-Investigator: Dr.V.P.Kale
- Harnessing the potential of stem cells: *In vitro* generation of megakaryocytes and dendritic cells. **DBT** (2005-2008).
  Co-Investigator: Dr.V.P.Kale

Dr. Nibedita Lenka

- *In vitro* differentiation of human embryonic stem cells into neural and non-neural lineages and understanding the underlying genetic basis. **DBT** (2003-2006).

- *In vitro* targeting and functional characterization of ES cell derived dopaminergic neurons and exploration of their therapeutic potential. **DBT** (2004-2008).
- Harnessing the potential of multipotent adult stem cells; Sub project: Exploring the potential of SP cells derived from umbilical cord blood and human bone marrow. **DBT** (2005-2008).

Dr. Vasudevan Seshadri

- Dual role of insulin in regulating the expression of IGFBP-1. **DBT** (2005-08).

Dr. Mohan Wani

- Studies on in vitro differentiation of osteoblasts from human adult stem cells. **DBT** (2005-2008).

Dr. Sharmila A Bapat

- Protein profiling of human ovarian tumor stem cells. **DBT** (2007-2009).

Dr. Anjali Shiras

- Identification and Characterization of Brain Tumor Stem Cells (BTSC) from a novel human cell line - HNGC-2 and elucidation of pathways for its differentiation. **DBT** (2005-2008).
- Program Support Project: Harvesting the potential of multipotent Stem cells Identification, Development and Characterization of long term Neural Stem cell-lines from adult brain tissue. **DBT** (2005-2008).

Dr. Gopal Kundu

- Received Funding from **DBT**, on "Role of Osteopontin, a chemokine like protein in regulation of vascular endothelial growth factor dependent tumor growth and angiogenesis in breast cancer" (2006-2009).
- Received Funding from **DBT**, on "Studies on role of Osteopontin in regulation of transcription factor-mediated matrix metalloproteinase-9 activation, cell motility, tumor growth and metastasis" (2006-2009).
- Received Funding from **DBT**, on "Silencing Osteopontin and its Downstream Oncogenic Molecules Suppress the Tumor Growth and Angiogenesis in Breast Cancer" (2008-2011)

Dr. Ramesh R Bhonde

- Islet Neogenesis from Adult Stem / Precursor cells A step towards regeneration therapy in Diabetes. Ghaskadbi S, ARI, Pune and RR Bhonde, NCCS, Pune. 2004-2007.**DBT**
- Islet immunoisolation with xenotransplantation and stem cell regeneration to islets as strategies for treatment of diabetes. Nair PD, SCTIMST, Thiruvananthapuram and RR Bhonde, NCCS, Pune. 2005-2008. **DBT**
- Characterization and differentiation of pancreatic progenitor cells/stem cells to insulin secreting cells from adult mice pancreatic tissue the role of specific nutrients. Venkateshan V, NIN, Hyderabad and RR Bhonde, NCCS, Pune. 2005-2008. **DBT**
- Harnessing the potential of adult human stem cells: Differentiation / transdifferentiation of stem cells from pancreatic and non pancreatic sources of human origin. RR Bhonde, NCCS, Pune. 2005-2008. **DBT**

Dr. Anandwardhan Hardikar

- Differentiation of human fetal pancreatic progenitor cells for potential use in cell replacement therapy for type 1diabetes. **DBT** (2007-2010).
- Biliary duct stem cells for cell replacement therapy in diabetes. **UKIERI** (2008-2011)

Dr. Yogesh Shouche

- Development of molecular techniques for identification and typing of indegenous probiotic cultures, **DBT** (2005-2008).
- Looking for evidence for life in outer space: Studies on meteor crators, **ISRO** (2003-2006).
- Molecular characterization of microbial and invertebrate diversity of Indian West Coast, **DBT** (2003-2006).
- Investigation of the microflora of insets of Western Ghats for potentially useful bioactive molecules, **DBT** (2004-2007).
- Identification, isolation and characterization of Azo dye degrading genes, **DBT** (2006-2009).

- Expressed Sequence Tag analysis of mosquito genome, **DBT** (2003-2006).
- Cloning, Expression and Production of haeme-proteins by yeast in fermenters for combating nutritional iron deficiency, **DBT** (2005-2008).
- DNA barcoding of butterflies from Western Ghat. 2007-2010.
- DNA Barcoding of Amphibians from Western Ghat 2007-2010.
- Screening for Bio-molecules from microbial diversity collected from different ecological niches, funded by **DBT** (2007-2010)

Dr. Debashis Mitra

- Identification of anti-viral compounds with potential for development of Microbicides to prevent HIV infection and transmission. **DBT** (2006 2009).

  Co-Investigator: Inderpal Singh and KK Bhutani
- Dissecting the CD40L-CD40 mediated signaling pathway in HIV infection.Funding Agency: **DBT-ICMR** (20062009).

  Co-Investigator: Bhaskar Saha and Sekhar Chakrabarti

Dr. Arvind Sahu

- Development of alternative pathway-specific complement inhibitors to block host cell damage. Funded by **DBT** (2006-2009).

  Co-Investigator: Dr. P B Parab.
- Role of vaccinia virus complement control protein in the viral pathogenesis. **DBT** (2007-2010).

  Co-Investigator: Dr. D. Mitra

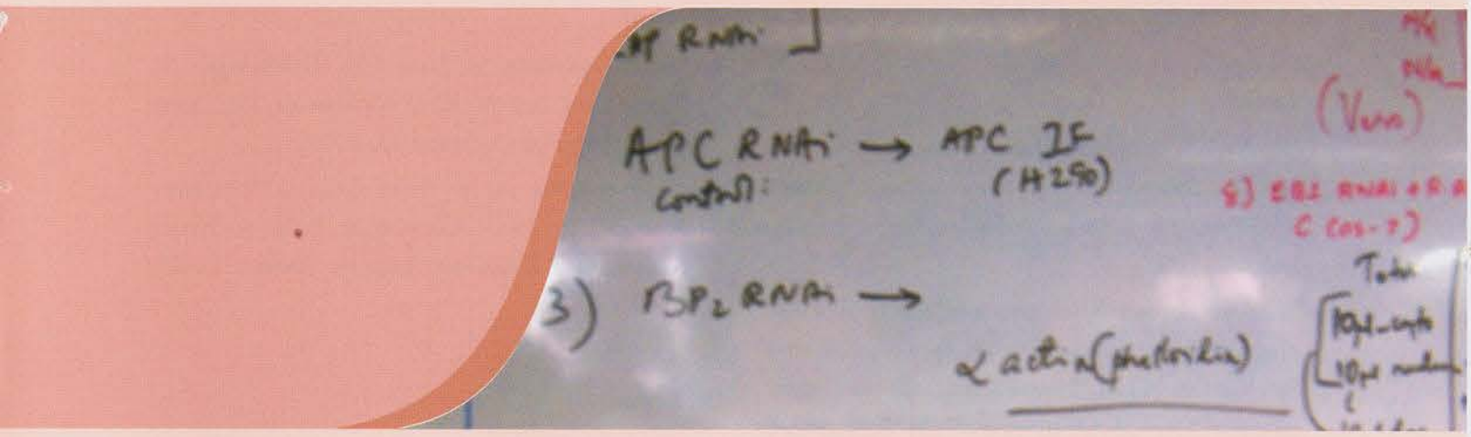
Dr. Samit Chattopadhyay

- Molecular switch in Th1-Th2 response by SMAR1: Its implications in Mycobacterium Tuberculosis infection. **DBT** (2007-2010).
- Regulation of HIV-1 LTR mediated transcription by MAR binding protein SMAR1. **DBT** (2007-2010).
- Indo-foreign Research project "Spatio-temporal expression of SMAR1 and Cux in the CNS. Indo-French project from **ICMR**, New Delhi. 2006-2008.

Dr. Sanjeev Galande

- Chromatin organization and signal transduction by SATB1. International senior research fellowship, **Wellcome trust**, UK. (2005-2010).
- Study of novel nuclear factors. **DBT** (2007-2010).
- SATB1 and T cell apoptosis. **DBT** Bioscience award (2007-2010).
- Systems biology of regulatory networks. **DBT** (2007-2010).

Seminars



Seminars delivered by Visiting Scientists

1. To be, or Not to be CD8+ DC: Role of IRF8 in Dendritic cell Biology by **Dr. Prafullakumar Tailor**, NIH, Bethesda, USA, 18th July, 2007.
2. Role of Transcriptiona Factor TonEBP in Osmoregulation of the Intervertebral Disc Cells, by **Dr. Makarand V. Risbud.**, Assistant Professor, Department of Orthopaedic Surgery and raduate Program in Tissue Eng. & Regenerative Medicine Thomas Jefferson University, USA, 16th August, 2007.
3. Quality control and trafficking of the prion protein and its mutants: implications for future therapeutics by **Dr. Aarthi Ashok**, National Institutes of Health, USA, 6th December, 2007.
4. Nutrition and Cancer: Critical elements for a roadmap by **Dr. John A. Milner**, Chief Nutritional Science Research Group, National Institutes of Health, USA, 7th December, 2007.
5. Curcumin: A journey from exotic spice to anti-cancer drug by **Dr. Gaurishankar Sa**, Scientist, Bose Institute, Kolkata, 17th Dec. 2007.
6. Insights from a reversible transgenic mouse model of RNA toxicity. **Prof. Mani S. Mahadevan**, M.D., FRCP(Canada), Department of Pathology, UVA-Health System, University of Virginia, USA. 10th January, 2008
7. *Drosophila* model for ALS: Human ALS associated mutation in VAPB identifies a dominant negative mechanism by **Dr. Anuradha Ratnaparkhi**, Dept. of Neurology, David Geffen School of Medicine, UCLA, CA, USA, 11th January 2008.
8. Distinct Nucleotide Bound States of the Tandem G-Domains of EngA Regulate Ribosome Binding by **Dr. Balaji Prakash**, Department of Biological Sciences & BioEngineering, Indian Institute of Technology, Kanpur 208016, India. 1st February, 2008.
9. Leishmania: Model For Microbial Virulence, **DR. K. P. Chang,** Department of Microbiology and Immunology Rosalind Franklin University of Medicine and Science, Chicago, USA. 1st February, 2008
10. Genetic association of macular degeneration with ELOVL4 a model approach. **Dr. Gautam Karan**, University of Utah, USA. 24th March, 2008.
11. Mitochondrial Role in Hematopoietic Stem Cell Death and Cancer by **Dr. Sudit S. Mukhopadhyay**, Advinus Therapeutics PVT. Ltd., Pune, 22nd August 2008.

Seminars delivered by NCCS Scientists

Dr. Joseph

- "Microtubules and Cell Polarity". National Institute of Virology, Pune, July 18, 2008.

Dr. Lenka

- "Stem Cells in Development & Therapy". Invited Speaker, Symposium on Stem Cells in Future Medicine. Pt. J.N.M. Medical College, Raipur, India.
- "The developmental enigma and the therapeutic prospects of stem cells". Invited Speaker, National Seminar on Cord Blood Stem cells and their potential Clinical Applications, NRI Academy of Sciences, Guntur, India.
- "Neurogenic and Cardimyogenic differentiation modulation from ES cells in vitro". Invited Speaker and Session Chair, The Joint Meeting for Indo-JSPS Exploratory Exchange under the Indo-Japan Co-operative Program between, RIKEN-CDB, Kobe, Japan.
- "The niche governing the lineage diversification from Embryonic Stem Cells *in vitro*". Invited Speaker, Annual Meeting of Society for Biotechnologists, India and National Symposium on Current Trends in Stem Cell Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India.
- "Envisioning Stem cells from the perspectives of development and therapy". Invited Speaker, Seminar in Advances in Animal Biotechnology, SRTM University, Nanded, India.
- "Extrinsic and intrinsic drivers during Embryonic stem cells differentiation and exploring the therapeutic efficacy of *in vitro* generated cells". Guest Lecture, Central Institute of Fisheries and Aquaculture, Bhubaneswar.

Dr. Seshadri

- "Translational Control in Eukaryotic cells" 13th March, 2008, Recent Trends in Modern Biology Department of Zoology, University of Pune.
- "Glucose Mediated Regulation of Insulin Biosynthesis "15 July, 2008, "Cell Replacement Therapy for Diabetes" A Discussion meeting sponsored by DBT and NCCS, Pune

Dr. Wani

- "Recent advances in Stem Cell Technology" on Foundation day of KNP College of Veterinary Science, Shirval, December 12, 2007.
- "Prospects for use of Stem Cells in Veterinary Practice" in ISVS Convention held at North Maharashtra University, Jalgaon, January 12-13, 2008.

Dr. Bapat

- Bapat S.A. Recruitment of Vascular Stem Cells by Human Ovarian Cancer Stem Cells towards Tumor Angiogenesis at Golden Jubilee Celebrations of Chittaranjan National Cancer Institute, symposium on "Recent Trends in Cancer Research and Treatment", on 1st 3rd November, 2007.
- Bapat S.A. Modeling Cancer Stem Cell Biology in Culture Systems at Panjab Univeristy, Chandigarh on 13th December, 2007.
- Bapat S.A. "Ovarian Cancer Stem Cells" at Maharashtra Institute of Technology, Pune on 21st February, 2008.
- S.A.Bapat. Histone Modification Regulated Gene Expression in Ovarian Cancer Stem Cells at International Symposium on Model Organism and Stem Cells in Development, Regeneration and Disease organized by National Centre for Biological Sciences, TIFR, Bangalore, India between February 23- 25 2008.
- Bapat S.A. "Ovarian Cancer Stem Cells" at Sinhagad College of Engineering, Pune on 8th March, 2008.
- Bapat S.A. "Ovarian Cancer Stem Cell Biology" at 27th Annual Convention of Indian Association of Cancer Research (IACRCON), February 6th 9th 2008 organized at Gujarat Regional Cancer Research Institute, Ahemadabad, India.
- Bapat S.A. Modulation of gene expression in ovarian cancer by active and repressive histone marks at the

AACR Special meeting on "Cancer Epigenetics" at Boston from 27th -30th May, 2008

Dr. Bhat

- Invited talk at Institute of Life Sciences, University of Hyderabad Campus, Hyderabad on 25th April 2008. Title of the presentation: Enhancement of the therapeutic index of chemotherapeutic drugs for improvement in cancer therapy
- Silver jubilee celebrations at Maharashtra Institute of Pharmacy, University of Pune sponsored National Conference on 'Current Scenario & Recent advances in Cancer Therapy' on 21st to 23rd February 2008. Title of the presentation: Enhancement of the therapeutic index of chemotherapeutic drugs for improvement in cancer therapy.
- National Seminar on "Apoptosis and Cancer" on 28th and 29th December, 2007, Department of Biochemistry, Faculty of Science, M. S. University of Baroda. Title of the presentation: Wild type p53: its functional role in cancer cell growth, molecular signaling and chemo sensitivity.
- National Conference on Diabetes and Cancer (Diacan 2007) on 15th and 16th, December, 2007, Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar. Title of the presentation: Enhancement of the therapeutic index of chemotherapeutic drugs for improvement in cancer therapy.

Dr. Shiras

- Recent advances in Nanotechnology at College of Pharmacy, Bharati Vidyapeeth, Pune March, 2008.

Dr. Hardikar

- Oral presentation entitled "Epigenetic changes in multigeneration undernourished rats cannot be reversed by 2 generations of nutrient supplementation". Presented at the Pennington Biomedical research symposia, New Orleans, USA (May 18-20, 2007)
- "MicroRNAs in pancreas development" oral presentation at the India-Australia research initiative meeting in Melbourne, Australia (June 12-14, 2007)
- "A unique microRNA seed sequence is involved in Epithelial-to-mesenchymal transition in human pancreatic islet cells" poster presentation at the 5th International Society for Stem Cell Research (ISSCR) meeting in Cairns, Australia (June 17-20, 2007)
- "Lineage restriction and commitment of pancreatic duct and islet-derived mesenchymal cells". Invited talk at RMIT University, Melbourne, Australia (June 15, 2007)
- "The Thrifty Jerry: A model of chronic multigenerational undernutrition". Oral presentation at the International meeting of Developmental origins of adult diseases held at CCMB, Hyderabad. (December 7-8, 2007)
- "Regulation of cellular plasticity in pancreatic progenitor cells" Oral presentation at International meeting on Phenotypic plasticity, Estuary Islands, Trivendrum (December 17-21, 2007)
- "microRNAs regulate epithelial-to-mesenchymal transition in human fetal islet cells" oral presentation at 12th ADNAT meeting, CCMB, Hyderabad (February 23-24, 2008)
- "Isolation of RNA and gene expression analysis from single cells using laser catapulting microscopy" presented at LPC meeting, Tübingen, Germany (March 12-16, 2008)

Dr. Shouche

- "Microbial Diversity of Lonar Lake", at Annual Conference of Association of Microbiologists of India, at Chennai, December 2007

Dr. Mitra

- Heat shock proteins: Role in HIV-1 gene expression and replication, Invited Talk delivered at Department of Pharmacology, Loyola University Medical Center, Chicago, USA, on 7th November 2007.
- Heat shock proteins: Role in HIV-1 gene expression and replication, Invited Talk delivered at Department of Biochemistry and Molecular

Biology, Uniformed Services University of Health Sciences, Bethesda, Maryland, USA on 21st November 2007.

- Identification of a novel anti-HIV-1 molecule from a Marine Bivalve, Invited Talk at National Conference on "Marine Biology to Marine Biotechnology: Current status, challenges and opportunities", 18-20 January 2008, The D. G. Ruparel College, Mahim, Mumbai, India.
- Heat shock proteins interact with viral proteins and regulate HIV-1 gene expression and replication, Invited Talk at Indo-Brazil Meeting On infectious diseases, January 25 27, 2008, JNCASR, Bangalore, India.**Dr. Sahu**
- "Complement: a viral target for immune evasion" UGC Sponsored National Seminar on Microbiological Developments and Biochemical Research, LAD College for Women of Arts, Commerce and Science, Nagpur, November 29, 2007.
- "Structural basis for potent complement regulatory activity of SPICE, the variola virus complement regulator", 16th Molecular Immunology Forum, Institute of Microbial Technology, Chandigarh,. March 16, 2008.

Dr. Samit

- Invited talk at ICPO, Noida, ICMR institute. Title "Cancer and Chromatin; Role of tumor suppressors", 25th February, 2008
- Invited speaker "Tenth Asian conferences on Transcription", Arranged by IISC, JNCASR and NCBS. January 12-16, 2008
- Invited speaker "Recent trends in cancer research and treatment". Chttaranjan National cancer institute, Kolkata, November 1-3, 2007
- Invited speaker " Emerging trends in Cytokeratin biology" International symposium on Intermediary filament proteins, ACTREC, Mumbai, 29th September, 2007
- Sreenath K presented talk on "Repression of HIV-1 LTR mediated transcription by SMAR1 promotes proviral latency" in 17th International AIDS conference, Mexico City, Mexico, August 3-8.
- Pavithra L presented talk on "Dual mechanism of p53 stabilization by tumor suppressor protein SMAR1" in XXXI All India Cell Biology Conference & Symposium on Stem Cells: Application and Prospects, December 14 - 16, 2007, BHU, Varanasi

Dr. Galande

- "Global regulator SATB1: From consensus sequence to consequence", July 3, 2007, Centre for Cellular and Molecular Biology, Hyderabad.
- "The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1" October 25, 2007, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore.
- "Recent Developments in Chromatin Biology and Genomics", December 12, 2007, Institute of Life Sciences, Bhubaneshwar.
- "The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1" December 14, 2007, Banaras Hindu University.
- "Global regulator SATB1 recruits •-catenin and mediates Wnt/•-catenin response". ACT IX meeting, January 2008, IISC/NCBS/JNCASR Bangalore.
- Lecture on "Molecular profiling" delivered at the Moving Academy of Medicine and Biomedicine, February, 2008.
- "Global regulator SATB1 recruits •-catenin and mediates Wnt/•-catenin response", International Meeting on 'Gene Regulation & Signaling in Immune System', Cold Spring Harbor Laboratory, New York, April 23, 2008.
- "The third dimension of gene regulation: It's all in the looping!" Invited talk delivered at the following institutions:
  i. Department of Biochemistry, Nagpur, Dec. 2007.
  ii. Epigenetics Interest group, University of California, Davis, February 22, 2008.
  iii. Indo-UK Frontiers of Science meeting, Ramoji city, Hyderabad, March 5, 2008.

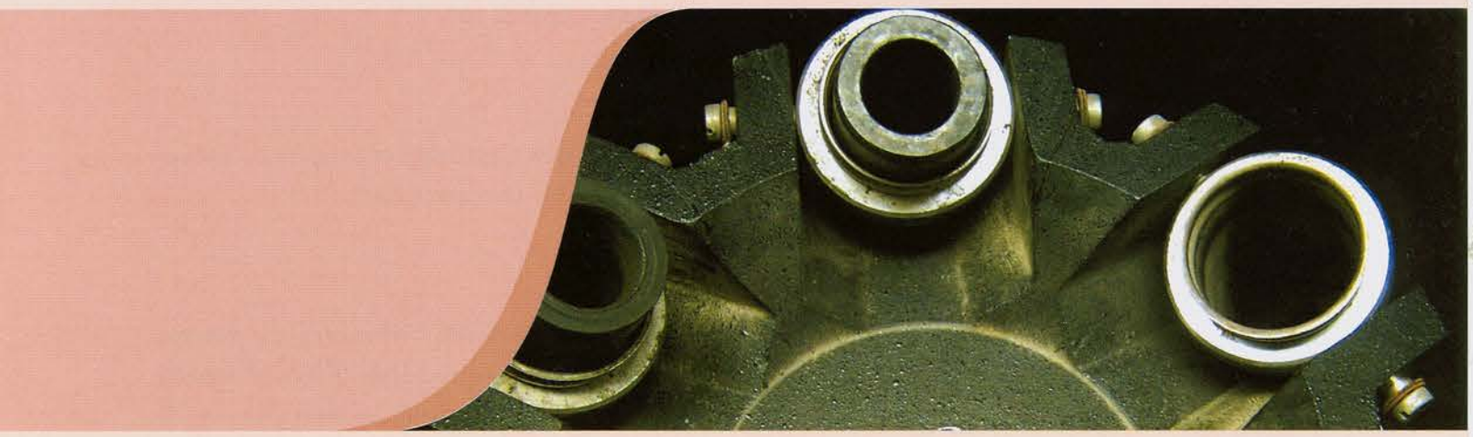
iv. Life Science Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, February 26, 2008.

v. Rockefeller University, New York, April 30, 2008.

Dr. Bhonde

- Pune Radio Talk on 'Stem cell therapy'. Jan 3, 2008.
- Symposium on Diabetes and Therapeutic Intervention held at Singhgad Institute of Pharmacy, Pune. Feb 2-5, 2008.
- 'Stem cells in islet neogenesis' at the symposium on Recent Trends in Pharmaceutical Sciences and Technology (Vision 2020) at the JSPM's Charak College of Pharmacy and Research, Wagholi. Feb 2-6, 2008.
- 'In vitro models for toxicity testing of metal nanoparticles' at the workshop on nanotechnology in Advanced Drug Delivery. Feb 6, 2008.
- 'Alternative to Animal Experimentation' at Pharmatech 2008, Departmnet of Pharmacology and Therapeutics, KEM Hospital, Mumbai. Feb 8, 2008.
- 'Role of Animal Tissue Culture in Tissue Banking and Engineering' at the Dept of Biotechnology, D.Y. Patil college, Pimpri. Feb 12, 2008.
- 'Unconventional therapies in diabetes' at the seminar on Drug Discovery, Development and Delivery. D.Y. Patil College of Pharmacy, Pimpri. Feb 15-16, 2008.
- 'Stem cell pool Trash to Treasure' at the National Symposium on Emergence of Mordern Techniques and Development in Business, Modern College. Feb 16, 2008.
- 'Stem cells and Regenerative medicine in "Science Day Talk" at DRDO, Dighi, Pune. Feb 28, 2008.
- 'Stem cells and regeneration' Sinhagad College of Engineering, Pune. March 8, 2008.

Conferences / Workshops



Conferences Attended

Dr. Limaye

Abstract accepted for a poster presentation entitled as "a simple method for generation of functional dendritic cells from cord blood CD34+ cells", B.Sreekumar, V. Kale and L. Limaye, National Centre for Cell Science, Pune, at the 6th ISSCR Annual Meeting held on June 11- 14, 2008, at Philadelphia USA.

Dr.Lenka

M. K. Verma, N. Lenka. 2008. Contextual modulation of BMP4 by Wnt and the specific threshold of each determining mesodermal and cardiac cell fate. International Society for Stem Cell Research (ISSCR) 6th Annual Meeting, Philadelphia, USA.

Dr. Wani

ISVS Convention held at North Maharashtra University, Jalgaon, January 12-13, 2008.

Dr. Shiras

Participated in the June -2007 : International Meeting on "Asia-Pacific Networks: promoting excellence in research" under the support of Japan Society for the Promotion of Science (JSPS) organized by Nature Publishing group at Tokyo, Japan.

Dr. Galande

1. Annual Cell Biology Meeting, December 14-16, 2007, Banaras Hindu University, Varanasi.
2. Guha Research Conference, December 18-22, 2007, Kolkata.
3. Graduate Students Meet, Trends in Life Sciences, December 29-30, 2007, ACTREC, Kharghar.
4. International conference "Asian conference on Transcription", January 11-13, 2008, IISc/NCBS/JNCASR, Bangalore.
5. Second Indo-American Frontiers of Engineering Symposium (IAFOE), held at The National Academies' Beckman Center in Irvine, California, February 27-March 1, 2008.
6. India-UK Frontiers of Science Symposium organized by the Royal Society, London and INSA, March 4-7 2008, Hyderabad.
7. Cold Spring Harbor Meeting on 'Gene Regulation & Signaling in Immune System' held during April 22-26, 2008, CSHL, New York.

Dr. Sahu

1. UGC Sponsored National Seminar on Microbiological Developments and Biochemical Research, LAD College for Women of Arts, Commerce and Science, Nagpur, November 29-30, 2007.

2. 16th Molecular Immunology Forum, Institute of Microbial Technology, Chandigarh, March 14-16, 2008.

Dr. Mitra

1. AIDS in India 2007 Workshop, 8th-13th July 2007, JNCASR, Bangalore, India.
2. National Conference on "Marine Biology to Marine Biotechnology: Current status, challenges and opportunities", 18-20 January 2008, The D. G. Ruparel College, Mahim, Mumbai, India.
3. Indo-Brazil Meeting On infectious diseases, January 25 27, 2008, JNCASR, Bangalore, India.
4. Microbicides 2008 International conference, 24-27th February 2008, Ashoka Hotel, New Delhi, India.

Dr. Bapat

Sharmila Bapat, Anjali Kusumbe, Avinash Mali. Ovarian cancer stem cells recruit CD133 expressing stem cells towards the establishment of an endothelial hierarchy that mediates long term tumor angiogenesis, at the ISSCR Annual meeting at Philadelphia from 10th 14th June, 2008.

Dr. Hardikar

1. Pennington Biomedical Research Conference, New Orleans, USA (May 18-20, 2007)
2. India-Australia research initiative meeting in Melbourne, Australia (June 12-14, 2007)
3. 5th International Society for Stem Cell Research (ISSCR) meeting in Cairns, Australia (June 17-20, 2007)
4. International meeting on Developmental Origins of Adult Diseases held at CCMB, Hyderabad. (December 7-8, 2007)
5. International meeting on Phenotypic plasticity, Estuary Islands, Trivendrum (December 17-21, 2007)
6. 12th ADNAT meeting, CCMB, Hyderabad (February 23-24, 2008)
7. LPC meeting, Tübingen, Germany (March 12-16, 2008)

Dr. Shouche

1. Second International Barcode of Life Conference and Regional Barcode meet in Taipei, Taiwan, September, 2007
2. Annual Meeting of Association of Microbiologists of India, Chennai, 2007

Dr. Samit

Guha Research Conference at Sundarban, Kolkata from 21st to 24th December, 2007.

Dr. Bhonde

1. Kadam SS, Bhonde RR, "Wharton's jelly jells into islets". Poster Presentation at the 5th ISSCR Meeting held at Cairns, Queensland, Australia, June 16-20, 2007.
2. Kadam SS, Bhonde RR, "Wharton's jelly jells into islets". Poster Presentation at the Beta Cell Workshop, University of Bath, U.K. June 26-28, 2007.
3. Phadnis SM, Joglekar MV, Ghaskadbi SM, Hardikar AA, Bhonde RR. Enrichment of islet like cells from human bone marrow for cell replacement therapy in diabetes. Poster presentation at 31st All India Cell Biology Conference held at Varanasi. Dec14-16, 2007.

Conferences / workshops attended by students

i. S. K. Ramasamy, N. Lenka. 2007. Notch orchestrates neural differentiation from mouse embryonic stem cells. Poster presentation during the International Symposium on Model Organisms and Stem Cells in Development, Regeneration and Disease, National Centre for Biological Sciences, Bangalore, India.

ii. Rupesh Srivastava: 34th Indian Immunology Conference held at National AIDS Research Institute, Pune, December 16-18, 2007.

iii. Navita Gupta: 34th Indian Immunology Conference held at National AIDS Research Institute, Pune, December 16-18, 2007.

iv. Sandeep Singh presented poster on "SMAR1 regulates Cytokeratin 8 expression by displacing p53 from cognate site" in International symposium on Intermediary filament proteins, 29th Spetember, 2007, ACTREC, Mumbai

v. Sandeep Singh presented poster on "SMAR1, a novel inhibitor of AKR1a4 enzymatic activity" in XXXI All India Cell Biology Conference & Symposium on Stem Cells: Application and Prospects, December 14 - 16, 2007, BHU, Varanasi

vi. Surajit Sinha presented poster in 10th Asian Conference on transcription 13th 16th January, IISc, Banglore "Functional cross-talk between tumor suppressor PML and SMAR1 regulates P53 acetylation and modulates cell cycle arrest and apoptosis through matrix attachment region."

vii. Sunil Kumar Malonia and Nidhi Chaudhry attended 4th winter school in Immunology, July 31st 5th August, Cochin, Kerala

viii. Amita Limaye: attended Hands on workshop on Multiphoton and Spectral Confocal Laser Scanning Microscopy held at the National Centre for Ultrafast Processes, University of Madras Taramani Campus, Chennai from August 20 22, 2007.

ix. Prabhat Kumar Purbey attended and presented a poster at the Abcam International conference on 'Chromatin structure and function', held in Antigua during 27th October to 3oth November 2007.

x. Dimple Notani and Kamal Gottimukkala attended Graduate Students Meet, Trends in Life Sciences, December 29-30, 2007, ACTREC, Kharghar. Dimple Notani delivered oral presentation at this meeting.

xi. Sunita Singh attended the 'Gene Expression and Analysis Workshop' arranged by ILS and Agilent Technologies at their Gurgaon facility during February 25-27, 2008.

xii. Kamal Gottimukkala attended the 'Winter School in Immunology' held at Ernakulum during July 30-August 5, 2008.

xiii. AIDS in India 2007 Workshop-Symposium, 8th-13th July 2007, JNCASR, Bangalore, India. Workshop Attended by Sudeep Sabde.

xiv. Symposium oral presentation by Pratima Rawat. Studies on the role of Heat Shock Proteins in HIV-1 pathogenesis.

xv. Swati P. Jalgaonkar, Nawneet K. Kurrey, Sharmila A. Bapat. Oral presentation entitled "Modulation of Gene Expression by Snail and Slug in Epithelial Ovarian Cancer" at 27th Annual Convention of Indian Association of Cancer Research (IACRCON), February 6th 9th 2008 organized at Gujarat Regional Cancer Research Institute, Ahemadabad, India.

xvi. Anjali Kusumbe, Avinash, Sharmila A. Bapat. Poster entitled "Identification and characterization of hierarchy established by Endothelial Stem Cell population that ensures long-term tumor angiogenesis" at International Symposium on Model Organism and Stem Cells in Development, Regeneration and Disease, February 23rd 25th 2008 organized by National Centre for Biological Sciences, TIFR, Bangalore, India

xvii. Nawneet K. Kurrey, Swati P. Jalgaonkar, Sharmila A. Bapat. Poster entitled "Modulation of cancer progression by Snail and Slug in epithelial ovarian cancer" at International Symposium on Model Organism and Stem Cells in Development, Regeneration and Disease organized by National Centre for Biological Sciences, TIFR, Bangalore, India between February 23- 25 2008.

xviii. "Clonally Derived Human Fetal Pancreatic Islet Cells Undergo Reversible Epithelial To Mesenchymal Transition (EMT) To Generate Islet-hormone Producing Cell Aggregates" " poster presentation by Mugdha V. Joglekar at the 5th International Society for Stem Cell Research (ISSCR) meeting in Cairns, Australia (June 17-20, 2007)

xix. "Differentiation of Adult Human Bone Marrow-derived Mesenchymal-like Cells to Islet-like Cell Aggregates" poster presentation by Smruti M Phadnis at the 5th International Society for Stem Cell Research (ISSCR) meeting in Cairns, Australia (June 17-20, 2007)

xx. 12th ADNAT meeting, CCMB, Hyderabad (February 23-24, 2008) attended by Mugdha V. Joglekar, Amaresh Kumar Ranjan and Vishal S. Parekh.

xxi. International meeting on Developmental Origins of Adult Disease held at CCMB, Hyderabad attended by Amrutesh Puranik and Sarang Satoor

Ph.D. awarded during 2007-2008

Ravi Shukla

Thesis Title: Studies on assessment of biocompatibility of gold and silver nanoparticles in cell culture for tissue engineering applications.

Guide: Dr. Ramesh R. Bhonde, Co-Guide: Dr. Murali Shastry

Meghana Kanitkar

Thesis Title: Regenerative and Protective mechanisms of maintenance of beta cell homeostasis.

Guide: Dr. Ramesh R. Bhonde, Co-Guide: Dr. Sanjeev Galande.

Shalini Gupta

Thesis Title: Studies on Immune Response to Human Immunodeficiency Virus -1 Proteins using DNA Immunization.

Guide: Dr. Debashis Mitra, Co-Guide: Dr. Bhaskar Saha

D. S. Ravi

Thesis Title: Identification and Characterization of Anti-HIV Activity from marine Bivalves.

Guide: Dr. Debashis Mitra

Archana Jalota

Thesis Title: Role of Tumor suppressor Protein SMAR1 as a cell cycle regulator and an Immuno-modulator in directing T-Helper (TH) Lineage Commitment

Guide: Dr. Samit Chattopadhyay

Kamini Singh

Thesis Title: P53 Mediated Regulation of SMAR1 and their Co-ordinated Roles in Tumorigenesis through modulation of NFkB and TGFB Target Gene Expression.

Guide: Dr. Samit Chattopadhyay

Varshiesh Raina

Thesis Title: "Regulation of Diabetes by stress: role of novel protein SMAR1 and p53 ".

Guide: Dr. Pradeep B Parab, Co-guide: Dr. Samit Chattopadhyay

Akhilesh Kumar Singh

Thesis Title: Molecular cloning, expression, characterization and structure-function analysis of Herpesvirus saimiri complement control protein homolog.

Guide: Dr. Arvind Sahu

Aslam Ali Khan

Thesis Title: Mechanism of Staphylococcal Enterotoxin B (SEB). Induced Toxic Shock Syndrome (TSS) in Bai B/C Mice

Guide: Dr. Bhaskar Saha

Varsha Shepal (Staff)

Thesis Title: Molecular and Cellular Studies of A Novel Non-coding RNA with Transforming Potential.

Guide: Dr. Anjali Shiras

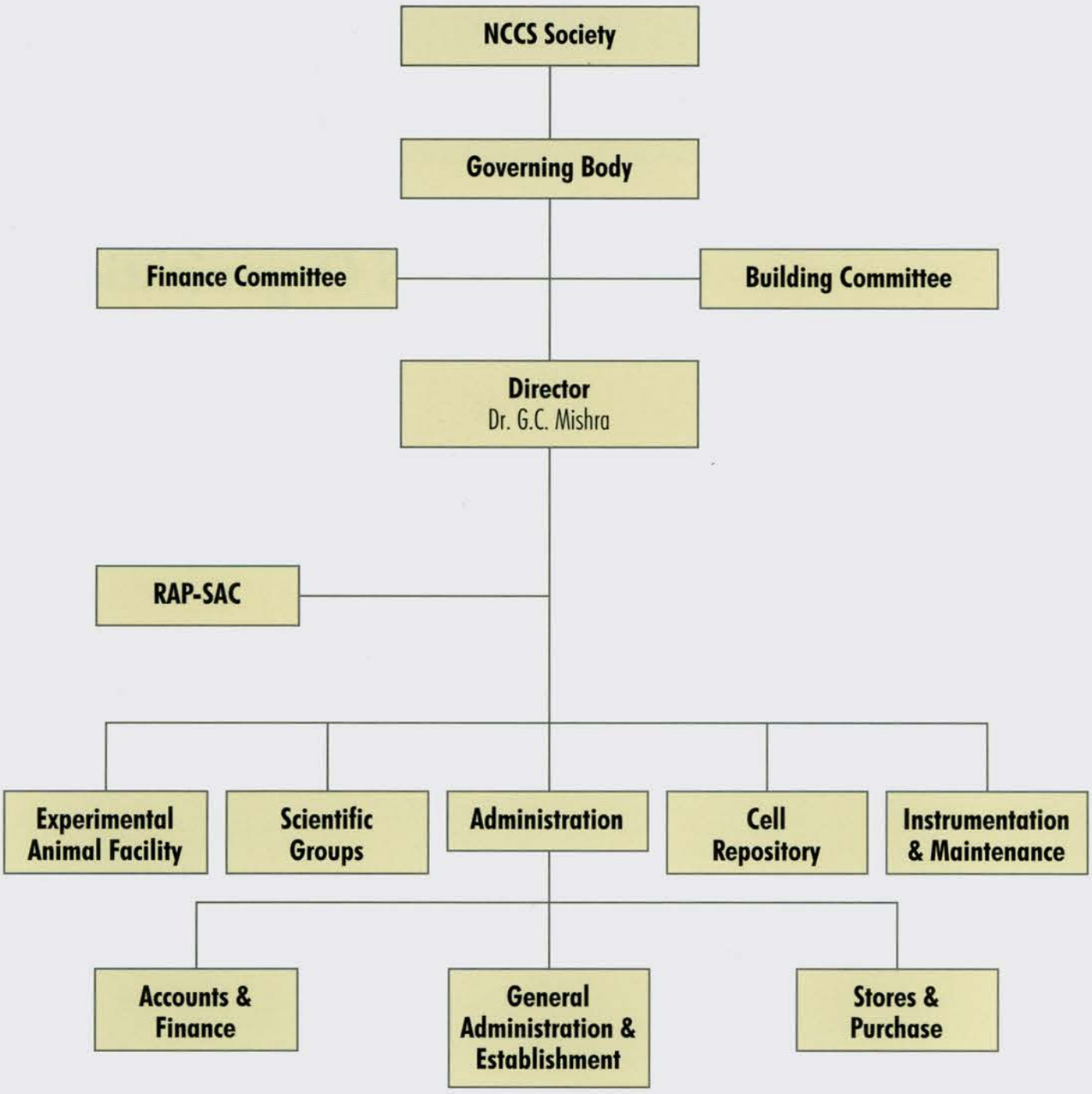
Samar Roy Chowdhury (Staff)

Thesis Title: A comparative study of Materials and Management practices in select biotech organizations under the Ministry of Science & Technology (GOI).

Guide: Dr. Anil Keskar, PUMBA, University of Pune

NCCS Organisation

NCCS Organisation



NCCS Committees

Society

1.	**Shri Kapil Sibal** Hon'ble Minister for Science & Technology & Earth Sciences, Anusandhan Bhawan, Rafi Marg, New Delhi - 110 001	President
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3.	**Prof. N.K. Ganguly** Distinguished Biotechnologist, National Institute of Immunology, (THSI), Aruna Asaf Ali Marg, New Delhi - 110 067.	Member
4.	**Dr. C.M. Gupta** Distinguished Biotechnologist, Central Drug Research Institute, Chattar Manzil, Post Box No. 173, Lucknow - 226 001	Member
5.	**Prof. Narendra Jadhav** Vice Chancellor, University of Pune Ganeshkhind, Pune - 411 007.	Member
6.	**Shri K.P. Pandian** Joint Secretary & Financial Adviser, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi - 110 003	Member

7.	**Shri Sukhdeb Sinha** Adviser, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi - 110 003	Member
8.	**Dr. Lal Krishna** Asst. Director General (Animal Health), Dept. of Agricultural Research & Education And Indian Council of Agricultural Research, Krishi Bhavan, Dr.Rajendra Prasad Road, New Delhi - 110 001.	Member
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10.	**Dr. Vijay Raghavan** Centre Director, National Centre for Biological Sciences, Tata Institute For Fundamental Research, GKVK, Bellary Road, Bangalore - 560 065	Member
11.	**Dr. Padma Shastry,** Scientist 'F' National Centre for Cell Science, Ganeshkhind, Pune - 411 007.	Member
12.	**Dr. G. C. Mishra** Director, National Centre for Cell Science, Ganeshkhind, Pune - 411 007.	Member Secretary

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   Secretary,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi - 110 003

2. **Prof. N.K. Ganguly** — Member
   Distinguished Biotechnologist ,(THSI),
   National Institute of Immunology ,
   Aruna Asaf Ali Marg,
   New Delhi - 110 029.

3. **Dr. C.M. Gupta** — Member
   Distinguished Biotechnologist,
   Central Drug Research Institute,
   Chattar Manzil, Post Box No. 173,
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7. **Dr. Lal Krishna** — Member
   Asst. Director General (Animal Health),
   Dept. of Agricultural Research & Education
   And Indian Council of Agricultural Research,
   Krishi Bhavan, Dr.Rajendra Prasad Road,
   New Delhi - 110 001.

8. **Prof. W.N. Gade** — Member
   Head,
   Department of Biotechnology,
   University of Pune,
   Ganeshkhind,
   Pune - 411 007.

9. **Dr. Vijay Raghavan** — Member
   Centre Director,
   National Centre for Biological Sciences,
   Tata Inst. For Fundamental Research,
   GKVK, Bellary Road,
   Bangalore - 560 065

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

11. **Dr. G. C. Mishra** — Member Secretary
    Director
    National Centre for Cell Science,
    Ganeshkhind,
    Pune - 411 007.

Finance Committee

1. **Shri K.P. Pandian** — Chairman
   Joint Secretary & Financial Adviser,
   Department of Science & Technology,
   Technology Bhavan,
   New Mehraulli Road,
   New Delhi - 110 016

2. **Dr. R.S. Paranjape** — Member
   Director,
   National Aids Research Institute,
   G-Block, P-73,
   Near Electronic Sadan,
   Bhosari,
   Pune - 411 026

3. **Dr. C.M. Gupta** — Member
   Distinguished Biotechnologist,
   Central Drug Research Institute,
   Chattar Manzil, Post Box No. 173,
   Lucknow - 226 001

4. **Dr. Akhil C. Banerjea** — Member
   Staff Scientist,
   National Institute of Immunology,
   Aruna Asaf Ali Road,
   New Delhi - 110 067

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   NCCS,
   Pune - 411 007

6. **Shri Sukhdeb Sinha** — Special Invitee
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodi Road,
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Building Committee

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   Dr. Ambedkar Road,
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   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
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   National Institute of Immunology,
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   Navi Mumbai

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   Block No. 2, 7th Floor,
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8. **Dr. G.C. Mishra** — Member
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2. **Prof. Avadhesha Surolia** — Member
   Director,
   National Institute of Immunology,
   Aruna Asaf Ali Marg,
   New Delhi - 110 067.

3. **Dr. A. N. Bhisey** — Member
   7, Yugprabhat Society,
   ST Road, Mahim,
   Mumbai - 400 016.

4. **Prof. Anil Tyagi** — Member
   Department of Biochemistry,
   University of Delhi South Campus,
   Benito Juarez Road,
   New Delhi - 110 021.

5. **Dr. Anuradha lohia** — Member
   Department of Biochemistry,
   Bose Institute,
   P-1/12, CIT Scheme, VII M,
   Kolkatta - 700 054.

6. **Dr. C. M. Gupta** — Member
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   Central Drug Research Institute,
   Chattar Manzil,
   Lucknow - 226 001

7. **Dr. J. Gowrishankar** — Member
   Director,
   Centre For DNA Fingerprinting & Diagnosis,
   ECIL Road, Nacharam,
   Hyderabad - 500 076

8. **Dr. Kanuri Rao** — Members
   International Centre for Genetic
   Engineering & Biotechnology
   NII Campus, Aruna Asaf Ali Marg
   New Delhi - 110 067

9. **Shri Sukhdeb Sinha** — Member
   Adviser,
   Department Of Biotechnology,
   Block-2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi - 110 003.

10. **Prof. Samir Bhattacharya** — Member
    Department of Zoology,
    Visva Bharati University,
    Shantiniketan - 731 235.
    West Bengal.

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    National Institute of Immunology
    Aruna Asaf Ali Marg
    New Delhi - 110 067

12. **Dr. Jyotsna Dhawan** — Member
    Scientist,
    Centre for Cellular and Molecular Biology,
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    Hyderabad - 500 007.

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    Director
    National Centre for Biological Sciences
    Tata Institute of Fundamental Research
    GKVK, Bellary Road,
    Bangalore - 560065

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    Director,
    Institute of Life Sciences,
    Nalco Square, Chandrasekharpur
    Bhubaneshwar - 751 023

15. **Dr. Soniya Nityanand** — Member
    Scientist,
    Sanjay Gandhi Post Graduate Institute of
    Medical Sciences,
    Immunology Division,
    Raebareli Road
    Lucknow - 226 014

Administration

The NCCS Administration consists of General Administration and Establishment, Accounts and Finance and Stores & Purchase sections. The centre has its Instrumentation & Maintenance unit as well. All these Sections are providing support services to the main scientific activities of the centre.

As on date the centre is having the following staff strength.

Scientists	:	26
Administrative	:	40
Technical	:	57
Total	:	123

In addition, NCCS has 146 Research Fellows / Project Assistants.

RESERVATION POLICY

NCCS is following Govt. of India orders on reservation matters. For the recruitment we follow respective rosters; 15% to SC, 7.5% to ST and 27% to OBC on All India Basis by open competition. The Centre is also observing Govt. 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 B.G. Acharya, Controller of Administration, as CPIO for Administrative matters and Dr. D. Mitra, Scientist 'E', as CPIO for Scientific matters. Shri A.D. Patil has been nominated as ACPIO and Dr. G.C. Mishra, Director, has been nominated as Appellate Authority.

IMPLEMENTATION OF OFFICIAL LANGUAGE

NCCS has constituted a Committee, which meets every month pursuing Govt. of India orders in the matter for implementation of official language in day to day work. Maximum staff members have passed Hindi Pragya Examination. The centre also observes Hindi Pakhawada every year. Essay and letter writing competitions were held and winners were given cash awards. Guest lecturers on Hindi day were arranged. Official language activities are strongly supported by the Director.

VIGILANCE MATTERS

Dr. Bhaskar Saha, Scientist 'E', is the Chief Vigilance Officer of the centre. Vigilance reports are sent to the nodal ministry i.e. Department of Biotechnology, New Delhi, regularly.

SECURITY

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

DISCIPLINARY MATTERS

The centre follows CCS (Conduct / CCA) rules and NCCS Bye-laws for maintaining the discipline at the centre.

COMMITTEES

The centre is also assisted by various committees such as;

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