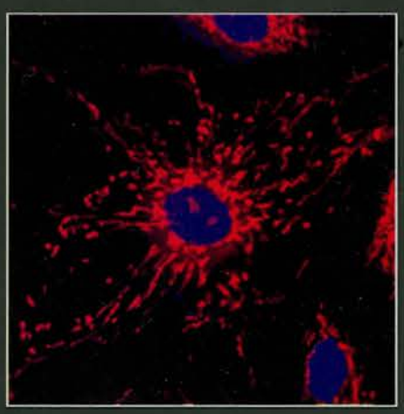
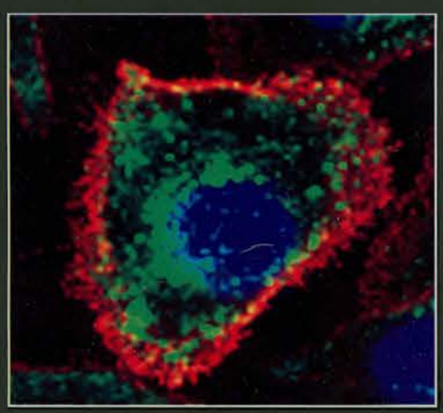
nccs

National Centre for Cell Science



Annual Report

2006 . 2007



National Centre for Cell Science

Annual Report 2006–2007

Contents

Index of Research Reports

The Terms of Reference

- 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 of the above facility.

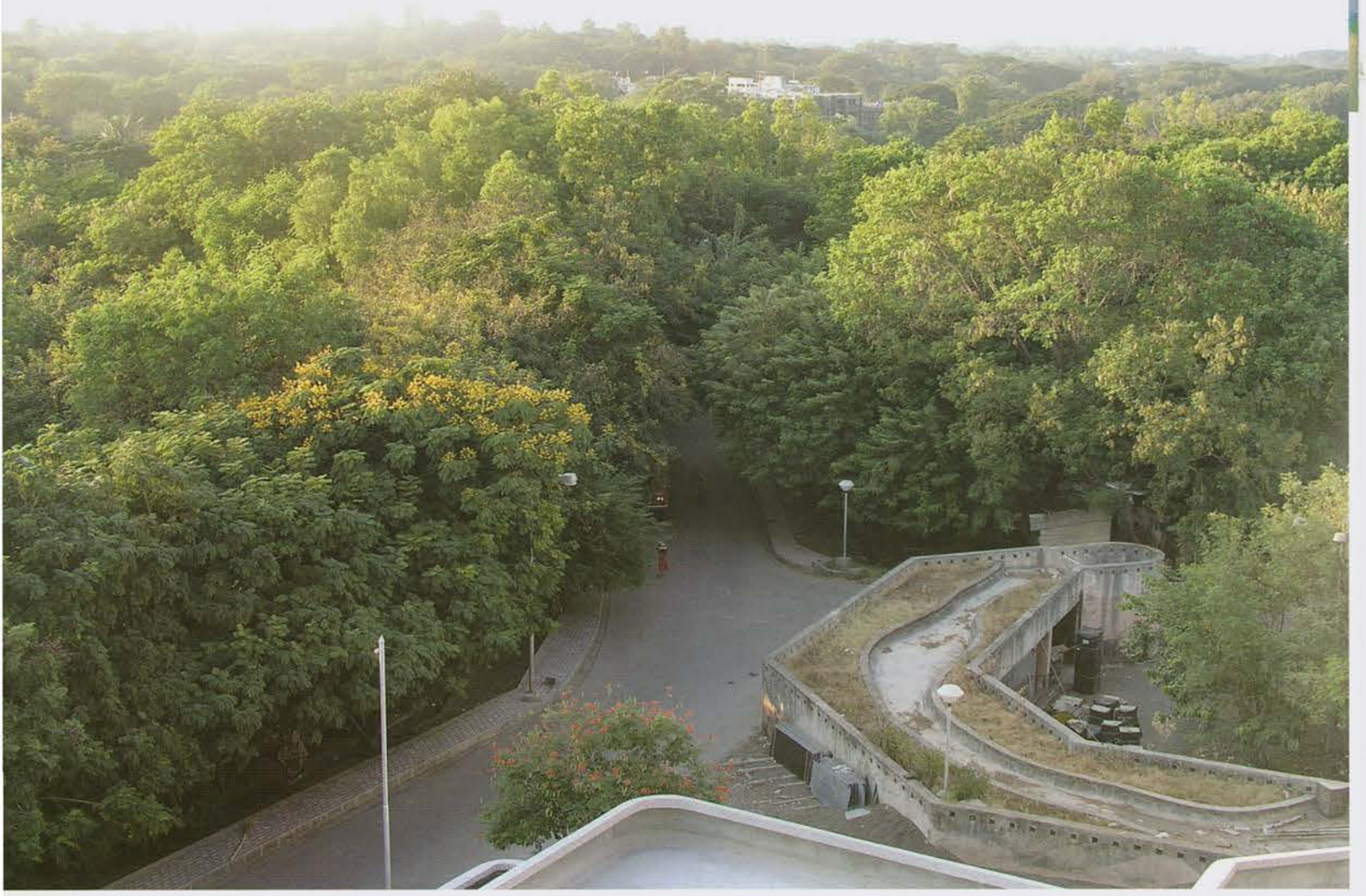
- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

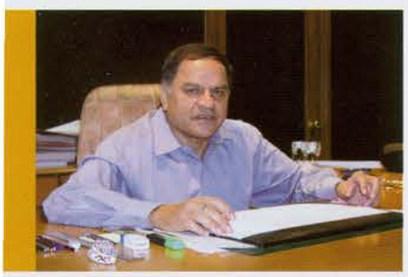
- To serve as National Reference Centre for tissue culture, tissue banking and cell products and data bank etc., and to provide consultancy services to medical, veterinary and pharmaceutical institutions, public health services and industries etc. in the country.

- 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 the Director's Desk...

It is time again to reflect on the progress we made during the last year . The Annual Report of the National Centre for Cell Science (NCCS) for the year 2006-2007 is an attempt to portray the recollections and reflections of activities in the previous year and the goals for the future. The NCCS mission encompasses tripartite functioning of the institute as; a National Cell Repository, Research & Development and Human Resource Development. Over the last year, the NCCS Repository has supplied 1400 cell lines comprising of 152 different cell types to 130 scientific institutions in India. Further, under our "Teaching and Training" programs, we have conducted workshops on "Basic Techniques in Animal Tissue Culture" on site for 70 researchers at Indore and Kolhapur.

In the area of Cell Biology, our work reveals that Nup358, a nuclear pore protein, associate with and regulate interphase microtubules. Functional significance of this unexpected connection is being explored. Investigation of the mechanism of glucose-stimulated activation of insulin mRNA translation has led to the identification of a minimal 29-nucleotide element in the 5'UTR of the insulin mRNA that is necessary and sufficient for its glucose-dependent translation regulation. Further, a 60 kDa protein that binds to this element in a glucose-dependent manner has been identified and its potential role in translation regulation is being investigated.

Nitric oxide (NO) is an important messenger that mediates various cellular responses. Endothelial nitric oxide synthase (eNOS), an enzyme involved in the generation of NO has been localized to the nuclei of marrow derived stromal cells and appears to have an important role in the cell's physiology. Its nuclear localization signal has been identified and characterized.

One of the major areas of research at NCCS is stem cell biology. The envisaged potentials of stem cells are being investigated at different levels. Embryonic stem (ES) cells have a unique property to differentiate into all three germ layer derivatives. ES cells have been successfully differentiated into dopaminergic neuronal cells. Transplantation of these cells into a hemi-parkinsonian rat model, that we developed, results in behavioral recovery, thus indicating the therapeutic potential of the ES-derived cells.

Use of stem cells has been envisaged in the treatment of osteoporosis and other skeletal diseases, where the regeneration potential of adult human bone is limited. In this direction, we have isolated mesenchymal stem cells (MSCs) from human bone marrow and standardized the procedure for differentiation into bone forming osteoblasts *in vitro*. At present, we are studying the role of parathyroid hormone in this differentiation process. An important aspect in the use of stem cells in therapy is the *in vitro* expansion

and cryopreservation of stem cells. We show that the anti-apoptotic agents, zVADfmk and calpain inhibitor, have a beneficial effect on ex-vivo expansion and recovery after cryopreservation of hematopoetic stem cells. Further, investigations into the conditions for enrichment, freezing and ex-vivo expansion of cord blood derived progenitors of megakaryocytes and dendritic cells are in progress. Results from these investigations will assist in betterment of thrombocytopenia therapy and dendritic cell tumor therapy.

The long-term survival and metastasis of tumors are determined by their potential to establish an efficient vasculature. Studies with an *in vitro* model of human ovarian cancer indicate that vascular progenitor cells recruited to the tumors establish a complete endothelial hierarchy, thus enabling tumor vascularization. Our studies on novel genes involved in transformation led us to the identification of mouse melanoma gene M3TR. We show that the human homologue of M3TR is involved in induction and maintenance of stemness in tumor stem cells. Similar to M3TR overexpression, siRNA-mediated silencing of M3TR also results in genomic instability thereby predisposing the cells towards transformation. In another study, in spheroids generated from gliomas, we demonstrate that the pro-survival pathway mediated by NF-kB and AKT are independently activated by TNF-α, which results in resistance to TNF-α mediated cytotoxicity. These findings emphasize the need for targeting multiple proteins on diverse pro-survival pathways for efficient anti-cancer treatments.

Our studies on breast cancer indicate that Osteopontin, a secreted chemokine-like protein, promotes vascular endothelial growth factor dependent tumor growth and angiogenesis via autocrine and paracrine mechanisms. Our results provide new insights into the mechanism underlying breast tumor angiogenesis that may form basis for novel therapeutic strategies. Induction of apoptosis in tumor cells is another form of intervention in cancer management. In depth study of the chemotherapeutic drug-mediated cell killing reveal that p53 may represent one of the upstream mediators of ERK-activated apoptosis in cervical cancers. This is the first report identifying a pro-apoptotic role for p53, which is otherwise considered to be a pro-survival protein.

Molecular analysis of interaction between the proteins of host and pathogen provides us novel drug targets for infectious diseases. Structure-function analysis of α-hemolysin (HL), a pore forming bacterial toxin, indicates that its caveolin-1 binding domain is important for membrane penetration and oligomerization. Mutations in α-HL that reduced the affinity for caveolin-1 led to changes in its membrane binding, oligomerization and penetration properties.

In the area of diabetes, we use divergent approaches for achieving control and management of experimental diabetes through enrichment of islets from unconventional sources of stem cells. In this regard, we have achieved islet neogenesis from stellate cells of chicken β-islets and from Wharton's jelly of human umbilical cord. Our preliminary studies show that transplantation of stellate derived islets into experimental diabetic mice, caused reduction in hyperglycemia. Alternatively, differentiated human fetal pancreatic progenitor cells can be used in cell replacement therapy for diabetes. We have successfully expanded human fetal pancreatic islet progenitor cells through epithelial-to-mesenchymal transition. Molecular characterization of the transition and differentiation processes is in progress.

Cardiomyopathy is among the most frequent complications of diabetes, which is caused by cytopathic effects of high glucose on cardiomyocytes. Previously, we showed that insulin protects cardiac cells against high glucose-induced damage by interruption of the mitochondrial apoptotic pathway and reduction of reactive oxygen and nitrogen species. We have delineated the mechanisms involved in this process and show that the protective role of insulin is mediated through inhibition p38 and activation of PI3K/AKT pathways.

A major focus of research at NCCS is in understanding the molecular intricacies of Infection and Immunity. We have identified and characterized the peroxizome targeting sequence-2 in hexokinase of Leishmania that targets it to glycobodies. Further, expression analysis of the selenophosphate synthetase [selD] gene from Leishmania indicate that it is constitutively expressed in both promastigote and amastigote stages. During our studies on the biology of the malarial parasite *Plasmodium falciparum*, we have established and characterized a novel population of extracellular erythrocytic parasite culture derived from

noninvasive merozoites. Current investigations are focused on merozoite formation in singly and multiply infected erythrocytes.

Studies on HIV pathogenesis have revealed that the cytokine IL-10 plays a crucial role in establishing Tat-mediated immuno-suppression. Further, we have identified and characterized a small molecule derived from green mussel that exhibited anti-HIV activity. We have developed a reporter system that could aid in the study of HIV-I subtype C, which is the most prevalent subtype in India.

NCCS has also been concentrating on identification of proteins important in viral pathogenesis. In this direction, we have shown that Vaccinia virus complement control protein (VCP), Herpervirus saimiri complement control protein (HVS CCPH) and Kaposica (HHV-8 complement control protein) target the host complement system and serve as virulence factors.

T-cells play an important role in tumor regression. We report that the CD40-induced effecter T-cell response against tumors depends on the CD40 expression levels *in vivo*. Further investigation suggests that a dose-dependent cross-linking of a co-stimulatory molecule dictates the effecter T-cell response. Apart from effecter T cells, we also studied the modulation and induction of cytotoxic T-cell response by different antigen presenting cells. These studies provide molecular insights in our understanding of $CD8^+$ T-cell mediated immunity. We have been successful in the isolation and characterization of dendritic cell types1 and 2 (DC1 & DC2). Activation through the T-independent and T-dependent modes reveals that the DC1 cells are stimulatory while the DC2 cells are regulatory in nature. Using dengue virus infected DCs, we have delineated the possible mechanisms by which this virus regulates T-cell response for its own survival and multiplication.

Chromatin architecture plays an important role in regulation of gene expression. SMAR1, a nuclear matrix attachment region (MAR)-binding protein, modulates many cellular processes through chromatin remodeling. We show that anti-cancer agents like prostaglandin-2 (PGA2) regulate the expression of SMAR1. We have characterized a stem-loop structure that is present in the 5'UTR of SMAR1 mRNA, which is critical for its stabilization in response to PGA2. Further, we show that restoration of SMAR1 function, which is reduced in many tumors, is essential for tumor regression. A potential role for SMAR1 in T-helper cell differentiation is also being investigated. SATB1, another MAR-binding protein, organizes the chromatin into distinct loops to regulate gene expression. We demonstrate that promyelocytic leukemia protein (PML) interacts with SATB1 and enhances its association with MARs. Also, individual PML isoform contributes to differential architecture and expression of MHC-I locus in response to IFNγ.

Research at NCCS also focuses on understanding the microbial community structure of unique ecosystems like insect gut, human colon and some extreme ecosystems. Our studies show a differential microbial distribution pattern between infants born through, normal or caesarean-section delivery. The assessment of microbial diversity in Lonar Lake, Maharashtra, has been completed and metagenomic analysis of this ecosystem is underway. Transcriptome analysis of mid-guts of Plasmodium-infected and uninfected *Anapheles stephensi* may provide better understanding of the vector-pathogen interactions.

NCCS is determined to provide state-of-the-art facilities to its scientists to perform modern biological research. We also have an excellent Animal Facility that procures and maintains experimental animals and provides technical support to the scientists who require use of animals for their research.

Over the last year, NCCS has over 50 publications in reputed peer-reviewed journals and books to its credit. In addition to the institutional research funds, our scientific endeavors are further substantiated by peer reviewed funding from various national and international funding agencies including DBT, DST, DRDO and Wellcome Trust.

We are confident that the research at NCCS provides greater insights into many biological processes that are important to health and disease.

G.C. Mishra
Director

Human Resource Development

During 2006-2007, 75 students joined for pursuing Ph.D under various Scientists. All student presentations were completed and their admission is confirmed by The University of Pune.

The Project Training programme is conducted twice 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 who attended these courses in the last year are :

Project Training - 10
Summer Training - 12

During this year, 59 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. We have conducted the following programs during the previous year.

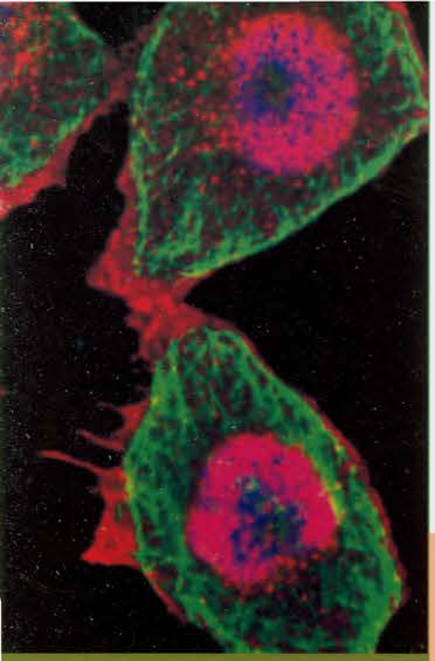
August 7-11, 2006.
School of Biotechnology,
DAVV, Indore (30 participants)

March 8-9, 2007
Department of Biochemistry,
Shivaji University, Kolhapur, (40 participants)

Ten individuals, comprising of technicians and Ph.D students from all over India were trained in basic animal tissue culture techniques at NCCS during the year 2006-2007.

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 35 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 2006-2007, we have supplied 1400 cell lines comprising of 152 different cell types to 130 research institutions in the country



Research Reports

Cell Biology

Non-traditional roles of nucleoporins: Nup358 in cytoplasmic functions

Jomon Joseph
josephj@nccs.res.in

Participants
Prayag Murawala, *JRF*
Mukesh Mani Tripathi, *JRF*
Pankhuri Vyas, *JRF*
Aparna Salunke, *Technician*

Background

Nup358 is a large mammalian nucleoporin that possesses multiple domains, including a cyclophilin homology domain, a domain that functions as a ligase for ubiquitin-like modifiers of the SUMO family, domains that bind the GTP- and GDP-bound forms of the Ran GTPase, a leucine-rich region (LRR) and a region that contains numerous motifs for binding to nuclear transport receptors. Nup358 localizes to metaphase spindles and kinetochores in a microtubule-dependent manner, where it is important for microtubule-kinetochore interactions. Nup358 binds throughout the cell cycle to RanGAP1, the GTPase activating protein for Ran. Ran controls mitotic microtubule dynamics and spindle assembly by regulating the binding and release of spindle assembly factors from Ran-GTP binding proteins that act as nuclear transport receptors during interphase. The Nup358-RanGAP1 complex is therefore likely to play a critical role in mitotic spindle assembly through regulation of Ran-GTP levels, but it is also possible that it can control spindle assembly through other mechanisms as well.

Aims and Objectives

1. Does Nup358 associate with interphase microtubules?
2. What are its cytoplasmic roles?
3. Identification of Nup358 interacting proteins and functional characterization

Work Achieved

Nup358's mitotic localization suggested that it interacts directly or indirectly with microtubules. While the bulk of Nup358 is associated to NPCs during interphase, immunostaining with anti-Nup358 antibodies consistently shows foci of Nup358 within the cytoplasm of interphase mammalian cells. To examine whether cytoplasmic Nup358 associates with microtubules in interphase cells, we immunostained COS-7 cells for Nup358 and tubulin (Fig. 1). Remarkably, the endogenous Nup358 often colocalized with microtubules, particularly at cell extensions (Fig. 1, arrow). Nup358 antibody that was neutralized with the antigen used for immunization showed no such staining indicating the specificity of the antibody used (Fig. 2). Interestingly, the cytoplasmic distribution of Nup358 varied under specific conditions; for example, Nup358 was enriched at the cell periphery when HeLa cells were serum-stimulated (Fig. 3), whereas it was more uniformly distributed within the cytoplasm under serum-starved conditions. This change is indicative of a dynamic population of Nup358 in the cytoplasm that may respond to external stimuli. In a microtubule pelleting assay using purified microtubule polymers, we show that endogenous Nup358 associates with microtubules. Together, these results suggest that in addition to its well characterized nuclear pore localization, Nup358 exists in a dynamic cytoplasmic pool that interacts with interphase microtubules *in vivo*.

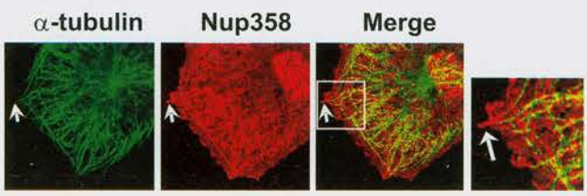


Fig. 1: Nup358 partially colocalizes with interphase microtubules. COS-7 cells were fixed and immunostained for endogenous Nup358 (red) and microtubules (green) using specific antibodies. Arrow indicates cellular extension where Nup358 partially colocalizes with microtubules.

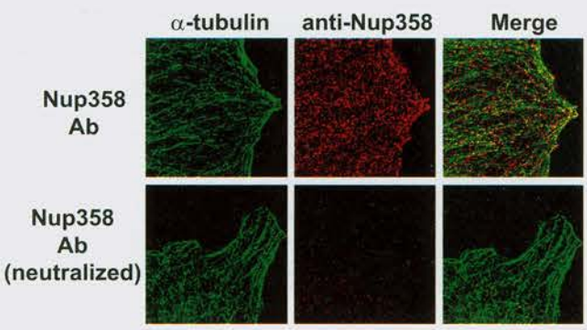


Fig. 2: Specificity of affinity purified anti-Nup358 antibodies used for endogenous Nup358 immunostaining. To determine the specificity of the Nup358 antibodies, immunostaining was performed with affinity purified Nup358 antibodies directly (top panel, red) or after neutralization (bottom panel, red) with the antigen used for immunization. Microtubules (green) were visualized with anti-α-tubulin antibodies.

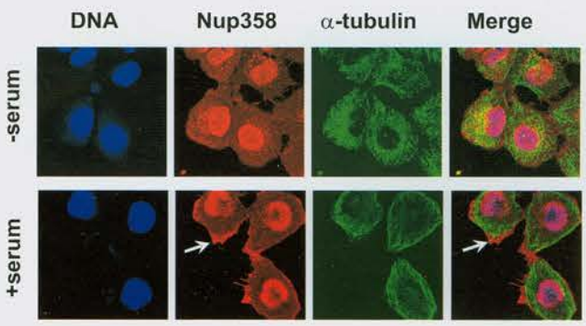


Fig. 3: Nup358 exists in the cytoplasm as a dynamic pool. HeLa cells were serum-starved (-serum) or serum-stimulated (+serum) and fixed and analyzed for endogenous Nup358 and microtubules (green) using specific antibodies. DNA (blue) was visualized with Hoechst staining. Arrow indicates Nup358 accumulation at cell edges upon serum stimulation.

Future Work

We are trying to identify and characterize the regions of Nup358 involved in association with microtubules and exploring different interacting partners to further our knowledge about the cytoplasmic function of Nup358, which may be coupled to, or independent of, its proposed role in nucleo-cytoplasmic transport of macromolecules across the nuclear envelope.

Experimental Hematopoiesis

Vaijayanti P. Kale
vpkale@nccs.res.in

Participants
Vasudha Laxman, *SRF*
Kavita Kumari, *SRF*
Anuradha Vaidya, *SRF*
Ashwini Hinge, *SRF*
Monika Sharma, *Project Assistant*
Prajakta Oak, *Project Assistant*
Manmohan Bajaj, *JRF*
Roli Mishra, *JRF*
George Fernandes, *Technician*

Collaborators
Dr. L.C. Padhy, *TIFR, Mumbai*
Dr. A. Surolia, Director, *NII, New Delhi*
Dr. L.S. Limaye, *NCCS, Pune*

Background

Steady state production of blood cells in a regulated manner throughout the life of an individual necessitates a precise control of this process at various levels. However, the intricacies of this process are still not clearly understood. Though the role of various growth factors and their mechanism of action on various progenitors have been worked out fairly well, it is still unclear as to how a stem cell decides to take up a particular option like self-renewal, commitment, migration etc. We have been interested in some of these fundamental issues and are trying to collect experimental evidence to address them.

Various experiments carried out by several workers over the years, especially using long-term cultures, have underscored the crucial role of stromal cells in the development of stem cells. However, the exact mechanism(s) involved in the effective cross talk is not yet known and the players involved in this process remain to be identified. We have earlier shown that TGFβ1 released from marrow mononuclear cells in response to erythropoietin stimulus is one such molecule that has the capacity to bring about effective cross talk between stromal cells and stem cells leading to stem cell amplification. We have further shown the involvement of nitric oxide signaling pathway emanating from activated eNOS in the process. NO is extremely reactive: it reacts with gaseous Oxygen and other reactive oxygen species, heavy metals, as well as with cysteine and tyrosine residues in proteins. In accordance, it is now widely accepted that once NO is generated, it is very short-lived and its diffusion is possible only over a short distances. This necessitates the local production of NO and the localization of the enzymes responsible for its generation, namely NO synthases, in close proximity of their downstream targets.

During the course of our studies we made an interesting observation that endothelial nitric oxide synthase (eNOS), the enzyme involved in the generation of NO in cells, was also localized to the nuclei of marrow derived stromal cells in addition to its usual known localization in the plasma membrane caveolae and endoplasmic reticulum-Golgi. This was a novel finding and, therefore, we decided to examine the physiological significance of this observation. Our further work has indicated that a sufficient quantity of active eNOS is present in the nucleus of mammalian cells in steady state condition and thus it appears that it may have an important role to play in the cellular physiology.

eNOS is localized in the cell surface structures known as Caveolae and its interaction with the structural protein Caveolin1 maintains it in inactive state. A stimulus that disrupts this interaction leads to the activation of eNOS and subsequent generation of nitric oxide. We have, therefore, focused our studies on these two molecules viz. eNOS and Caveolin1 in order to get better insight in their regulation and function. Such studies would enable us to modulate the eNOS activity in the stromal environment to stimulate stem cells. A very prominent signaling molecule that is related to Caveolin expression is estrogen receptor. Therefore, we decided to examine regulation of Caveolin 1 expression by estrogen mediated signaling. Even though several systems including the marrow stromal cells are known to express estrogen receptors, we have chosen a standard estrogen receptor positive breast cancer cell line, namely MCF 7, as a starting point to examine the issue.

Aims and Objectives

The principal aim of this study was to determine the mechanism by which eNOS may partition and distribute itself into cellular compartments like nucleus, cytoplasm or membrane and to determine the possible physiological consequences that may follow from such a distribution.

Work Achieved

During the process of studying the nuclear localization of eNOS we developed a specific plasmid construct expressing a stretch of sequences cloned from cDNA of eNOS encompassing the putative nuclear localization sequence and GFP as the reporter protein. We observed that this construct takes GFP to nucleus and nucleolus. This observation confirmed the biological activity of the NLS sequence. We developed several independent stable clones using this plasmid construct. Since MCF 7 cells which were used to generate these clones are of breast cancer origin, we studied the biology of these clones for their tumorigenic properties. We observed that these clones show reduced anchorage independent growth and reduced matrigel invasion. It appears that the construct suppresses the tumorigenic properties of MCF-7 cells (Fig. 1).

We have shown that transfection of MCF-7 cells with a plasmid construct expressing dominant negative estrogen receptor resulted in significant upregulation of Caveolin 1. Since Src is a down stream effector of estrogen mediated signaling we examined the effect of transfection of a plasmid construct expressing dominant negative Src. We observed a significant up-regulation of Caveolin-1 protein

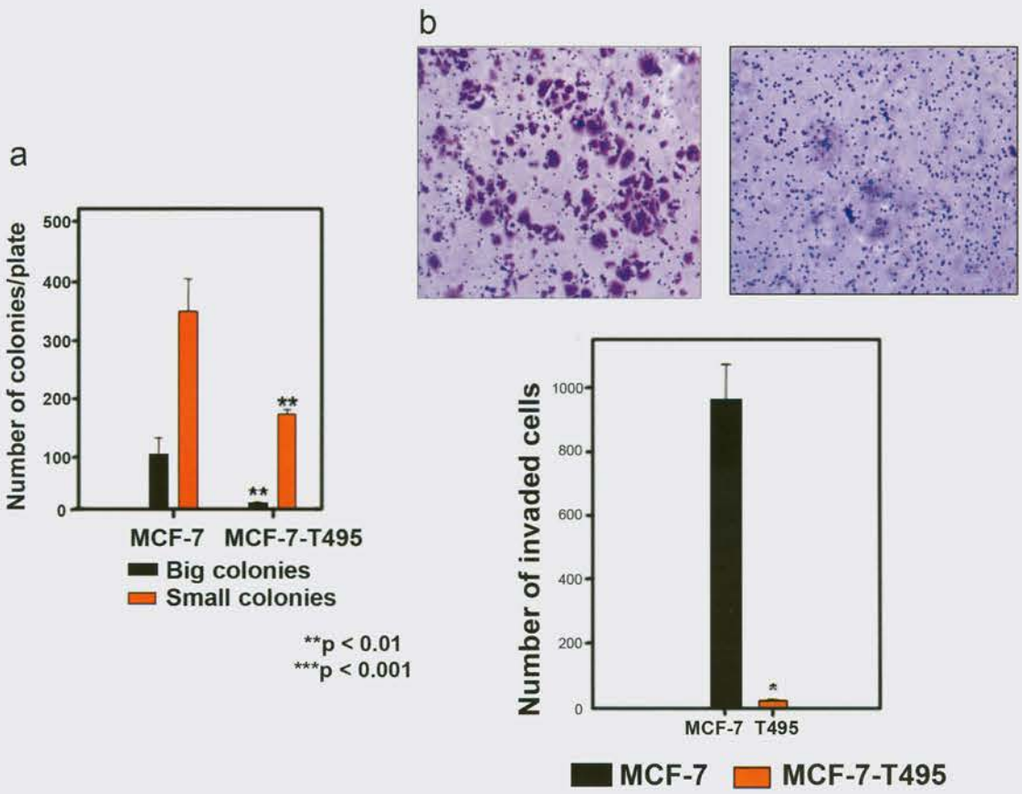


Fig. 1: Transfection of a plasmid construct expressing truncated eNOS protein reduces *in vitro* tumorigenic properties of breast cancer cells: Graphical representation of anchorage independent growth of MCF7 and MCF-7-T495 clone in soft agar (a) Reduced matrigel invasion by MCF-7-T495 clone as compared to MCF-7 (b).

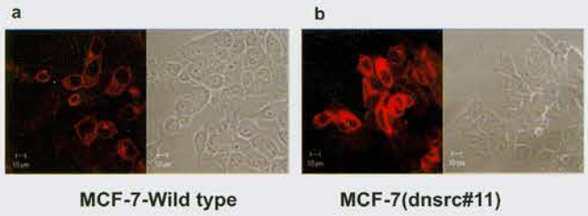


Fig. 2: Transfection of MCF-7 cells with dominant negative Src plasmid construct leads to upregulation of Caveolin synthesis: Figure shows image of MCF 7 (a) and MCF-7 stably transfected with DN-SRC (b) immunostained with anti Caveolin 1 antibody.

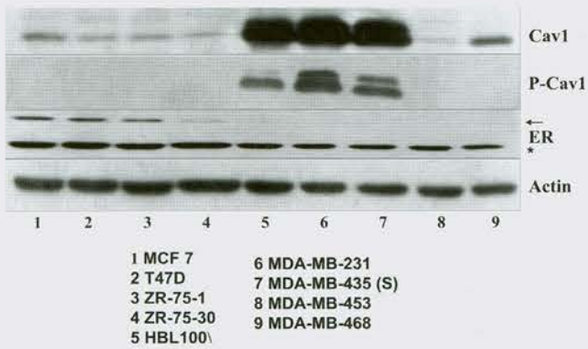


Fig. 3: Inverse expression of estrogen receptor and Caveolin 1 in breast cancer cell lines: The figure shows western blot analysis of lysates prepared from a battery of cell lines of breast cancer origin. The same blot has been probed with antibody to Caveolin1, estrogen receptor and beta-actin. Note that all cell lines expressed a truncated form of ER (indicated by *) irrespective of the expression of normal ER (indicated by arrow).

expression in MCF 7 cells after stable expression of this construct indicating the involvement of Src in the process (Fig. 2). We also checked expression profile of Caveolin in a series of ER positive (e.g.: MCF7, T47D, ZR-75-1, ZR-75-30) and ER negative (e.g.: HBL-100, MDA-MB-231, MDA-MB-435(S), MDA-MB-453, MDA-MB-468) breast cancer cell lines by western blotting, immunofluorescence, and semi-quantitative RT-PCR. Low level of Caveolin-1 expression was detected in estrogen positive cell lines and high level of caveolin expression was found in estrogen negative cell lines supporting our working hypothesis (Fig. 3). Thus our data indicate that Caveolin-1 expression is reciprocally regulated by estrogen receptor signaling involving activation of c-Src.

Future Work

Nitric oxide also plays an important player in tumor formation and it acts at various stages. Since the transfection of specific eNOS sequence in MCF-7 cells resulted in compromised *in vitro* correlates of tumorigenic properties, we believe that this system is an excellent experimental paradigm to study the role of eNOS in tumorigenesis. We propose to examine this aspect in greater details using various other cell lines of neoplastic origin. A major issue in estrogen signaling is whether it is a genomic action or a non-genomic one emanating from the membrane localized ERs. We propose to address this issue in near future.

Studies on expansion, cryopreservation and differentiation of hematopoietic stem cells

Lalita S. Limaye
lslimaye@nccs.res.in

Participants
Sangeetha V M, *SRF*
Sreekumar Balan, *SRF*
Namrata Shabrani, *Project Assistant*
Nikhat Firdaus Siddiqui, *Technician*

Collaborators
Dr. R.L. Marathe, Jehangir Hospital, Pune
Dr. (Mrs.) Vaijayanti P. Kale, NCCS, Pune

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. The role of Arachidonic acid (Omega 6) and its metabolites in regulation of haematopoiesis is well documented. We plan to use them as supplements in media for *in vitro* expansion of $CD34^+$ cells to see whether they reduce apoptosis. To date a body of evidence has been compiled, which attests to the hypothesis that cold shock can induce apoptosis. Thus detection of the level of apoptosis during preservation and expansion and to develop ways and means to prevent it is an important aspect of haematopoietic stem cell research.

Megakaryocyte generation:

Umbilical cord blood (CB) 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 currently are underway to expand the number of CB stem cells and MK progenitor cells *ex vivo,* which may facilitate platelet production during post transplant nadir and decrease the time of thrombocytopenia. The optimal conditions for *ex vivo* expansion and cryopreservation of MK progenitor cells have not been established. Here we propose to define the conditions of *ex vivo* expansion of MK progenitor cells from umbilical cord Mononuclear Cells (MNCs) and CD34+ cells using various cytokines and serum free media and nutrient supplements. Attempts will be 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* expanding CB MK progenitor cells.

Dendritic cell generation:

Dendritic cells (DC) 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 and in the outcome of clinical transplantation. In recent clinical studies DC were used as a vaccine for cancer patients and showed induction of anti tumor effect. The total number of DCs available for immunotherapy remains limited. DCs have been shown to be generated from $CD34^+$ cells from Bone Marrow (BM), Umbilical Cord Blood (UCB) and Granulocyte Colony Stimulating Factor (GCSF) mobilized Peripheral Blood Stem Cells (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.

Aims and Objectives

In vitro expansion of hematopoietic stem cells:

The aim is to expand and cryopreserve $CD34^+$ hematopoietic cells by preventing apoptosis. We have used a class of compounds like antiapoptotic agents, nutraceuticals, antioxidants etc. in the expansion and freezing media.

Harnessing the potential of stem cells: *In vitro* generation of megakaryocytes and dendritic cells

The aim is *in vitro* generation, characterization and cryopreservation of the two cell types i.e megakaryocytes and dendritic cells from cord blood cells.

Work Achieved

Expansion of hematopoietic stem cells:

$CD34^+$ cells were expanded with and without antiapoptotic agents and expansion was quantitated by long term culture assays (Fig. 1a and b) and chemotaxis of expanded cells towards SDF1alpha (Fig. 1c). The expanded cells were cryopreserved in conventional freezing medium and revival efficacy was tested by viability and CFU assays (Fig. 1d). All these parameters show that indeed the antiapoptotic agents ZVADFMK and calpain inhibitor have a beneficial effect on *ex vivo* expansion.

Differentiation of hematopoietic stem cells to Megakaryocytes:

Having standardized the culture conditions for generation of megakaryocytes from Cord blood $CD34^+$ cells, we then tried to test the effect of a nutraceutical omega 6 fatty acid i.e arachidonic acid (A.A.) as additive in the culture medium. We found that when cells were grown with (Test) and without A.A. (Control) in the medium the fold increase in cell number was significantly higher in test set as compared to control. It was found by morphological quantitation by Wright's Giemsa staining that Arachidonic acid favored the production of megakaryocytes. This observation was further confirmed by phenotypic analysis of megakaryocyte-platelet specific markers like CD61, CD41 and CD42, on a flowcytometer and capturing images on confocal laser scanning microscope.

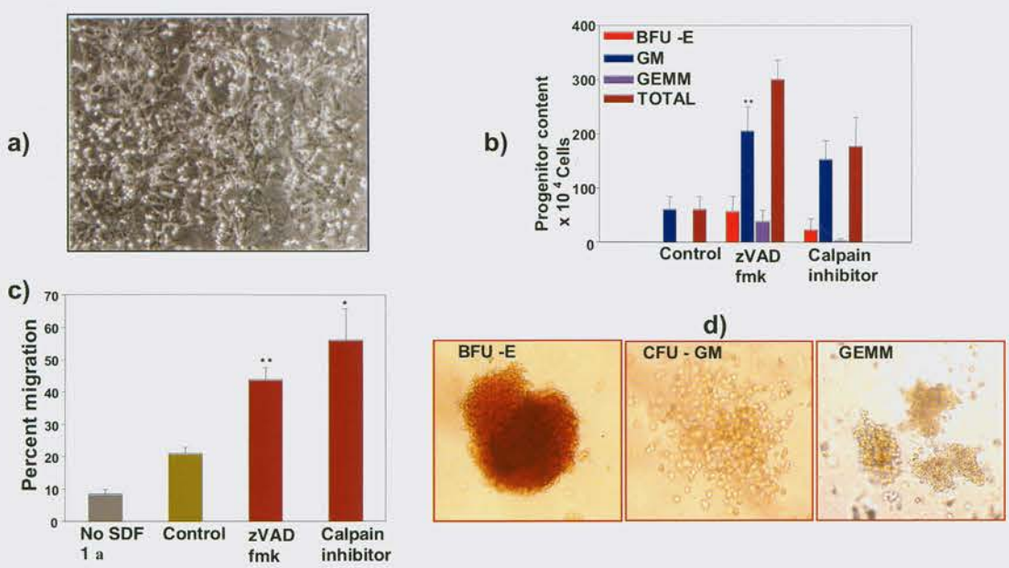


Fig. 1: *Ex vivo* expansion of hematopoietic stem and progenitor cells with and without anti-apoptotic agents: a) Phase contrast image of the expanded cells in Long Term Culture (LTC) b) Colony Forming Unit (CFU) assay of the expanded cells after LTC c) Chemotaxis of the expanded cells towards SDF1α and d) CFU colonies formed from the expanded cells after cryopreservation and revival.

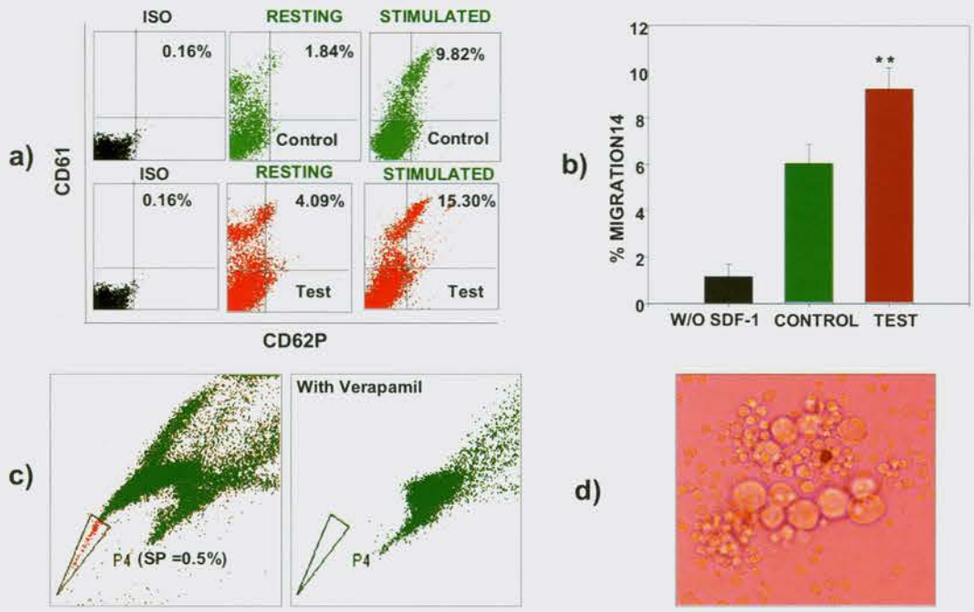


Fig. 2: Generation of Megakaryoctes from cord blood CD34⁺ cells/MBM SP cells. a) Platelet Activation b) Chemotaxis [Control = -Omega 6 and Test = +Omega 6] c) sorting of SP cells d) Megakaryocytes from SP cells

The functionality of *in vitro* generated megakaryocytes was tested by CFU Meg assay, chemotaxis towards SDF1 alpha (Fig. 2b) and platelet activation by thrombin (Fig. 2a). We also generated megakaryocytes from Side population cells sorted from mouse bone marrow. (Figs. 2c and 2d)

Generation of Dendritic cells in autologous plasma containing culture Expansion and differentiation:

CD34⁺ cells were cultured in the presence of Flt-3 , SCF and TPO in IMDM supplemented with 5% autologous plasma. The cultures were maintained for 14 days, with a weekly harvest and reseeding with fresh media and growth factors. Both the adherent and non adherent cells were harvested. Harvested cells were further used for sorting or were cryopreserved.

Sorting on flow cytometer:

Expanded cells were stained with anti-CD14-PE conjugated antibodies and sorted on FACS Aria. Both Positive and negative fractions were collected and used for inducing dendritic cell differentiation. Subsequently the cells were reanalyzed for purity. The reanalyzed positive fraction showed 98.66 ± 0.585 % purity and negative fraction was 98.18 ± 0.238 % pure (n = 5). Sorted cells were cultured in IMDM supplemented with 5% autologous plasma and cytokines. On day 7 the cells were harvested and labeled with a panel of dendritic cell surface markers and analysed. Surface antigen expressions of the cell after expansion and inducing differentiation of the positive and negative fractions were compared.

Phenotypic characterization and functional assays:

Dendritic cells generated from expanded sorted CD14⁺/CD14⁻ fractions were further subjected to maturation by stimulation with LPS, CD40-L and TNF-α. Expression of dendritic specific markers were then analysed on immature and mature cells (Fig. 3a). The combined treatment with different stimuli gave completely matured dendritic cells characterized by the high level of expression of DC specific CD83 and co stimulatory molecules (CD40, CD80, CD86 and MHC II). This results show that the dendritic cells generated are functionally active and they are able to respond to the inflammatory signals and become mature dendritic cells. The dendritic cells generated from CD14⁺ fraction showed a better response, may be due to the homogenous nature of the cells.

Mixed Lymphocyte reaction:

Mature stimulated dendritic cells generated from CD14 positive and negative fraction in autologous plasma containing medium were tested for functionality by Mixed Lymphocyte Reaction (MLR). They were mixed in different ratios with allogeneic T cells and the T cell proliferation was tested by thymidine assay. Results show that the dendritic cells are functionally active (Fig. 3 b and c).

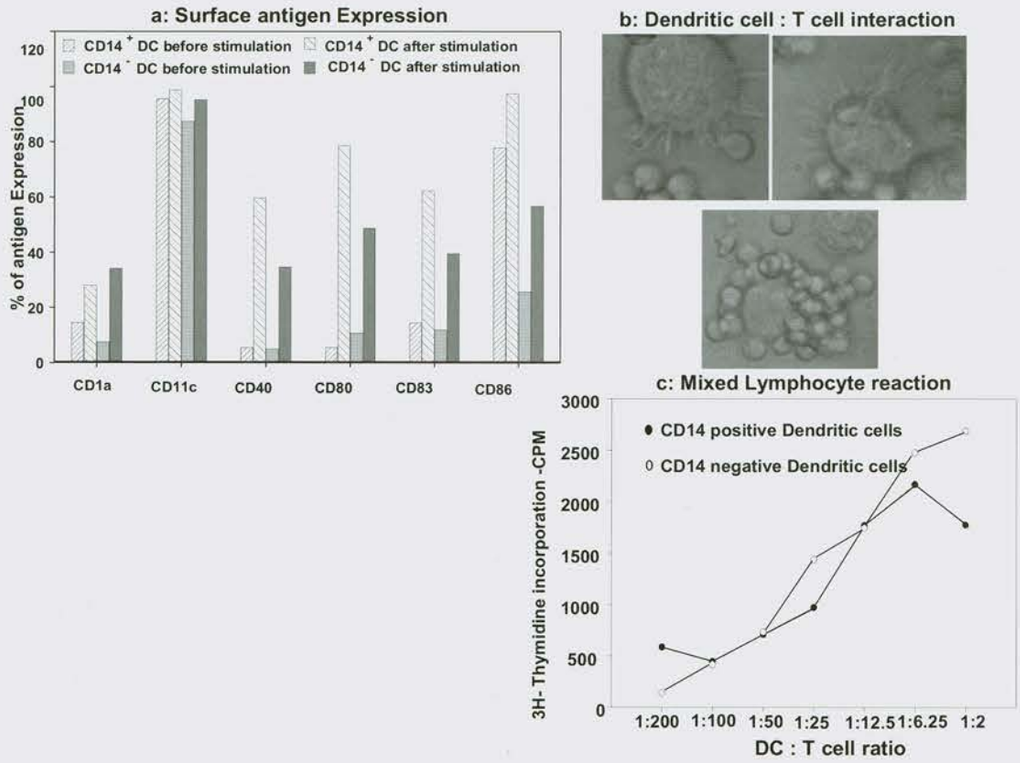


Fig. 3: *In vitro* generation of Dendritic cells from Cord blood hematopoietic stem cells. a) Phenotypic characterisation. b) Phase contrast image of dendritic cell – T-cell interaction. c) Allogeneic T-cell stimulation by dendritic cells.

Future Work

Expansion of hematopoietic stem cells

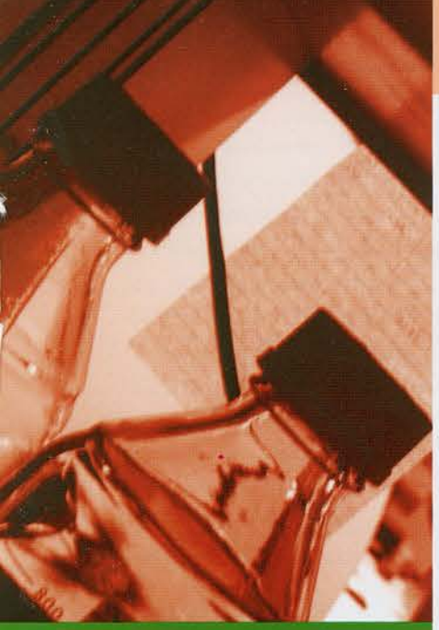
1. *In vivo* engraftment assays on expanded cells using NOD/SCID mice
2. Cryopreservation of expanded cells with and without the selected additives
3. Expansion of cryopreserved $CD34^+$ cells will be undertaken.

Megakaryocytes

1. Use of nutraceutical omega3 as additive in megakaryocyte expansion medium
2. Identification of megakaryocyte specific molecular markers like NFE2, GATA1, Platelet glycoprotein etc. by PCR and Western blot techniques.
3. Characterization of Proplatelets generated during in vitro megakaryocyte production.

Dendritic cells

1. Quantitation of IL-6, IL-12 in culture supernatants by ELISA
2. Optimization of cryopreservation and revival protocols for the in vitro generated Dendritic precursors
3. Standerdization of *in vitro* and *in vivo* assays to test antitumor activity of dendritic cells.



Nibedita Lenka
nibedita@nccs.res.in

Participants
Ranju Nair, *SRF*
Mahesh Kumar Verma, *SRF*
Saravana Kumar Ramasamy, *SRF*

Collaborator
Dr. K.P. Mohanakumar, IICB, Kolkata

Embryonic stem cells: Differentiation into dopaminergic neurons *in vitro*

Background

The embryonic stem (ES) cells possess the unique property of differentiation into all the three germ layers derivatives recapitulating the *in vivo* development in a relatively precise manner. Hence, these cells serve as a potent model for investigating early embryonic development and hold promise for their usage in cell replacement therapy. Our group's interest lies in differentiating the ES cells into neural lineage with special reference to the dopaminergic neuronal subtypes, enriching those and studying their therapeutic efficacy upon transplantation into animal models.

Aims and Objectives

The major focus of our group has been,

1. To establish stable transgenic ES cell clones using live reporter gene expression under the regulatory control of neural specific promoters/enhancers.

2. To differentiate the ES cells into neural lineage and understand the underlying molecular basis of lineage commitment and specification.

3. Manipulate extrinsic factors for the efficient generation of proliferative neural progenitors, differentiated neurons with special reference to the dopaminergic neuronal subtypes from ES cells *in vitro*.

4. To explore the efficacy of these *in vitro* generated cells in cell replacement therapy using animal models.

Work Achieved

The progressive degeneration of the dopaminergic neurons leads to Parkinsonism, one of the major neurodegenerative disorders associated with movement disorder and emotional disturbances. This disease condition is characterized by bradykinesia and akinesia caused by the loss of dopamine – the neurotransmitter produced by the dopaminergic neurons. Cell-replacement therapy has been considered as a suitable alternative to the present mode of treatment with administration of L-DOPA that gives symptomatic relief only. Accordingly, *in vitro* generation of the dopaminergic neurons in substantial quantity has been a prime necessity for transplantation therapy. As reported earlier, we have used the promoter mediated cell trapping approach to demarcate and characterize the dopaminergic neurons among the differentiating heterogeneous cell population from the ES cells. We have been successful in devising the strategy for enriching the same from the ES cells *in vitro*. Further, we developed the hemi-parkinsonian rat model and injected these *in vitro* generated cells into the striatum of these rats to asses their efficacy in replacing the damaged dopaminergic neurons and thereby leading to the functional restoration. The cells were used at different time points during differentiation along with the undifferentiated ES cells to determine the stage specific influence, if any. The survival and the successful integration of the transplanted cells were verified by monitoring EGFP expression and immuno-histochemical characterizations in tissue sections obtained by sacrificing the animals at various time points. The immuno-fluorescence studies on cryo-sections of the perfused brain show the extent of the TH-EGFP expressing cells that survived and integrated following the transplantation (Fig. 1A). Moreover, the immuno-histochemistry with anti-TH (Tyrosine Hydroxylase) indicated the presence of dopaminergic neurons in a localized area near the site of the transplantation as shown by the infusion mark as compared to that in the HBSS control (Fig. 1B).

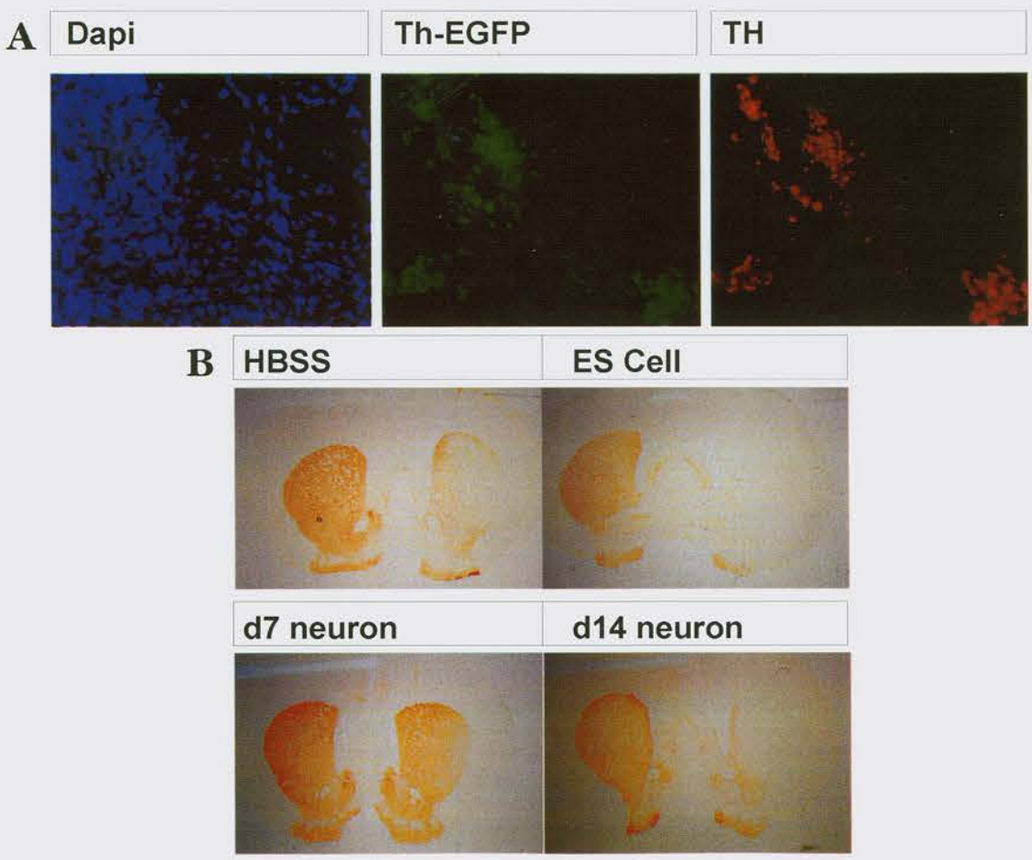


Fig. 1: The immuno-histochemical analysis on the brain sections of hemi-parkinsonian rat model subsequent to the transplantation of the ES cell derived dopaminergic neurons. A: The immuno-fluorescence detection of the co-localized expression of EGFP and TH indicating the integration of transplanted cells into the host brain. B: The immuno-histochemical staining of TH in both transplanted and normal hemispheres of the rat brain 74 days after infusion of 6-hydroxydopamine in single hemisphere. (Left hemisphere: normal)

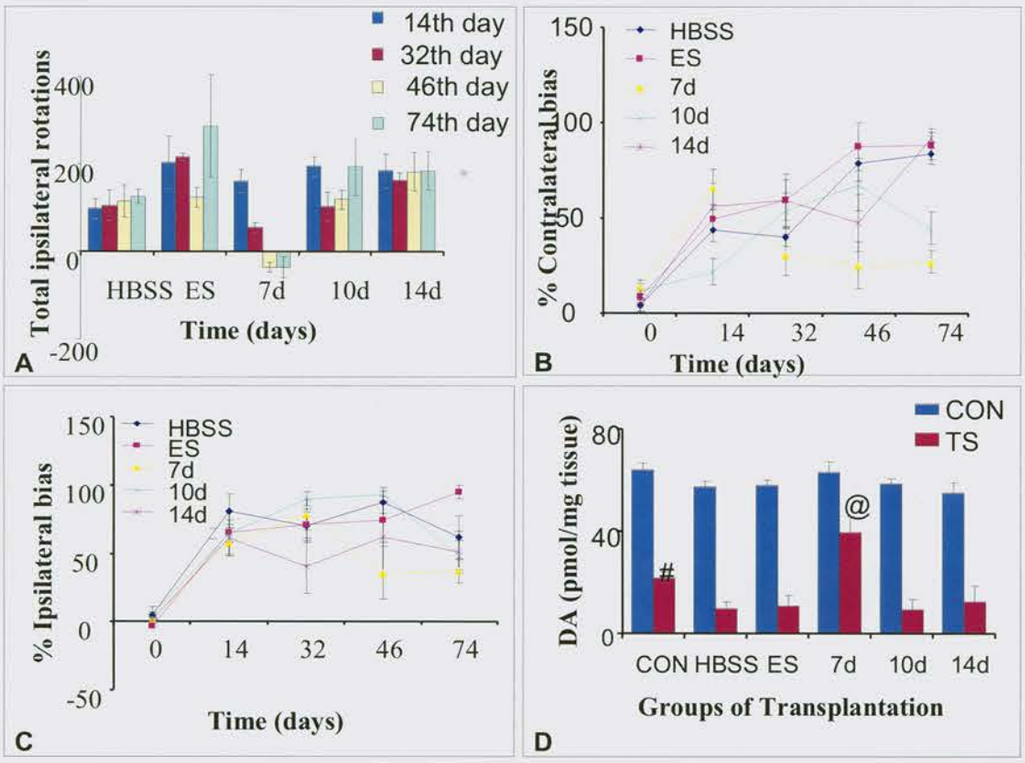


Fig. 2: The functional restoration of hemi-parkinsonian rat model following tranplantation of the ES cells derived dopaminergic neurons taken at varying stages during differentiation. A: Amphetamine induced rotational behavior monitored at 2-, 4- and 8 weeks after transplantation. B, C: The recovery in contalateral (B) and ipsilateral biased behaviour (C) conducted before and after infusion of 6-hydroxydopamine. D: The estimation of dopamine by HPLC to quantify the recovery in terms of functionality following the transplantation.

The rotational behaviour was monitored at 2-, 4- and 8 weeks after transplantation and was assessed for behavioural recovery in each group of these rats with respect to the amphetamine/apomorphine induced rotations in those. Our observation revealed a behavioural recovery in rats injected with ES cells derived neural cells compared to the HBSS/sham control (Fig. 2A). Similarly, the results from the elevated body swing test (Fig. 2B) and the forelimb upper asymmetry test (Fig. 2C) indicated the significant recovery following the transplantation of these cells in the respective contralateral and ipsilateral bias developed after infusion. The dopamine estimation by HPLC in the transplanted brain tissues could demonstrate the successful integration of the transplanted cells into the host and their physiological activity (Fig. 2D). Together, our investigation could delineate the therapeutic potential of the *in vitro* generated dopaminergic neurons from ES cells that could functionally integrate into the host brain.

Future Work

Further we would like to perform electrophysiological characterizations on these *in vitro* generated dopaminergic neurons to authenticate their physiological behaviour.

Translational Regulation of Insulin mRNA

Vasudevan Seshadri
seshadriv@nccs.res.in

Participants
Bhavana M, *SRF*
Baskar B, *SRF*
Amaresh C.P., *JRF*

Background

The pancreatic β-cell contains a large pool of cytoplasmic insulin mRNA (10–15% total mRNA), which is translationally quiescent at hypoglycemic (<3 mM) glucose concentrations. Recruitment of this mRNA to polysomes and the activation of translation occur in response to higher glucose levels, leading to a 50-fold increase in biosynthesis. It has been shown that the 5'- and 3'-UTRs act cooperatively to markedly increase glucose-induced insulin biosynthesis. The mechanism by which this regulation occurs remains unclear. Translational regulation occurs mostly at the initiation step, due to the interaction of specific proteins that bind to the 5' UTR along with the translation initiation machinery. It is likely that elements within the 5'- and 3'-UTRs of insulin mRNA interact with specific proteins, to confer glucose-regulated insulin synthesis. Our hypothesis is that protein(s) bind to the UTR and increase the rate of translation initiation. The mechanism by which these proteins interact with the UTRs needs to be described to gain an understanding of glucose stimulated translational regulation of insulin. We synthesized RNA corresponding, to the 5'-UTR of rat insulin mRNA and have shown by RNA gel-shift assays, that a complex is formed upon incubation with RIN cell extract. We have also observed that a similar complex is formed by the protein extracts prepared from Rat pancreatic islets, and the complex formation is increased when the extracts are prepared from the islets treated with high glucose levels. Our preliminary RNA secondary structure analysis identified conserved structural elements including a stem loop structure in the 5'UTR of Rat insulin mRNA that is important for the complex formation. We have characterized the protein that binds to the 5'UTR by UV-cross-linking studies. We have purified the RNA binding protein by RNA affinity column and identified it by protein mass spectroscopy. The characterization of the RNA binding protein and its interaction with the insulin 5'-UTR are under investigation.

Aims and Objectives

1. To identify the sequence and secondary structure elements in the 5'UTRs that are necessary for the RNA-Protein complex formation and translation regulation.
2. Isolation and characterization of the insulin mRNA UTR binding protein or protein complex by RNA affinity chromatography.
3. To understand the basic mechanism of translation regulation of insulin and the role of the 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 but the mechanism of this induction in translation is not completely understood. There have been suggestions, that the un-translated regions (UTR) of the insulin mRNA is 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 is also formed with extracts from rat pancreatic islets. The complex formation is induced by high glucose treatment of the islets. We further synthesized 5'UTR with various deletions, and used them as cold competitors in gel-shift assays to identify the sequences that are essential for the formation of the complex. We identified a minimal 29 nucleotide element that is necessary and sufficient for the protein factor binding (Fig. 1). Our analysis shows that the predicted stem loop structure is important for the complex formation. We further performed mutational analysis of the stem region to show that the stem structure is important for the formation of the complex but the sequence per se does not seem to be important. We performed in vitro translation assay, using the luciferase reporter gene flanked by the rat 5' and 3'UTRs, to show that the 5'UTR as well as the minimal 29 nucleotide fragment are functional as a translational activator in presence of the high glucose treated extracts (Fig. 2). UV-cross linking experiments reveal that a 60 kDa protein binds to the 5'UTR. We synthesized biotinylated insulin 5'UTR and purified the binding factors associated with the RNA. We were able to purify a 60KDa protein as a major component of specific insulin 5'UTR binding protein (Fig. 3). We identified this protein by MS and further confirmation and analysis of the RNA-protein interaction are in progress.

The mechanisms that underlie nutrient-induced translational regulation of insulin biosynthesis are likely to be crucial in 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.

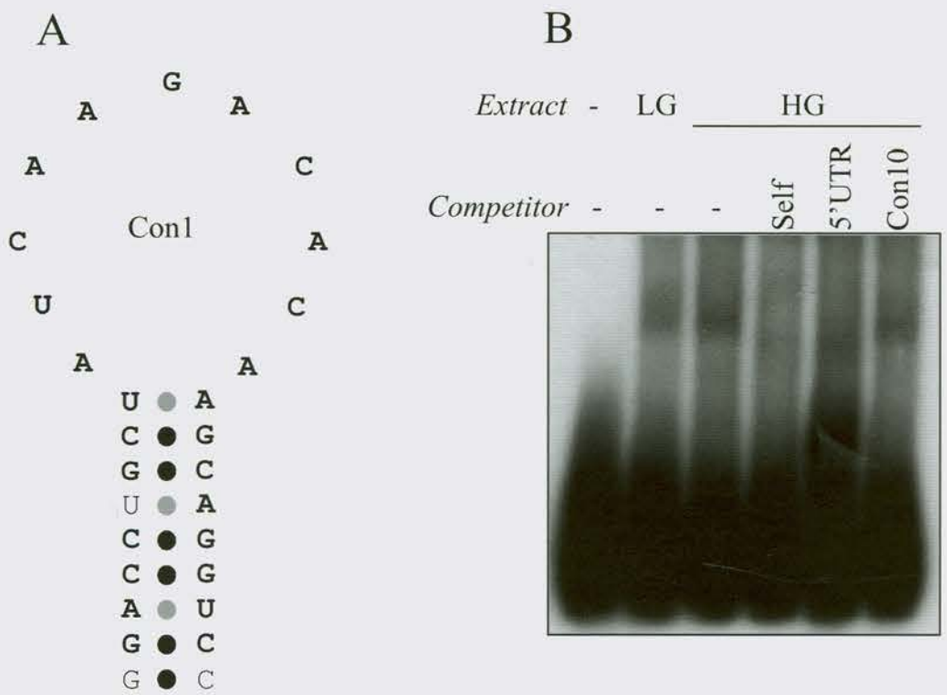


Fig. 1: **A.** Secondary structure of the rat insulin 5'UTR gene1 minimal 29 nucleotide element (Con1) that is necessary for the binding as predicted by mfold algorithm. The nucleotide substitution in the Con1 is indicated by light lettering. **B.** RNA-EMSA using the minimal insulin 5'UTR (Con1) fragment as probe and pancreatic extract. The competitors used were Con1 (self), 5'UTR and Con10.

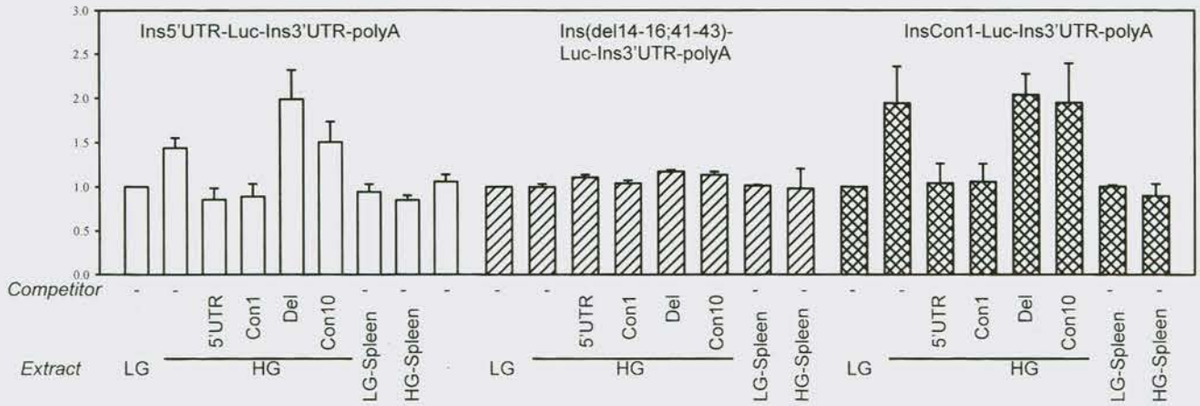


Fig. 2: Translation regulation mediated by insulin 5'UTR. A, *In vitro* translation of capped Ins5'UTR-Luc-Ins3'UTR-polyA, Ins(del14-16; 41-43)-Luc-Ins3'UTR-polyA, or InsCon1-Luc-Ins3'UTR-polyA RNA in presence of LG or HG extracts. The decoys used are, insulin 5'UTR, Con1, del14-16; 41-43 (del), or Con10. Translation was quantitated by dual luciferase assay and the graph shows the fold induction of normalized luciferase activity, with activity in presence of LG extracts set to 1. The graph represents the average of experiments with three different sets of extracts, and the error bars represent the standard error.

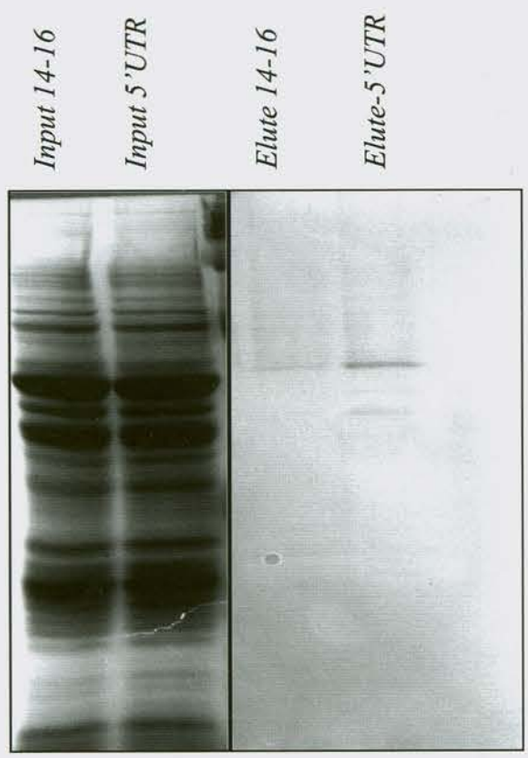
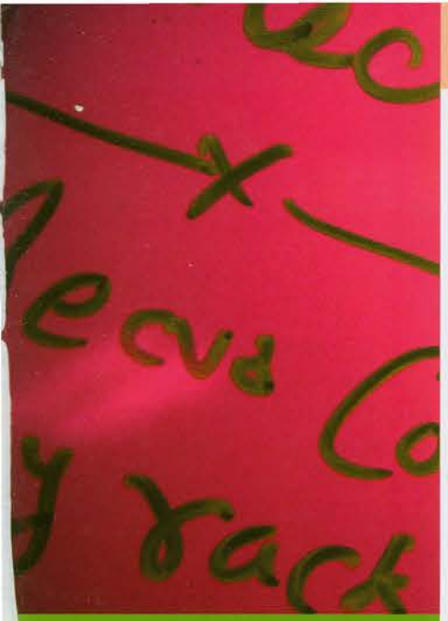


Fig. 3: Purification of the insulin mRNA 5'UTR binding proteins. 5 mg of pancreatic extract was purified with biotinylated insulin 5'UTR or the insulin 14-16; 41-43 deletion fragment. The RNA bound proteins were purified with magnetic streptavidin beads. Proteins bound to the RNA was eluted with TE-1%SDS and resolved on 10% PAGE.

Future Work

We would be characterizing the 5' UTR complex by gel filtration chromatography to identify the molecular weight of the complex. We have identified the insulin 5'UTR binding protein and we will be characterizing this protein for its role in translation regulation of insulin mRNA.



Studies on regulation of osteoblast differentiation from human adult stem cells

Mohan Wani
mohanwani@nccs.res.in

Participants
Geetanjali Joshi, *SRF*
Amruta Barhanpurkar, *JRF*
Rupesh Srivastava, *JRF*
Navita Gupta, *JRF*
Satish Pote, *Technician*

Collaborators
Dr. G.C. Mishra, NCCS, Pune

Background

Bone homeostasis and skeletal integrity are maintained by co-ordinated activities of bone forming osteoblasts, and bone resorbing osteoclasts. In osteoporosis, the number and life span of bone resorbing osteoclasts are increased whereas the number and life span of bone forming osteoblasts are decreased. Therefore, the regenerating potential of adult human bone is limited in osteoporosis, and other skeletal diseases. Recent studies suggest the potential of human adult mesenchymal stem cells (MSCs) for treatment of these disorders. Osteoblast differentiates from bone marrow-derived MSCs. Better understanding of the molecular mechanisms of differentiation of MSCs into functional osteoblasts is very important. How local and systemic factors control human osteoblast differentiation from MSCs is not clearly understood. Parathyroid hormone (PTH) is the most important endocrine regulator of calcium homeostasis. In bone, PTH stimulates the release of calcium and phosphate. PTH has complex and only partially understood actions on bone because endogenous PTH functions to maintain normal extracellular calcium levels in the adult in part by enhancing osteoclastic bone resorption and liberating calcium from the adult skeleton. Exogenous PTH has been shown to exert significant skeletal anabolic effects in the adult when administered intermittently as a pharmacologic agent. Intermittent PTH favors bone formation instead of increasing bone resorption. We are investigating the role of PTH on differentiation of human MSCs into osteoblasts.

Aims and Objectives

1. To standardize the isolation and purification of MSCs from human bone marrow.

2. To develop the optimal culture conditions for growth of MSCs and maintenance of their multi-potential activity for differentiation.

3. Characterization and differentiation of human MSCs into functional osteoblasts.

4. To investigate the role and mechanism(s) of parathyroid hormone (PTH) action on differentiation of MSCs into osteoblasts.

Work Achieved

Isolation and characterization of MSCs from human bone marrow

For isolation of human MSCs we received several bone marrow samples from various hospitals in Pune. Human bone marrow was harvested from the posterior iliac crest of the pelvic bone of normal volunteers. Volunteers were negative for HIV, hepatitis B and had no clinical signs of illness at the time of harvest. Bone marrow samples were first examined for normal hematological values and all values were within normal limits. Myeloid and erythroid ratios were within normal limits and no extraneous cells or parasites were detected. Mononuclear cells were isolated with a density gradient (Ficoll-Hypaque) and incubated (1 to 3 x 10^5 cells/cm^2) in DMEM with low glucose and 10% FCS. MSCs were purified by their adherent properties and well spread elongated fibroblast-like morphology (Fig. 1). Cultures become 80% confluent between 12-14 days. After comparing different media we found optimal growth of these cells in DMEM with low glucose (1.5 g/L) and 10% heat inactivated FCS. In this medium, cells preserved their characteristics and multilineage differentiation potential.

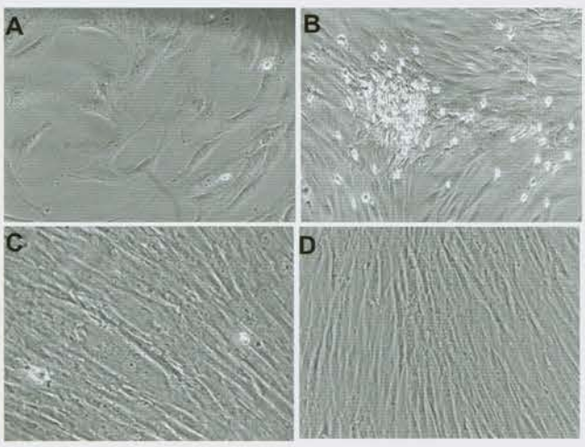


Fig. 1: Mesenchymal stem cells isolated from human bone marrow displays homogenous population of fibroblast-like cells. A) Primary cultures on day 7, B) Primary cultures on day 14, C) Cells at Passage 5, D) Cells at passage 6.

From second passage we characterized human MSCs for the presence of cell surface antigens such as CD44, CD29, CD73, CD90, CD105 and absence of CD34, CD45, CD14 and CD11 by flow cytometry analysis. We observed that expression of MSC markers increased from 3rd passage indicating purity of MSCs. Figure 2 shows the strong expression of CD44, CD29, CD90, CD105 and very low expression of CD34 and CD45 at passage five and six. Both the cell lines have maintained the characteristic of MSCs and displayed homogenous population of fibroblast-like cells. Cells at different passages are cryopreserved and some cells were evaluated for cell viability and morphology after revival. Our cells showed 60-70% viability after revival and morphology was unchanged.

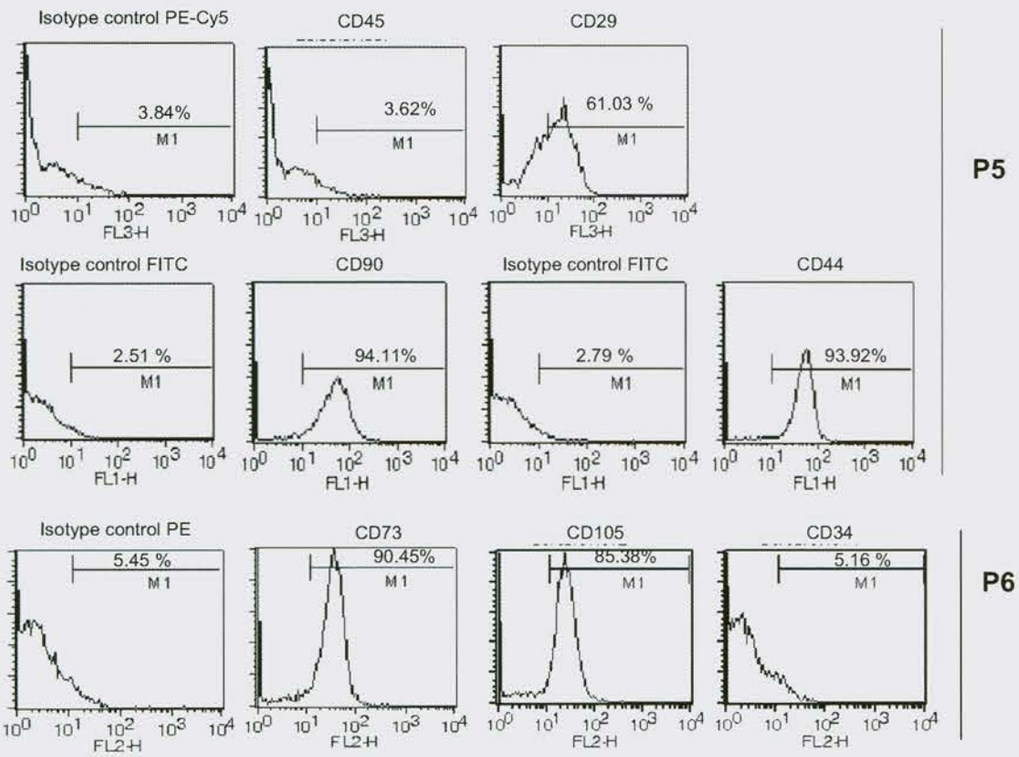


Fig. 2: Characterization of human MSCs for surface antigens by FACS. Cells showed strong expression of MSC markers such as CD29, CD73, CD105, CD90 and CD44, and very low expression of negative markers such as CD34 and CD45.

Differentiation of human MSCs into functional osteoblasts

Human MSCs were tested for in vitro differentiation into functional osteoblasts by incubating cells for 21 days in αMEM and 10% FCS containing osteogenic factors such as ascorbic acid (50 μg/ml), β-glycerophosphates (10mM) and dexamethasone (100 nM). In the presence of these osteogenic factors, MSCs showed formation of many mineralized bone nodules. Calcium mineralization was assessed by Von Kossa staining. Figure 3 shows the formation of calcium mineralization. These osteoblasts also showed expression of other markers such as collagen type I, osteopontin, and Cbfa 1 and osterix transcription factors.

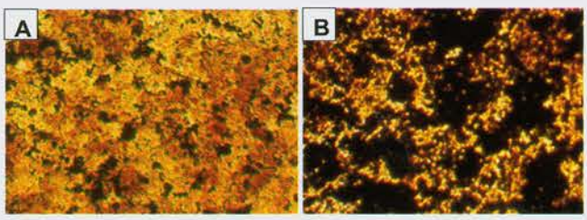
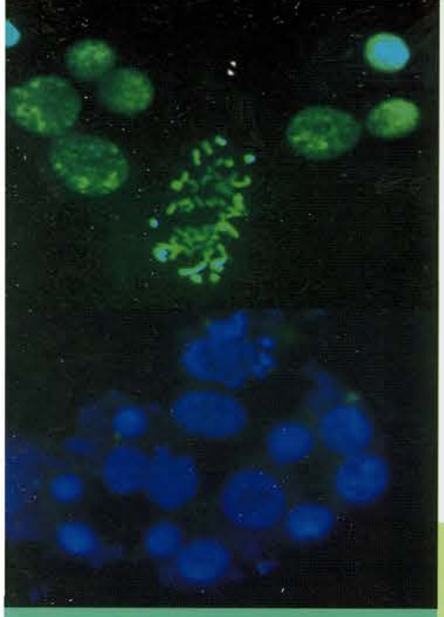


Fig. 3: Differentiation of human MSCs into functional osteoblasts. Functional osteoblasts were detected by Von Kossa staining. A) MSCs without osteogenic factors B) MSCs with osteogenic factors show calcium deposits.

Future Work

To investigate the role and mechanism(s) of parathyroid hormone (PTH) action on differentiation of MSCs into osteoblasts.



Research Reports

Cancer Biology

Recruitment of Vascular Stem Cells by Ovarian Tumor Stem Cells

Sharmila Bapat
sabapat@nccs.res.in

Participants
Nawneet Kurrey, *SRF*
Neeti Sharma, *SRF*
Anjali Kusumbe, *JRF*
Avinash Mali, *Technician*

Background

The long-term survival, growth and metastasis of the majority of tumors within the host is determined by their potential to establish an efficient vasculature. Tumors often adapt more than one mechanism to accomplish this crucial vascular development. Apart from angiogenesis, vasculogenesis, which involves the recruitment and subsequent endothelial differentiation of either bone marrow-derived progenitors or tissue-derived stem cells, appears to be an indispensable mechanism adapted by some tumors to ensure their survival. However the cellular interactions involved in the process remains unexplored as yet. Moreover, the exact nature of these stem/progenitors contributing to vascularization of the primary and metastatic tumors and the underlying mechanisms by which these are recruited remains vague.

Aims and objectives

1. Exploring the possibility of vasculogenesis as an alternative or indispensable mechanism to angiogenesis for ovarian tumor vascularisation.

2. Deciphering the phenotypic signature and hierarchal organization of the stem/progenitor cells participating in this process.

Work Achieved

Establishment of an *in vitro* model of human ovarian cancer

Earlier work in our lab provided the first evidence of maintenance of human ovarian tumors by the notorious stem/progenitor cells, through the establishment of a novel, *in vitro* model system consisting of nineteen immortalized stem/progenitor clones derived from an ovarian tumor spheroid (Fig 1a). Further detailed studies included profiling of mutations in the mitochondrial genome that revealed lineage demarcation within these clones into two groups, namely; a group consisting of Tumor Stem Cells (TSCs) and pre-TSCs (in the pre-tumorigenic stage), that expressed a distinct mutant profile highly divergent from the germline and a second group comprising of non-tumorigenic clones that retained the germline mitochondrial genome mutational profile (Fig. 1b).

Identifying the functionality of the germline clones

With the objective of demarcating the mutant clones from the germline clones, extensive surface phenotyping was carried out that provided a distinct demarcation between the mutant and germline clones surface phenotypes, and also provided a hint towards the putative lineage committment of the germline clones. All the 14 germline clones isolated from the ovarian tumor spheroid were found to express CD133 (a promising marker for the endothelial precursors) while the mutant clones lack the expression of the same (Fig. 2a). As a step towards the investigation of endothelial differentiation potential of these CD133 positive clones, cells of each individual clone were subjected to matrigel tube formation assay (an *in vitro* endothelial differentiation assay). Of the fourteen clones, twelve exhibited differential tube formation capacities, yet another clone (E2) which normally lacked tube formation potential on the matrigel, gained a capability to do so, subsequent to VEGF stimulation (Fig. 2b). Thus except one clone, all the germline clones were capable of undergoing endothelial differentiation (vasculogenesis); these germline clones were termed as vascular stem cells (VSCs).

Dechiphering the cellular hierarchy established by vascular stem cells which contributes towards development of the ovarian tumor vasculature

Stage-specific immunophenotyping during differentiation was adapted as a strategy for deciphering further characteristics of these VSCs and the consequent hierarchy established by them. This approach revealed that each lineage expresses a characteristic signature. The primary recruited stem cells were slow-cycling and displayed an extremely immature surface expression ($CD133^+$, $CD44^+$, $ckit^+$, $VEGFR1^+$, $VEGFR2^+$, $VEGFR3^+$, $ABCG2^+$, $Nestin^+$, Bmi^+, $Nanog^+$), thus termed by us as VSCs. After a brief span of

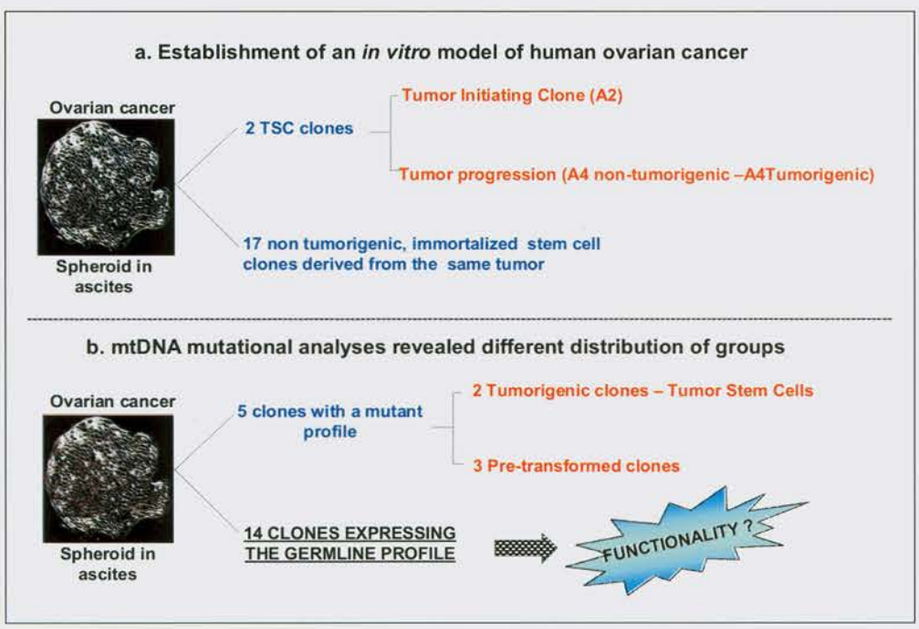


Fig. 1: Schematic representation of the *in vitro* ovarian cancer model system. **(a)** *In vitro* ovarian cancer model system comprising of 19 immortalized stem/progenitor cell clones, one of which was found to be tumorigenic termed TIC (tumor initiating clone); while yet another one (A4) underwent transformation (A4-T) after a few passages the other 17 clones remained non-tumorigenic. **(b)** Mitochondrial genome profiling revealed lineage demarcation within these clones into, TSCs and pre-TSCs expressing a distinct mutant profile, while the other 17 non-tumorigenic clones retained the germline mitochondrial genome profiles.

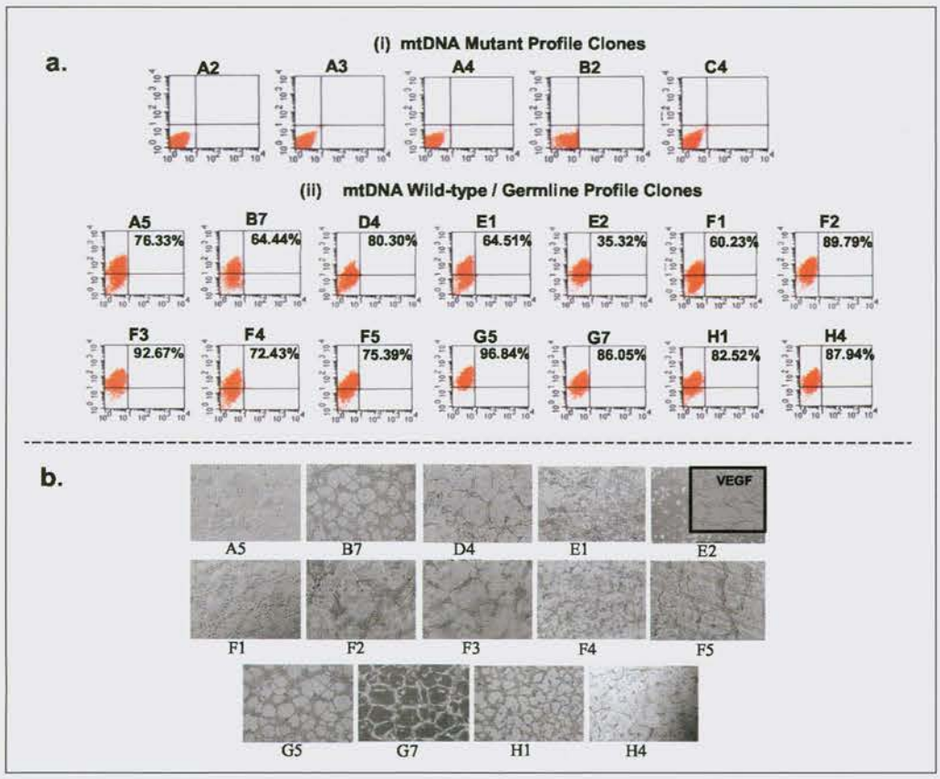


Fig. 2: Expression of CD133 in the germline clones. **(a)** (i) FACS analysis of CD133 showed that the mutant clones lacked the CD133 expression (ii) All the germline clones expressed different levels of CD133 **(b)** Tube formation assay on induction by matrigel. Twelve of the fourteen germline clones expressed the capability for tube formation on matrigel; clone E2 required an additional induction of VEGF, while clone A5 totally lacked this capacity.

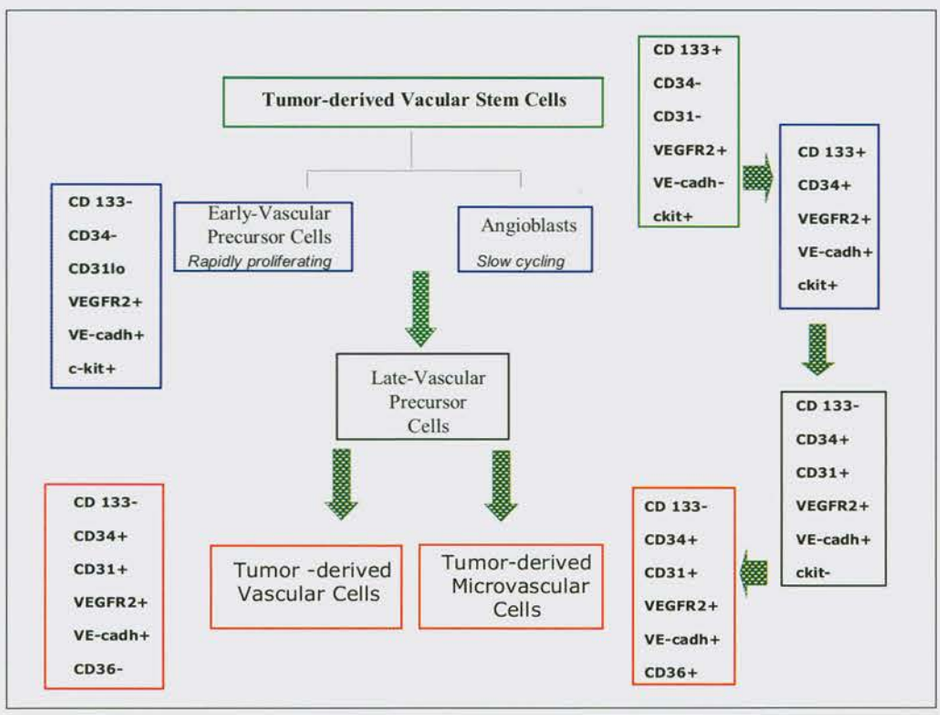


Fig. 3: Schematic representation of the hierarchy established by the vascular stem cells.

in vitro culture, these VSCs lost their CD133 expression and generated rapidly proliferating CD144+ early vascular progenitor cells (VPCs), which exihibited minimal surface expression specific for endothelial lineage. Expression of CD144 by these early-VPCs can be considered to symbolize their commitment towards endothelial lineage. Maturation of these early-VPCs into late-VPCs ($CD34^+$, $CD31^+$) required matrigel induction which finally on terminal differentiation yielded the Tumor-derived Microvascular Cells (TMVCs) expressing endothelial specific markers including CD36, CD62E and CD106 (Fig. 3).

The above results indicate:

(i) the involvement of a more immature stem/ progenitor cell population besides the earlier reported progenitors for the vascularization of primary ovarian tumors and also during their distant metastases,

(ii) these recruited/infiltrating cells perhaps establish a complete endothelial hierarchy within the tumor thus ensuring long- term vascularization to the tumor tissue,

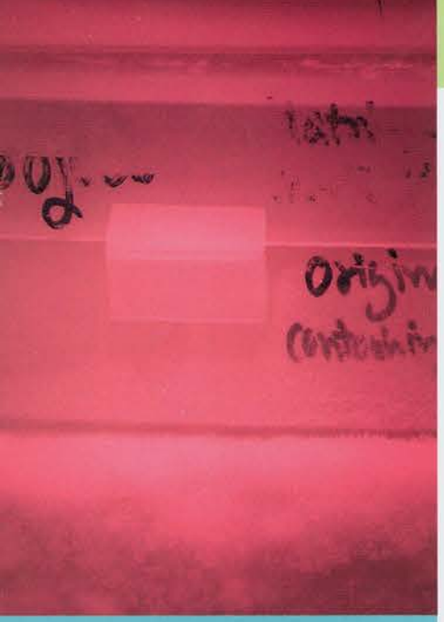
(iii) hint towards a definite mechanistic role of ovarian tumor stem cells in the recruitment process.

Future Work

1. Identification of factors involved in recruitment of the vascular stem cells by the TSCs.

2. Interactions of TSCs with the VSC lineage members during tumor neo-vascularisation.

3. Deciphering the functional differences between neo-vascularisation in normal vs. tumor microenvironment.



Chemosensitivity of cancer cells to drugs: Elucidation of mechanism of cell death

Manoj Kumar Bhat
manojkbhat@nccs.res.in

Participants
Ankur Kumar Upadhyay, *SRF*
Amrendra Kumar Ajay, *SRF*
Ratna Kumari, *JRF*
Vimal Pandey, *JRF*
Aanchal Sharma, *JRF*
Vijay Kumar M.V., *Officer A*

Background

The importance of apoptosis induction in cancer therapy has been recognized and the ongoing discovery of numerous apoptosis-regulating proteins provides new potential targets for molecular cancer therapy. DNA-damaging drugs are commonly used chemotherapeutic agents for the treatment of various cancers including human papillomaviruses (HPVs)-positive cancers. In general, these drugs inhibit proliferation of sensitive tumor cells by the induction of apoptosis. A numbers of key factors are involved in the regulation, coordination and execution of apoptotic response by the cells. Drug sensitivity may depend on the relative intactness of these pathways in individual tumor cells. The necessary information about the proteins and the pathways involved in the cytotoxic action of a given drug may provide a better and more rational approach for the treatment of individual tumor type. These studies may further provide the understanding of the molecular basis of drug resistance in non-responsive tumors.

Carboplatin (Carb) is among the most effective and widely used chemotherapeutic agents employed for the treatment of human cancers including human cervical carcinoma. Carb induced cytotoxicity is generally considered to be the result of its ability to damage DNA. In response to DNA-damage stresses, various molecular and cellular processes are activated, that coordinate and transduce incoming signals from stress and result in the induction of apoptosis. Like other members of MAP-kinase family, ERK is reported to be activated in response to stresses for the induction of apoptosis. Interestingly, these findings have reported a contrasting role for ERK, as the activation of ERK cascade is originally thought to be associated with proliferation, differentiation and survival of the cells. The upstream regulations of ERK cascade in response to DNA damage stresses which assign the apoptotic role to ERK have remained unclear.

In response to DNA damage stress, p53 is reported as a central mediator of the cellular responses. Since, almost all cervical cancer cases are found to be infected with high-risk HPV, p53 is rapidly degraded by E6 mediated ubiquitin-dependent proteolysis in these cells. Therefore, p53 expression is very low in cervical cancer cells. However, in response to DNA damage stress, p53 is stabilized and induced to exert its various cellular responses including apoptosis.

Since, there are similarities between ERK and p53 in the cellular response to DNA damage stress; we sought to explore the relationship between activation of ERK and p53 expression in Carb induced apoptosis in cervical cancer cell lines SiHa

and CaSki. We demonstrate here that Carb activates ERK and p53 in a time dependent manner. Both, ERK activation as well as p53 transactivation activity leads to Carb induced apoptosis. By applying chemical as well as genetic strategies to manipulate p53 transactivation activity, our data provides the evidence that Carb induced p53 leads to the activation of ERK in SiHa and CaSki cells. We for the first time report that p53 may represent one of the upstream mediators of Carb induced ERK activation responsible for the induction of apoptosis in cervical cancer cells.

Aims and objectives

To understand mechanisms of chemotherapeutic drugs mediated cell killing and to investigate the molecules and molecular events that contribute to drug induced cell death.

Work Achieved

Carboplatin activates ERK in SiHa and CaSki cells which is associated with the induction of apoptosis

The ERK signaling pathway has been shown to be activated in response to a variety of DNA-damage stresses. To investigate whether Carb induced DNA damage-stress also leads to the activation of ERK, SiHa and CaSki cells were treated with indicated doses of Carb and lysates were processed for western blot analysis to detect phosphorylation of ERK. (Fig. 1). As shown in Fig. 1A, and B, SiHa and CaSki cells were treated with indicated concentrations of Carb. SiHa cells showed constitutive activity of ERK which decreased subsequently at 50 and 200 μM of Carb treatment. However, 500 and 1000 μM of Carb treatment resulted in strong activation of ERK in SiHa cells (Fig. 1A). On the other hand, CaSki cells demonstrated a dose dependent increase in ERK activation in response to Carb treatment (Fig. 1B). Next, the time kinetics of ERK activation in response to 500 μM Carb was studied in SiHa and CaSki cells and results are shown in Fig. 1C, D. SiHa cells showed a biphasic response of ERK activation in a time dependent manner. The basal activity of ERK decreased till 12 h of treatment. Carb reactivated ERK after 18 h of treatment which further increased till 30 h of treatment (Fig. 1C). On the other hand, in CaSki cells, the activation of ERK was apparent after 6 h of treatment and sustained at almost similar level till 18 h following treatment with 500 μM Carb. However, a strong ERK activation was observed at 24 h which further increased at 30 h following treatment (Fig. 1D). To explore the functional involvement of ERK activation in Carb induced apoptosis, ERK inhibitor (U0126) was utilized. U0126 is a highly selective inhibitor of ERK pathway which inhibits the MEK mediated ERK activation. SiHa and CaSki cells were treated with 500 μM of Carb for 30 h with or without pretreatment of U0126. Pretreatment with U0126 suppressed Carb induced PARP cleavage (as demonstrated by decrease in p85 fragment of PARP) in a dose dependent manner as it suppressed the activation of ERK (Fig. 1E and F; lanes 3 and 4 as compared to lane 2). Overall, these results indicated that the Carb induced DNA damage stress activates ERK and the activation of ERK is associated with the induction of apoptosis in SiHa and CaSki cells.

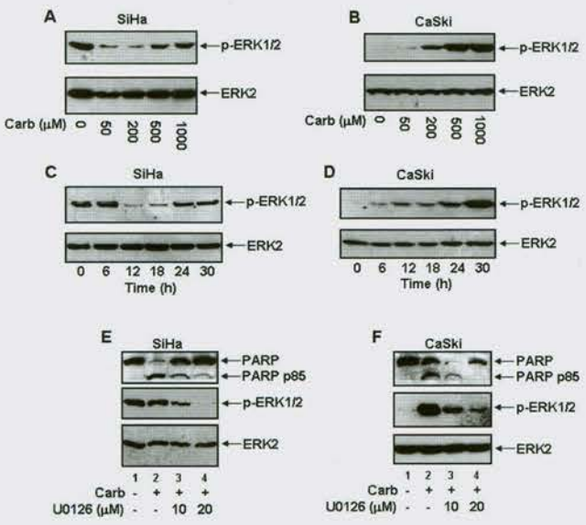


Fig. 1: Carb induces ERK phosphorylation which is associated with apoptosis. (A and B) SiHa and CaSki cells were treated with increasing concentration of Carb for 24 h. (C and D) SiHa and CaSki cells were treated with 500 μM of Carb for indicated time. In all the panels, cells were harvested and equal amount of protein was processed for western blot analysis to detect phosphorylation of ERK1 and 2. Blots were stripped and reprobed to detect total ERK2. (E and F) SiHa and CaSki cells were treated with 500 μM of Carb for 30 h with or without indicated concentration of U0126. Cells were harvested and equal amount of protein was processed for western blot analysis to detect the cleavage of PARP, phosphorylation of ERK1 and 2 as well as for total ERK.

Carboplatin induces p53 transactivation activity which is associated with the induction of apoptosis

In response to DNA damage stress, p53 plays a major role in the induction of apoptosis. We next studied the response of p53 and its functional significance in Carb treated cells. SiHa and CaSki cells were treated with 500 μM Carb for increasing time and lysates were processed for western blot analysis to detect p53. Both SiHa and CaSki cells showed a time dependent response of p53 after Carb treatment. p53 upregulation was apparent after 6 h of treatment and it further increased after 12 h. SiHa cells showed sustained p53 levels up to 24 h which decreased after 30 h of treatment (Fig. 2A). On the other hand, CaSki cells showed the maximum p53 protein levels by 12 h of treatment which decreased at later time points studied (Fig. 2B). Next, the transactivation activity of upregulated p53 was checked

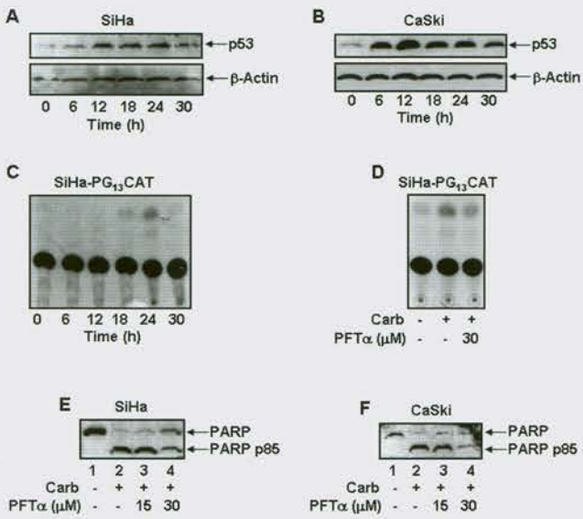


Fig. 2: Carb induces p53 transactivation activity which is associated with apoptosis. (A and B) SiHa and CaSki cells were treated with 500 μM of Carb for indicated time. Cells were harvested and equal amount of protein was processed for western blot analysis to detect the p53 levels. Blots were stripped and reprobed with β-Actin antibody to confirm equal loading. (C) SiHa-PG13CAT cells were treated with 500 μM of Carb for indicated time and p53 transactivation activity was detected by CAT assay. (D) Carb induced p53 transactivation activity was suppressed by the treatment of PFTα and demonstrated by CAT assay. (E and F) SiHa and CaSki cells were treated with 500 μM of Carb for 30 h with or without indicated concentration of PFTα. Cells were harvested and equal amount of protein was processed for western blot analysis to detect the cleavage of PARP.

in Carb treated cells. SiHa-PG_{13}CAT cells, carrying p53-CAT reporter construct (PG_{13}CAT) were treated with 500 μM of Carb. Cells were harvested at indicated time (Fig. 2C) and CAT reporter activity was measured. SiHa cells showed an increased p53 transactivation activity after 18 h of Carb treatment which further increased after 24 h of treatment. However, p53 transactivation activity decreased after 30 h of treatment. Overall, these results indicated that in SiHa and CaSki cells, Carb treatment not only results in p53 protein upregulation but also increases p53 transactivation activity. We next studied the functional role of induced p53 in response to Carb treatment in SiHa and CaSki cells. A cell permeable and potent inhibitor of p53 transactivation activity, pifithrinα (PFTα) was chosen to inhibit the activity of induced p53 in Carb treated cells. SiHa-PG_{13}CAT cells were treated with Carb in the presence or absence of PFTα. Pretreatment with PFTα for 1 h before the addition of 500 μM of Carb resulted in significant decrease in CAT activity as compared to Carb alone treated cells (Fig. 2D). Next, to check the significance of upregulated p53 transactivation activity in Carb induced apoptosis, SiHa and CaSki cells were treated with 500 μM of Carb for 30 h with or without the pretreatment of PFTα at indicated concentrations. In both SiHa and CaSki cells pretreatment with 30 μM PFTα significantly suppressed Carb induced PARP cleavage as detected by the decrease in the levels of p85 fragment of PARP (Fig. 2E and F; lane 4 as compared to lane 2). Overall, results so far indicated that Carb induced p53 as well as ERK activation and both of these are associated with the induction of apoptosis in SiHa and CaSki cells.

p53 regulates the activation of ERK in Carboplatin treated cells

p53 transactivation activity has been linked with the activation of ERK and to explore the possible role of induced p53 in the activation of ERK in response to Carb treatment, SiHa and CaSki cells were pretreated with indicated doses of PFTα for 1 h followed by the addition of 500 μM Carb for 30 h in the presence of inhibitor. In both SiHa and CaSki cells, PFTα pretreatment inhibits the Carb induced ERK activation as compared to its activation in Carb alone treated cells (Fig. 3A and B; lane 3 or 4 as compared to lane 2). To confirm whether the Carb induced p53 transactivation activity is indeed essential for Carb induced ERK phosphorylation, p53 transactivation activity was abrogated by a dominant negative mutant of p53. A stable cell line of SiHa cells was

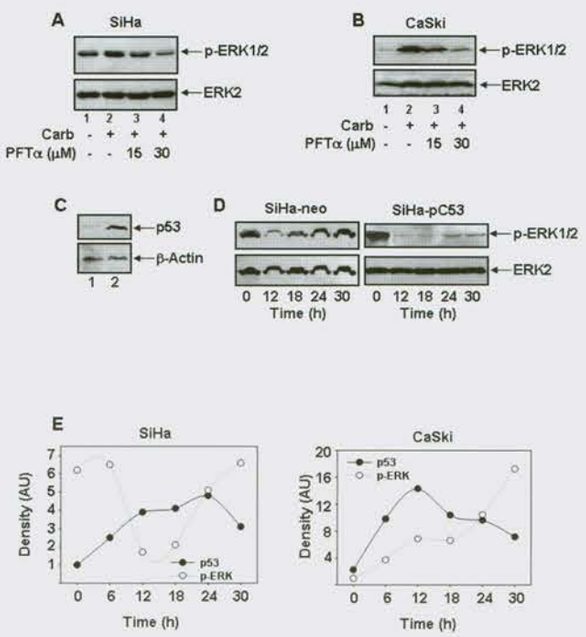


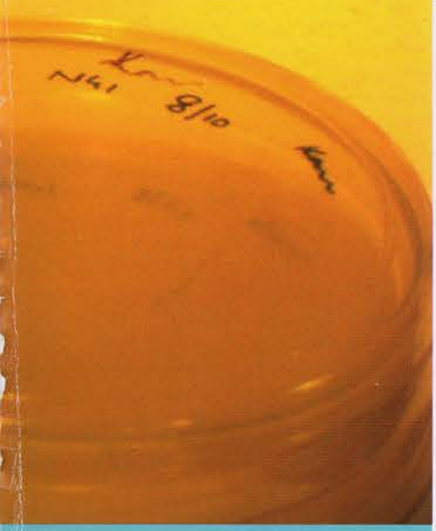
Fig. 3: p53 transactivation activity regulates Carb mediated ERK activation. (A and B) SiHa and CaSki cells were treated with 500 μM of Carb for 30 h with or without indicated concentration of PFTα. Cells were harvested and equal amount of protein was processed for western blot analysis to detect the activation of ERK1 and 2. Blots were stripped and reprobed to detect ERK2. (C) Equal amount of protein from SiHa-neo (lane 1) and SiHa-pC53 (lane 2) cells was detected for the expression of p53 in the cells. Blots were stripped and reprobed for β-Actin to confirm equal loading. (D) SiHa-neo and SiHa-pC53 cells were treated with 500 μM of Carb for indicated time. Cells were harvested and equal amount of protein was processed for western blot analysis to detect the Tyr204-phosphorylation of ERK1 and 2. Blots were stripped and reprobed to detect ERK2. These results are representative of three independent experiments. (E) Expression of activated ERK and p53 (Fig. 1 and 2) was determined by quantitation of bands using densitometry scanner analysis. Average of the density of the bands are plotted against time of treatment from three independent experiments and represented as line graph.

established by transfecting pC53-SCX3 expression vector to express a dominant-negative mutant of p53. Vector transfected (SiHa-neo) and pC53-SCX3 transfected (SiHa-pC53) cells were analyzed by western blot analysis for the expression of p53 in these cells. As shown in Fig. 3C, SiHa-neo cells (lane 1) demonstrated the minimum expression of p53 where as SiHa-pC53 cells (lane 2) showed a very high expression of p53, which is a mixture of wild-type as well as expressed mutant p53. Both these cells were treated with 500 μM of Carb for various time points and time depended response of ERK activation was analyzed. As shown in Fig. 3D, SiHa-neo cells demonstrated an almost similar time dependent response as shown by SiHa parental cells (Fig. 1C). In contrast to these observations, SiHa-pC53 cells failed to demonstrate the strong activation of ERK even after 30 h of Carb treatment (Fig. 3D). These results clearly indicate that Carb induced p53 transactivation activity is required for the activation of ERK in cervical cancer SiHa and CaSki cells.

In summary, our data demonstrates that in response to Carb treatment, both p53 and ERK are activated in a specific manner. Abrogation of Carb induced p53 transactivation activity by chemical as well as genetic inhibitors results in decreased activation of ERK in SiHa and CaSki cells. We suggest for the first time that p53 acts as upstream regulator of DNA damage induced ERK activation which assigns the apoptotic role to ERK instead of its conventional survival functions. Further characterization for the factors which link p53 to the activation of ERK may contribute to better understanding of ERK activation mediated induction of apoptosis in response to DNA damage stress.

Future work

Studies on effects of various chemotherapeutic drugs on cancer cells will be continued and these investigations will be expanded to cell lines derived from various human solid tumors.



Signaling pathways in receptor mediated cell death in 2D and 3D cultures derived from human glioma cells

Padma Shastry
padma@nccs.res.in

Participants
Sudheer Kumar. P, *SRF*
Vandna Prasad, *SRF*
Sri Gowry Das. G, *JRF*
Jayashree C. Jagtap, *Technician*

Collaborator
Anjali Shiras, NCCS, Pune

Background

Gliomas are the most common of the primary brain tumors in adults. In gliomas, the tumor associated macrophages constitute a substantial mass. The activated macrophages secrete various cytokines including TNF-α, FasL and TRAIL that affect diverse functions of tumors. TNF-α is a classical activator of NF-κB. The most common form of NF-κB comprises of a heterodimer of p50 and p65 subunits and is normally retained in the cytoplasm in association with inhibitor proteins, IκBα and IκBβ. Activation of NF-κB occurs in response to various stimuli and cytokines and constitutive activation of NF-κB is documented in different cancers including high-grade gliomas. Akt/PKB, a serine-threonine kinase, is activated by PI3-kinase and promotes cell survival in response to cytokines or growth factors. Upregulation and activation of PI3K/Akt pathway confers resistance to tumors against cytotoxic effects of anti-cancer drugs. Akt and NF-κB are constitutively expressed and intimately involved in progression of gliomas and confer resistance to conventional treatments including chemotherapy. While PI3K/Akt is shown to regulate the NF-κB activation in diverse systems, other studies place NF-κB upstream of Akt activation. Delineating the TNF-α mediated signaling will lead to better understanding of the precise role of this cytokine in progression of gliomas.

Aims and Objectives

1. To study the role of cell death receptor ligands in gliomas with reference to proliferation and cell death using monolayers and spheroids as models.

2. To decipher the TNF-α mediated signaling pathways in monolayer and spheroids generated from gliomas.

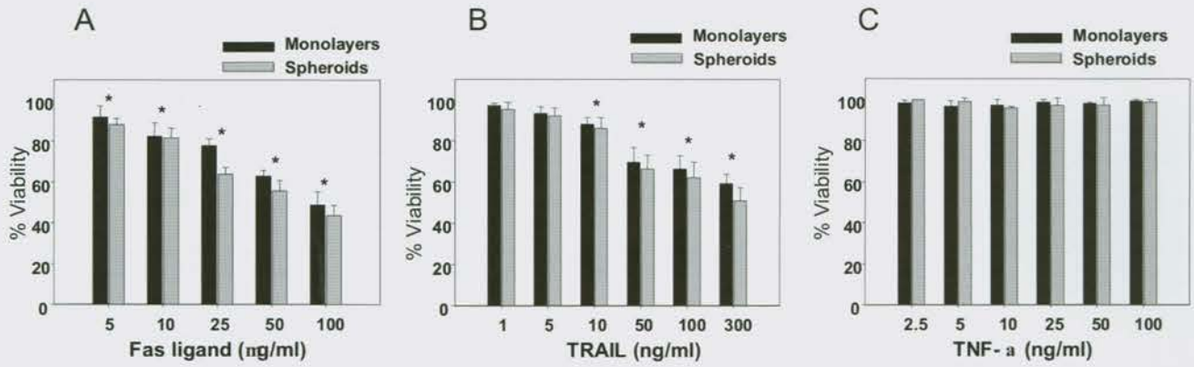


Fig. 1: Cell viability assessed by MTT assay in LN-18 cells cultured as monolayers and spheroids. Viability was determined in cells treated for 24 h with serial concentrations of (A) FasL, (B) TRAIL and (C) TNF-α.

Work Achieved

The human glioma cell lines – LN-18, LN-229 and U373MG were used for the study. LN-18 cells were sensitive to FASL and TRAIL-mediated cell death but resistant to TNF-α-induced cytotoxic effect (Fig. 1). Studies have indicated that activation of NF-κB induced by TNF-α is dependent and functions down stream of Akt. Using a stable IκBα mutant LN-18 cell line and pharmacological inhibitors to PI3K/Akt (LY294002) and Akt, we have demonstrated that NF-κB and Akt are independently activated on stimulation with TNF-α in human glioma cells and contribute towards resistance to TNF-α-induced cytotoxicity. The key events in NF-κB activation-degradation of IκBα, translocation of p65 to nucleus and NF-κB promoter activation were unaffected in the presence of LY294002 suggesting that PI3K/Akt pathway was not involved in the activation of NF-κB (Fig. 2). TNF-α-induced NF-κB activation, independent of PI3K/Akt pathway was also confirmed in human glioma cell lines – LN-229 and U373MG suggesting

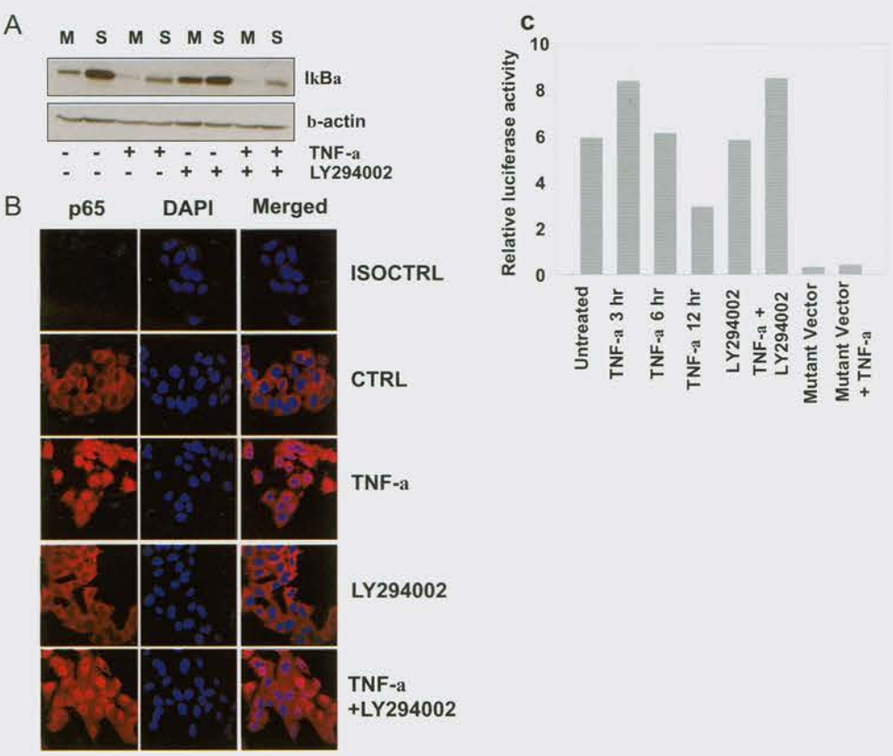


Fig. 2: (A) Effect of PI3K/Akt inhibitor LY294002 on IκBα degradation, (B) p65 nuclear translocation, (C) luciferase activity in LN-18 cells treated with TNF-α.

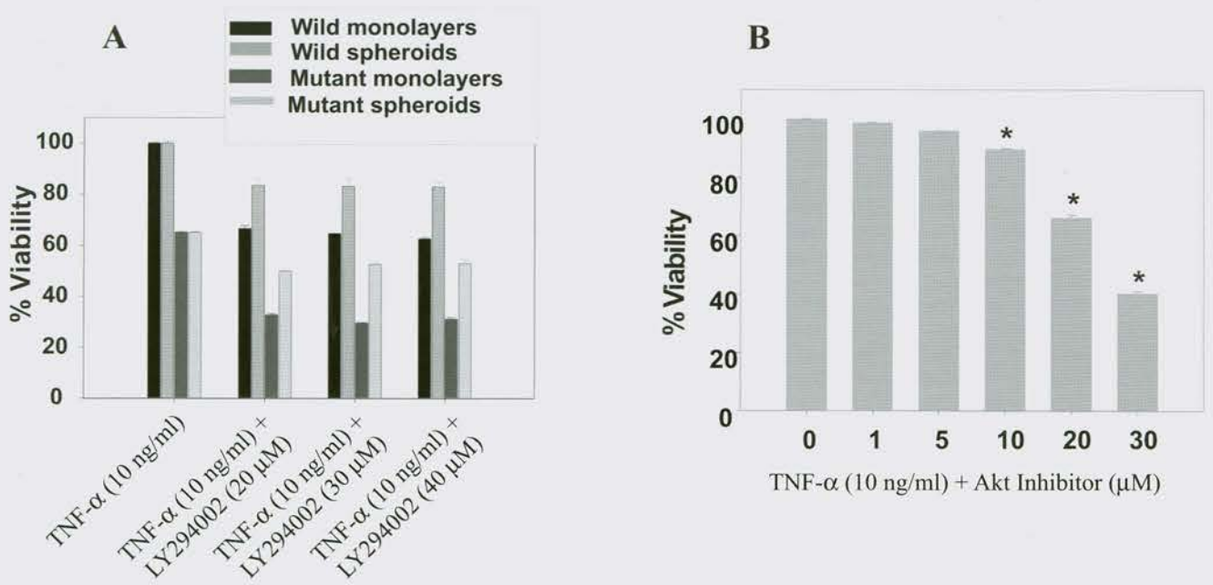
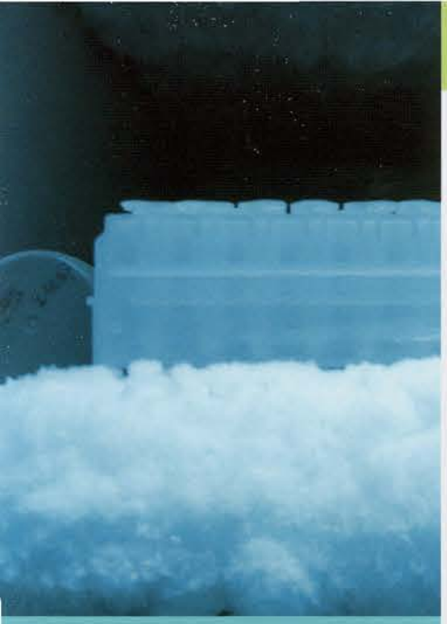


Fig. 3: Cell viability determined by MTT assay in wild and IκBα mutant LN-18 cells preincubated with LY294002 (A) and LN-18 cells pretreated with Akt2 (B) followed by TNF-α treatment for 24 h.* $p < 0.05$, comparison between viability in TNF-α treated cells in the presence and absence of inhibitor in corresponding culture models.

that this may be an essential feature of gliomas for survival. Our data also revealed that LN-18 cells constitutively expressed Akt1 and Akt2 and spheroidogenesis enhanced their expression in wild type as well as mutant cell lines. Inhibition of the PI3K/Akt pathway using LY294002 and Akt specific inhibitor-Akt2 rendered the LN-18 cells sensitive to TNF-α-induced death confirming the survival role of Akt (Fig. 3B). We also show that NF-κB pathway is activated during spheroidogenesis and is further enhanced on stimulation with TNF-α, implicating its involvement in resistance to cell death. These findings underscore the importance of multicellular spheroids as an appropriate *in vitro* model for studying the signaling pathways in response to chemotherapeutic drugs. Though gliomas are sensitive to FasL and TRAIL, the high degree of resistance to TNF-α associated with activation of the two prosurvival pathways–Akt and NF-κB in response to TNF-α may facilitate immune evasion of gliomas cells. These findings lead us to conclude that strategies to target multiple proteins on diverse prosurvival pathways should be considered for development of efficient anti-cancer drugs.

Future Work

Further work will be focused on characterization of the signaling process downstream of Akt and NFκB pathways that might contribute to resistance to TNF-α-mediated cell death in gliomas.



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

Anjali Shiras
anjalishiras@nccs.res.in

Participants
G. Rajendra Prasad, *SRF*
E. Maheshwara Reddy, *SRF*
Sivaranjan Chettiar, *SRF*
Ganeshkumar Rajendran, *SRF*
Nagesh Shanbagh, *Project Assistant*
Varsha Shepal, *Technicial Officer A*

Collaborators
Dr. L.C. Padhy, TIFR, Mumbai
Dr. Padma Shastry, NCCS, Pune

Background

In an endeavor to study novel genes in melanoma genesis, an expression cDNA library was generated from mouse melanoma cells - Clone M3. From the Clone M3 cDNA a new kind of molecule – M3TR was identified that was unconventional in the sense, that it was a non-coding RNA and yet displayed transforming ability. The last decade has seen an explosion of discovery of functional RNAs known as Non-coding RNAs (ncRNAs). These transcripts contain large number of stop codons and therefore lack extensive ORFs (Open Reading Frames), and function at RNA level. They basically range from small miRNA/siRNAs (21nt) to large ncRNAs (>10 kb). The various non-coding RNAs regulate gene expression by novel mechanisms such as RNA interference, gene co-suppression, gene silencing, imprinting and DNA demethylation.

Aims and Objectives

1. Cloning and molecular characterization of the noncoding RNA – M3TR from human cells.

2. Investigating the biological function of M3TR by ectopic expression studies.

3. Understanding the molecular mechanisms and signaling pathways elicited by M3TR.

Work Achieved

SK-N-MC, a pseudodiploid cell line developed from the neuroepithelioma of human female brain, showed high expression of M3TR and was used in studies aimed to understand the role of human M3TR. The human homologue was amplified from SK-N-MC cDNA and cloned into pTarget mammalian expression vector by TA cloning strategy in both sense and reverse orientations. On BLAST analysis the human sequence was found to be identical to mouse M3TR sequence. Expression studies revealed its presence in hematopoetic cell-lines like Raji and Jurkat. The expression of M3TR was found in human fetal brain, fetal lung and testis, implying the role of M3TR during development. The also M3TR transcript length in human cells was found to be 1.9 kb by Northern analyses. Ribonuclease protection assay (RPA) indicated that M3TR was expressed as a natural anti-sense transcript (NAT) (Fig. 1). The chromosomal localization of M3TR transcript was assessed by transfecting the fluorescently labeled M3TR RNA transcripts into NIH3T3 cells followed by chromosomal preparations using calyculin A. The M3TR sense transcript was found to be localized to the chromatin as shown in Fig. 2. The major focus of the study was to understand and identify the mechanisms that may be leading to transformation caused by M3TR at genetic, epigenetic and RNA (i.e., RNAi) levels. Since, the antisense expression of M3TR was observed; we looked into the possibilities of M3TR acting through RNAi mechanism, i.e. functioning as a siRNA. For this a Renilla luciferase assay was designed by cloning M3TR in pRL-CMV vector at the C terminus of the Renilla luciferase vector. M3TR siRNA was synthesized *in vitro* by digesting the dsRNA with Dicer (RNase III endonuclease) enzyme and transfected in NIH 3T3 cells. We found that the Renilla luciferase activity was found decreased up to 100 fold in presence of the M3TR siRNA signifying that M3TR could be acting as a siRNA through RNAi mechanism. We now have experimental evidence to show that M3TR siRNA induces genomic instability with over-expression of molecules involved in DNA damage repair and checkpoint arrest and thus predisposes the cells towards cell transformation (Fig. 3). The M3TR-siRNA effects on genomic instability were similar to that observed with M3TR transfected stable cell-line. In a simplistic manner, we propose the possible ways through which M3TR induces cellular transformation and functions as a novel oncoRNA (Fig. 4).

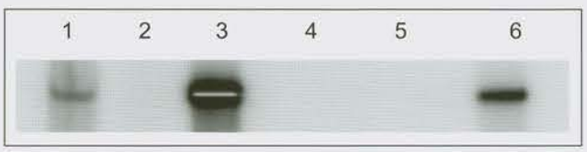


Fig. 1: Ribonuclease Protection assay (RPA) with SK-N-MC cells, showing protection of RNA ~600bp with M3TR sense probe (lane 1). Lanes 2 and 3 show sense riboprobe with and without RNase A/T1, while lanes 5 and 6 shows reverse riboprobe with and without RNase A/T1. The reverse probe failed to protect any RNA (lane 4), indicating that M3TR is synthesized as Natural Antisense Transcript (NAT).

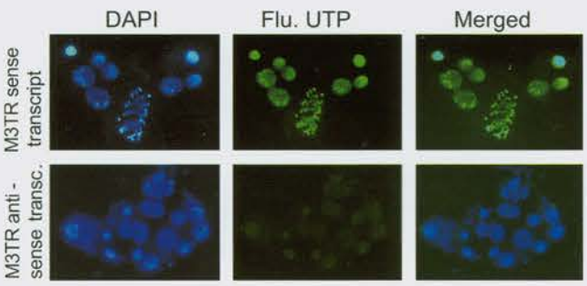


Fig. 2: Localization of M3TR; The M3TR sense transcript was localized to the chromatin in NIH 3T3 cells. The chromosomes and nuclei show up intensely with M3TR sense transcript and staining co-localizes with DAPI. The cells transfected with M3TR anti-sense transcript show weak staining in chromosomal spreads and counterstained with DAPI.

Future Work

1. Identifying the transcriptional targets of M3TR
2. Understanding the epigenetic mechanisms evoked by M3TR acting as a transforming gene

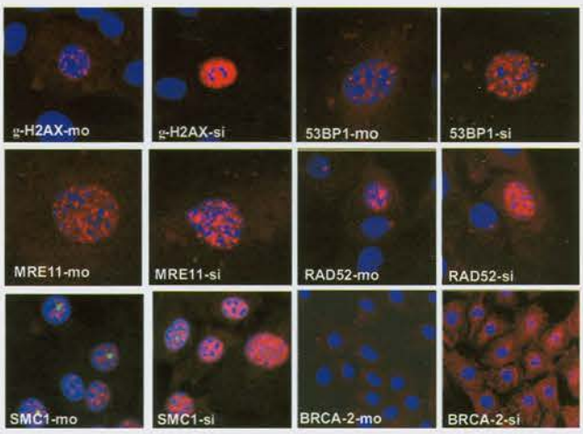


Fig. 3: Activation of DNA damage respone modifiers in M3TR-siRNA transfected NIH3T3 cells.

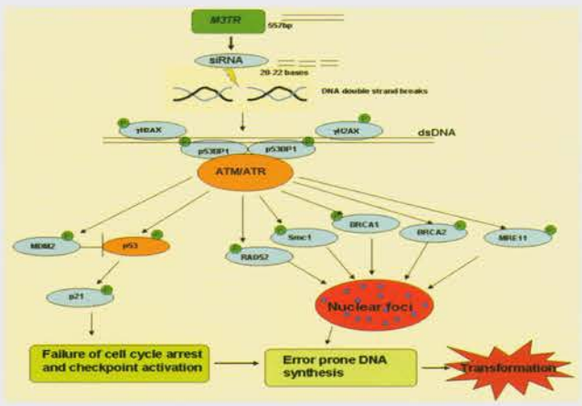
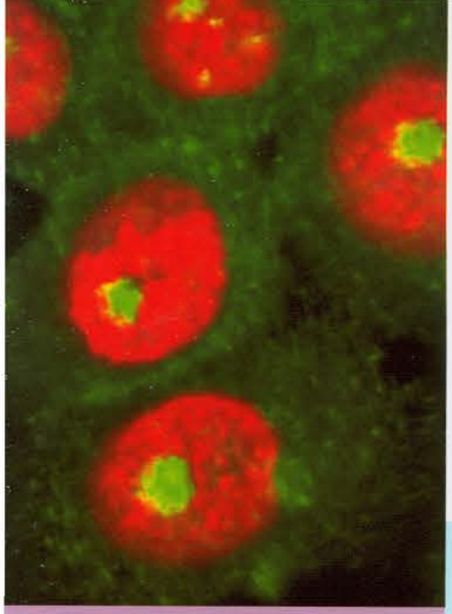
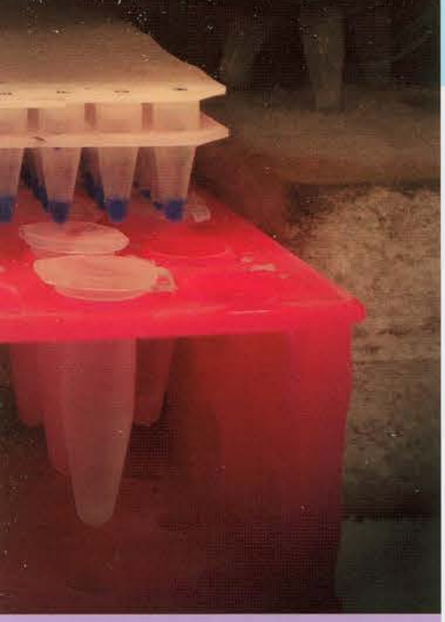


Fig. 4: A proposed model implicating the role of defective checkpoint control in cellular transformation induced by M3TR



Research Reports

Signal Transduction



Gopal C. Kundu
kundu@nccs.res.in

Participants
Goutam Chakraborty, *SRF*
Shalini Jain, *SRF*
Reeti Behera, *SRF*
Mansoor Ahmed, *SRF*
Priyanka Sharma, *JRF*
Vinit Kumar, *JRF*
Rajinder Kaur, *JRF*
Remya Raja, *JRF*
Smita Kale, *JRF*
Santosh Kumar, *JRF*
Rosalin Mishra, *JRF*
Anuradha Bulbule, *Technician*

Osteopontin promotes vascular endothelial growth factor dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms

Background

Angiogenesis, the formation of new blood vessels from the existing one is the key step for tumor growth and metastasis. A large number of proangiogenic factors and their cognate receptors have been identified. Vascular endothelial growth factor (VEGF) is one of the well characterized proangiogenic cytokine which turn on the 'angiogenic switch'. In last several years, the role of VEGF in regulation of tumor angiogenesis has been under intense investigation. Alternative exon splicing results in four isoforms of VEGF ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$) and among them $VEGF_{165}$ is the predominant one that plays major role in tumor angiogenesis. Recent data demonstrates that the functions of VEGF may not be limited to endothelial cells but it also plays important role in survival, proliferation and migration in tumor cells.

Osteopontin (OPN), a secreted non-collagenous, sialic acid rich chemokine like protein and also a member of SIBLING (small integrin binding ligand N-linked glycoprotein) family, plays important role in determining the oncogenic potential of various cancers. OPN exerts its prometastatic effects by regulating various cell signaling events through interaction with integrins and CD44 receptors that ultimately lead to tumor progression. 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 COX-2 and uPA expressions and activation of matrix metalloproteinase (MMP) in various cancer cells. Earlier reports also suggested that OPN produced either from tumor or stromal cells has been shown to enhance the metastatic ability of tumor cells. Breast tumor kinase (Brk/PTK6/Sik) is a non-receptor tyrosine kinase expressed mostly in metastatic breast tumors. Recent data revealed that alternative transcription factor-4 (ATF-4/CREB-2) regulates VEGF expression. However, the molecular mechanism by which OPN regulates Brk activation and Brk dependent ATF-4 activation that ultimately augment the VEGF expression and tumor angiogenesis through autocrine and paracrine mechanisms are not well understood.

Aims and Objectives

1. To examine whether OPN induces $\alpha_v\beta_3$-mediated VEGF expression in breast cancer cells
2. To study whether OPN regulates Brk/NIK dependent NFκB activation and VEGF expression in these cells
3. To investigate whether OPN controls Brk dependent ATF-4 activation and whether there is any cross talk between NFκB and ATF-4, which leads to VEGF expression in breast cancer cells
4. To delineate whether OPN-induced VEGF regulates cell motility, angiogenesis and tumorigenesis of breast cancer through both autocrine and paracrine mechanisms and whether the increased expressions of OPN and VEGF correlate with oncogenesis and angiogenesis in breast cancer specimens of higher grades.

Work Achieved

In this study, we provide both *in vitro* and *in vivo* experimental evidences to demonstrate the molecular mechanism, by which OPN regulates Brk/NFκB/ATF-4 signaling cascades that ultimately augment the VEGF expression and tumor angiogenesis through autocrine and paracrine mechanisms. We have further substantiated the crucial roles of tumor derived, endogenous OPN in tumor angiogenesis using siRNA based approach in *in vitro* and *in vivo* models. Most importantly, the orthotopic tumors generated in OPN$^{-/-}$ mice suggested that both tumor as well as stromal OPN plays crucial role in regulation of VEGF expression and tumor angiogenesis. Furthermore, clinical data revealed that the enhanced expressions of OPN and VEGF correlate with NRP-1, Brk, NFκB and ATF-4 levels in breast carcinoma of higher grades. These data provide new insights into the mechanism underlying the regulation of VEGF expression by OPN in breast tumor angiogenesis and understanding these mechanisms may form the basis of new therapeutic regimens for the management of breast cancer.

Future Work

The roles of ILK/eIF-2α/ATF-4 on OPN-induced VEGF dependent breast tumor growth and angiogenesis will be further studied. Moreover, the potential role of OPN in stromal-tumor interaction that leads to tumor progression will be addressed.

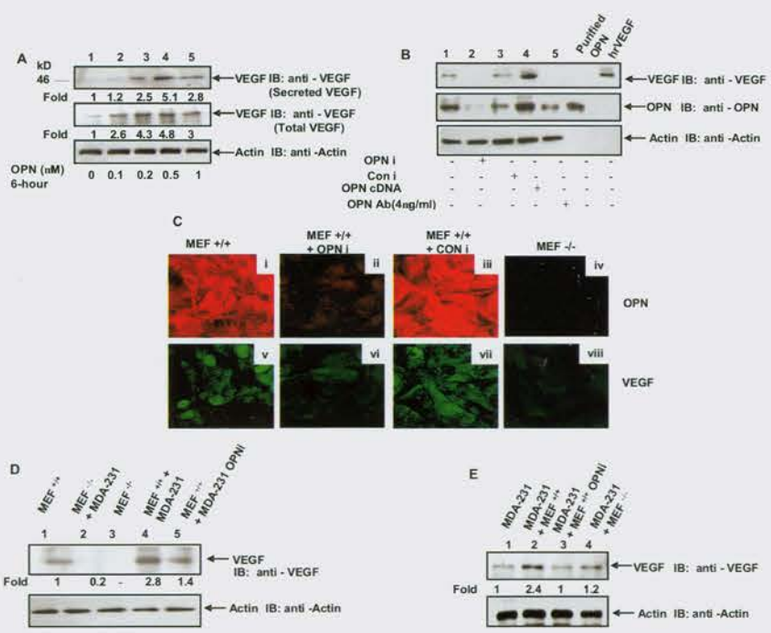


Fig. 1: Tumor and stroma derived OPN augments VEGF expression. **A**, MDA-MB-231 cells were incubated with 0-1 μM OPN for 6 h at 37°C and conditioned media and cell lysates were analyzed by western blot using anti-VEGF antibody. **B**, Cells were transfected with OPN cDNA or OPNi or Coni or treated with OPN blocking antibody. The expressions of VEGF and OPN in cell lysates were analyzed by western blot using their specific antibodies. Human recombinant VEGF and purified OPN were used as control. **C**, Expressions of OPN and VEGF in wt and OPN knockout mice derived MEF cells were detected by immunofluorescence studies. Wt MEF cells were also transfected with OPNi or Coni and levels of OPN and VEGF were detected. **D & E**, WT and knockout MEF cells were seeded on the upper chamber whereas MDA-MB-231 cells were used in the lower part of modified Boyden chamber. Cells were also transfected with OPNi. VEGF expression in both these cell lysates was detected by western blot. Actin was used as loading control..

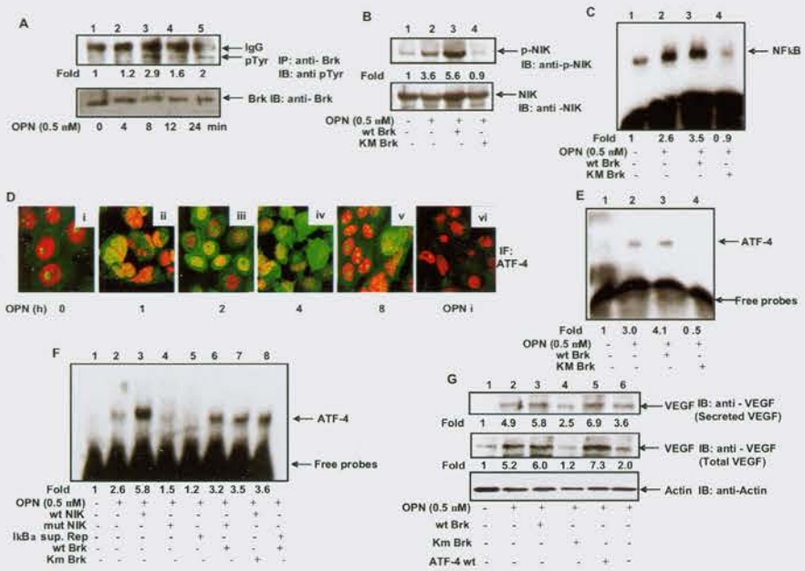


Fig. 2: **A**, OPN regulates the tyrosine phosphorylation of Brk. OPN treated or untreated cell lysates were immunoprecipitated with anti-Brk antibody and analyzed by western blot using anti-phosphotyrosine antibody. The same blots were reprobed with anti-Brk antibody. **B**, OPN-induced Brk-mediated NIK phosphorylation were determined by transfecting the cells with wt or KM Brk and then treated with OPN. Cell lysates were analyzed by western blot using anti-phospho-NIK antibody. **C**, Brk plays crucial role in OPN-induced NFκB-DNA binding. Cells were transfected with WT or KM Brk followed by treated with OPN. NFκB-DNA binding activity was analyzed by EMSA. **D**, OPN regulates ATF-4 cellular localization. Cells treated with OPN or transfected with OPNi were subjected to immunofluoresence study using anti-ATF-4 antibody. ATF-4 was stained with FITC (green) whereas nuclei were stained with PI (red). **E** and **F**, Brk regultes OPN dependent ATF-4-DNA binding and crosstalk between NFκB and ATF-4. Cells were transfected with wt or KM Brk and treated with OPN. In separate experiments, cells were transfected with wt NIK or mut NIK or sup. rep. IκBα or wt Brk and mut NIK or KM Brk and wt NIK or wt Brk and IκBαsup. rep followed by treatment with OPN. ATF-4-DNA binding activity was analyzed by EMSA. **G**, Roles of Brk and ATF-4 in OPN-induced VEGF expression. Cells were individually transfected with wt and KM Brk or wt and dn ATF-4 and then treated with OPN. VEGF expressions (secreted and total) were analyzed by western blot.

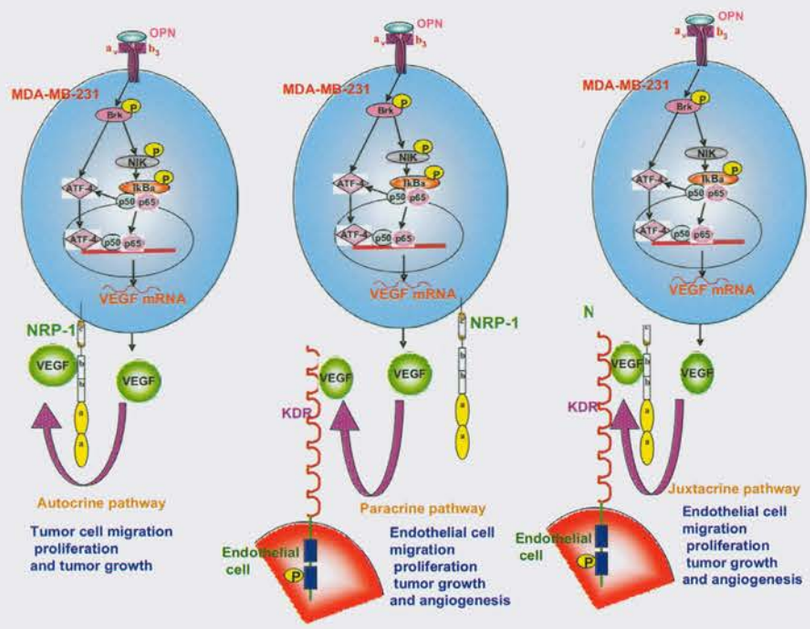
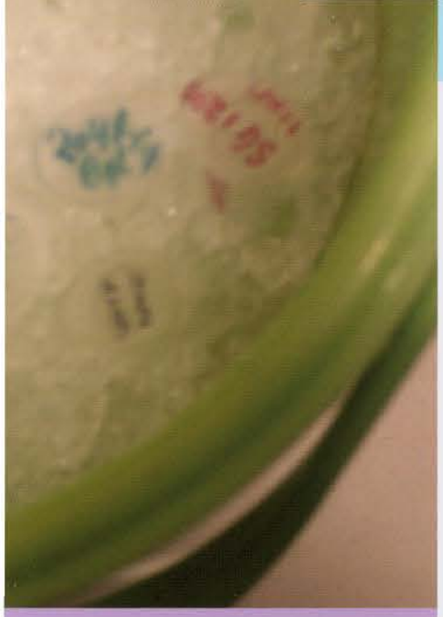


Fig. 3: A schematic representation of OPN-induced Brk/NF-κB/ATF-4-mediated VEGF expression through activation of various upstream kinases leading to tumor growth and angiogenesis through autocrine, paracrine and juxtacrine mechanisms.



Intrinsically Disordered Caveolin-1 Binding Motif of α-Hemolysin: Implication in assembly, membrane penetration and cell dynamics

Musti Krishnasastry
mvks@nccs.res.in

Participants
Satyabrata Pany,*SRF*
Saumya Srivastava,*SRF*
Neesar Ahmed,*SRF*
Aejaj-ur Rahman,*SRF*
Anil Lotke, *Technician*

Background

In recent years bacterial pore forming toxins offered many practical aspects in modern biology such as nano-technology (nano-robotics, nano-DNA sequencing, analyte identification at single molecule level), cell biology, immunobiology (antigen pathway tracking), and cancer biology (cancer cell targeted therapy) etc. In most respects bacterial pore forming toxins differ widely among themselves but some strategic host targeting mechanisms appeared to emerge with recent research. β-barrel pore forming toxins, a subgroup of bacterial pore forming toxins, offer a strategic advantage for understanding protein assembly and folding at cell membranes as well as their pathogenic pathways. Among many β-barrel pore forming toxins, α-Hemolysin (α-HL) of *Staphylococcus aureus* is major virulence factor and one of the few pore forming toxins studied in detail at molecular level. The pore formation on susceptible cells appears to go through a novel pathway, in which α-HL assembles from a hydrophilic water soluble monomer to form a membrane-bound heptameric β-barrel across a target membrane by undergoing several conformational changes. Upon assembly, α-HL's intrinsic hydrophobic part (transmembrane domain) is inserted into membrane for pore formation, which leads to cell permeabilization. However, the thermodynamic cost of transferring transmembrane segment (β-barrel) into the hydrophobic interior of bilayer membranes is very high during the conversion with the help of hydrophilic-hydrophobic segments. These hydrophobic-hydrophilic segments may initiate and trigger a series of conformation changes for a successful insertion of transmembrane β-barrel.

Recently in our laboratory it has been observed that α-HL interacts with Caveolin-1, the marker protein of caveolae, and also resulted in bidirectional clustering of Caveolin-1 and α-HL at cell-cell contact in A431 carcinoma cells. The physical interaction between Caveolin-1 and α-HL has been speculated to occur through the scaffolding domain of Caveolin-1 and Caveolin-1 binding motif of α-HL shown by us earlier. This physical interaction might be favored by π-π stack interaction of aromatic amino acids of Caveolin-1 and α-HL. But the question remained unsolved is how the water soluble Caveolin-1 binding motif of α-HL establishes the contacts with Caveolin-1, which is present in cytoplasmic side of the cell membrane? as what is the mode of penetration with respect to pore formation? A detailed understanding of structure-function relationship of this Caveolin-1 binding motif is required with special reference to its membrane insertion to delineate the modality of α-HL assembly and the susceptibility of various cells such as human monocytes, lymphocytes, erythrocytes, platelets, etc., to α-HL attack.

Aims and objectives

1. Characterization of the 9 amino acids motif in α-HL by cysteine scanning mutagenesis and site directed chemical modification. Studies will include membrane impermeant reagent and fluorescent molecules.
2. Generalization of Caveolin-1 binding motif penetration of soluble proteins and effects cell dynamicity and signaling.

Work Achieved

At initial stage of the study we predicated that the α-HL might possess an intrinsically disordered polypeptide chain which can penetrate the membrane bilayer. The available data suggested that Caveolin-1 binding motif has strong preference to be an intrinsically disordered region (Fig. 1). We, therefore, have investigated the structural importance of the Caveolin-1 binding motif of α-HL by mutating important aromatic amino acids of Caveolin-1 binding motif to cysteine viz., W179C, Y182C and W187C by site directed mutagensis. The logic behind cysteine scanning mutagenesis is that we can carry out site specific modification with an environmentally sensitive fluorophore to study conformational changes, if any.

To analyze of the importance of the Caveolin-1 binding motif, we performed indirect limited proteolysis in presence

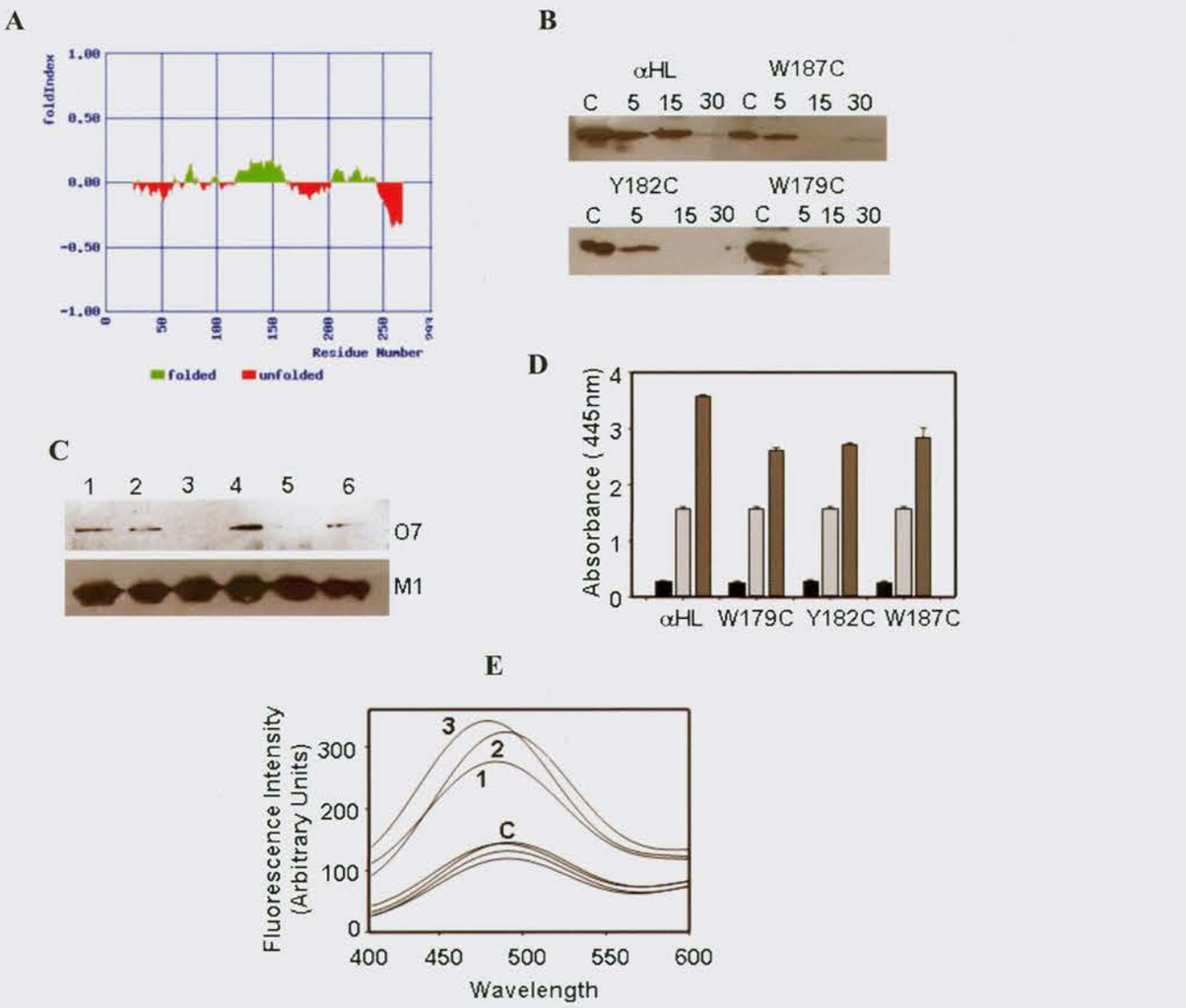


Fig. 1: **(A)** The Caveolin-1 binding motif of α-HL is between amino acids 150 and 200 which is in red color indicating the unfolded nature or flexible region of α-HL. **(B)** Proteinase K susceptibility of membrane bound single cysteine mutants on RBCs membrane. Numerical number represents time period of digestion and C-control **(C)** Membrane Binding and oligomer formation on RBCs membrane by single cytosine: Various lanes represent: Lane1: α-HL (50°C), lane 2: W179C (50°C), lane 3: W179C (90°C), lane 4: Y182C (50°C), lane 5: Y182C (90°C), lane 6: W187C9 (50°C). O7 corresponds the heptameric oligomer formed mutants and M1 represents the monomers of corresponding mutants. **(D)** In vitro binding assay of mutants to Caveolin-1: Black bar (background of α-HL), grey (background of Caveolin-1) and dark grey (single cysteine mutant proteins of α-HL). **(E)** Cysteine modification with Badan fluorophore and membrane penetration assessment: Various spectra represents controls(C), W179CBd (1), Y182CBd (2) and W187CBd (3). Notice that the increase in fluorescence of the spectra shown for 1, 2 and 3 against C (control group of spectra) which represent the same proteins and buffer controls in the absence of the cell membrane.

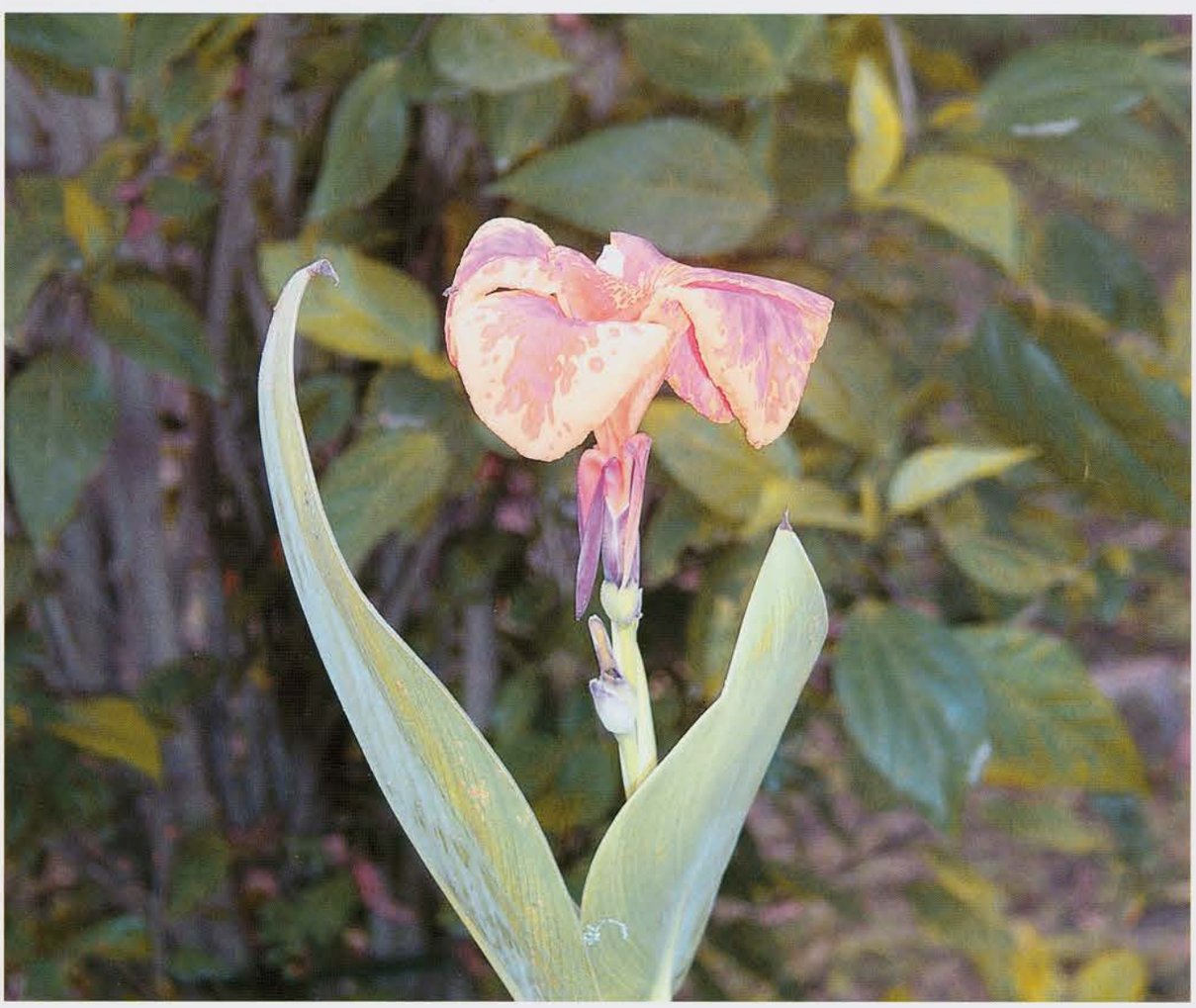
of Proteinase-K and direct conventional spectroscopic methods such as intrinsic fluorescence and ANS binding. Proteinase-K sensitivity assays have revealed the absence of major conformational changes or alterations in single cysteine mutants of α-HL as they are fairly resistant to digestion like its wild type counter part (Fig. 1B). Apart form Proteinase-K digestion study, hydrophobic packing by spectroscopic measurements also suggested that mutants are well folded as they exhibit almost similar intrinsic fluorescence spectra and ANS biding pattern to that of α-HL. This observation was in agreement with our previous structural and functional analyses data of two loop deletion mutants, where absence of Caveolin-1 binding motif caused drastic increase in intrinsic and ANS fluorescence and increased susceptibility to Proteinase-K digestion but without alteration of fluorescence emission (λ_{max}). These comparative results support the view that mutants are reasonably folded and the motif is structurally flexible.

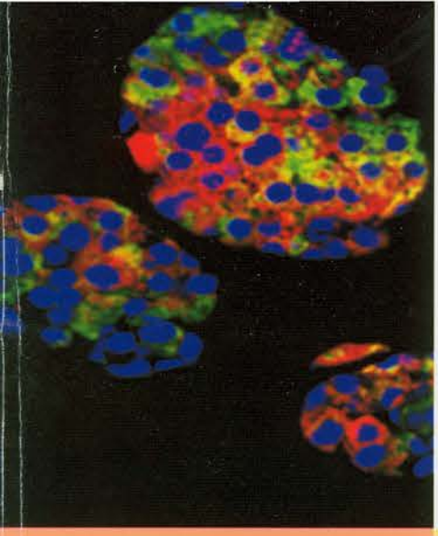
To investigate the functionality of these single cysteine mutants, we performed hemolytic assays on highly susceptible rabbit erythrocytes. Quantitative hemolysis assays showed a complete loss of hemolytic activity in the time course of the experiment but surprisingly, they bind and oligomerize normally on RBCs membrane very much like the wild type α-HL (Fig. 1C). We have also examined the direct binding of these mutants to Caveolin-1 by direct ELISA and it is clear from Fig. 1D that these mutants have much reduced affinity for Caveolin-1. These results also suggested that entire oligomerization loss is not dependent on a single residue rather sum of all the residues. To examine, how the mutants differ in their degree of oligomerization, oligomer stability assay was also performed and it became clear that the stability of the oligomer differs considerably. To know where these mutants are really halted in the assembly processes, Proteinase-K digestion assay was performed after the mutants were allowed to assemble on RBC membranes. The assay revealed that W179C only trapped at pre-pore stage but the other two assembled beyond pre-pore stage but could not make the final pore formation. In order to assess membrane penetration of transmembrane domain with vertical collapse Caveolin-1 binding motif, we modified mutants with environmentally sensitive fluorophore Badan and studied the relative membrane penetration by these residues (Fig. 1E). The degree of penetration was assayed as function of increase in intensity and blue shift of the spectra. The observed differences in the spectral intensity are indicative of the degree of penetration into the red cell membrane. Clearly, the relative distance penetrated into the membrane by theses mutants were in the order: W187C>Y182C>W179C.

In summary, we examined the nature of the Caveolin-1-binding motif and the relevance of α-HL binding to membrane or Caveolin-1 for oligomerization and membrane penetration. Reduction in intrinsically disordered Caveolin-1 binding motif affinity, effected residue dependent membrane binding, oligomerization and penetration. Interestingly, the Caveolin-1 binding motif of α-HL is water soluble but appears to penetrate the cell membrane in dramatic fashion.

Future Work

We will be investigating the dynamics of caveolae before and after docking of α-HL on the cell membrane and the consequences of the docking.





Research Reports

Diabetes

Divergent approaches for control and management of experimental diabetes

Ramesh R. Bhonde
rrbhonde@nccs.res.in

Participants
Sachin S Kadam, *JRF*
Swetha G, *JRF*
Vikash Chandra, *JRF*
Smruti Phadnis, *JRF*
Malati Umrani, *Technician*

Collaborators
Dr. S.B. Padhye, Department of Chemistry, University of Pune
Dr. Murali Sastry, Tata Chemicals, Pune
Dr. Sanjeev Galande, NCCS, Pune
Dr. Anandwardhan Hardikar, NCCS, Pune

Background

Enrichment of islets through neogenesis from unconventional stem cells

Diabetes is a degenerative disease that results from the selective destruction of pancreatic beta-cells. These cells are responsible for insulin production and secretion in response to increases in circulating concentrations of nutrients, such as glucose, fatty acids and amino acids. The total absence or low production of insulin by beta-cells imposes inadequate glycemia forcing diabetic people to daily insulin injection for survival. Pancreatic islet transplantation represents an attractive approach for the treatment of diabetes. However, the limited availability of donor islets has largely hampered this approach and the finding of renewable sources of cells could circumvent this problem. In this respect, embryonic or adult stem cells represent an interesting alternative. Stem cells display robust proliferation along with the required plasticity to differentiate into various other cell types, including insulin-containing cells. In this respect, the use of alternative sources of islets such as the ex-vivo expansion and differentiation of functional endocrine cells for treating diabetes has become the major focus of diabetes research. The present report summarizes the work carried out on isolation, expansion, characterization and differentiation of pancreatic and extra pancreatic stem cells (from different sources) into islets so as to enrich beta cell mass for subsequent transplantation.

Aims and Objectives

To test the potential of adult stem cells derived from various pancreatic and extra pancreatic sources such as pancreatic stellate cells, human umbilical cord and bone marrow, for differentiation into islets and acinar cells.

Work Achieved

Islet neogenesis through Chick β-islet derived stellate cells.

While evaluating the susceptibility of vertebrate islets to a known diabetogen streptozotocin (STZ) we found a striking similarity between chick and human islets as both were found to be insensitive to STZ action. It is assumed that islets isolated from other vertebrates are likely to be less immunogenic to mammals because of the ancestral history. It has been reported that transplantation of pancreatic explants from embryonic chick reverses diabetes in mice. However, adult chick pancreatic islets are non-responsive to glucose, indicating their unsuitability for transplantation. Our earlier studies demonstrate that islets isolated from 5-6 days old chick were responsive to glucose and tolbutamide, emphasizing the importance of age of chick in determining the glucose responsiveness. Therefore we hypothesized that the newly generated islets from stem cells would be ideal candidates for islet transplantation. Since, pancreatic stellate cells from mammals have been reported to differentiate into islets we prospectively isolated stellate cells from the population of cells proliferating from the chick β-islet monolayer. These were successfully passaged, in a defined nutrient medium without spontaneous differentiation into any lineage. These cells stained positive for stellate specific cell markers such as vimentin, desmin, fibronectin, alkaline phosphatase and Cytokeratin-7 (CK7) as well as for fat storage (Fig. 1). When these stellate-like cells were grown in serum free media containing specific nutrients and differentiating agents, they gave rise to two distinct cell populations viz; single cells and islet like cell clusters. Islet like cell clusters stained positive for DTZ and C-peptide as well as showed insulin secretion in response to glucose and arginine suggesting their identity as islets. Our preliminary studies on transplanting these newly generated islets into experimental diabetic mice indicated

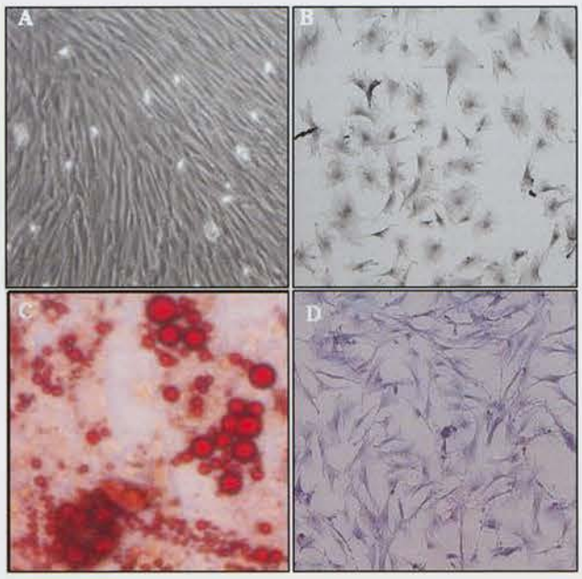


Fig. 1: Characterization of chick pancreatic stellate cells. A: Chick Embryo Fibroblasts (for morphological distinguishing from chick stellate cells). B: Chick pancreatic stellate cells; C: Stellate cells show presence of fat droplets (stained using Oil-Red-O); D: Stellate cells stained positive for Alkaline Phosphatase

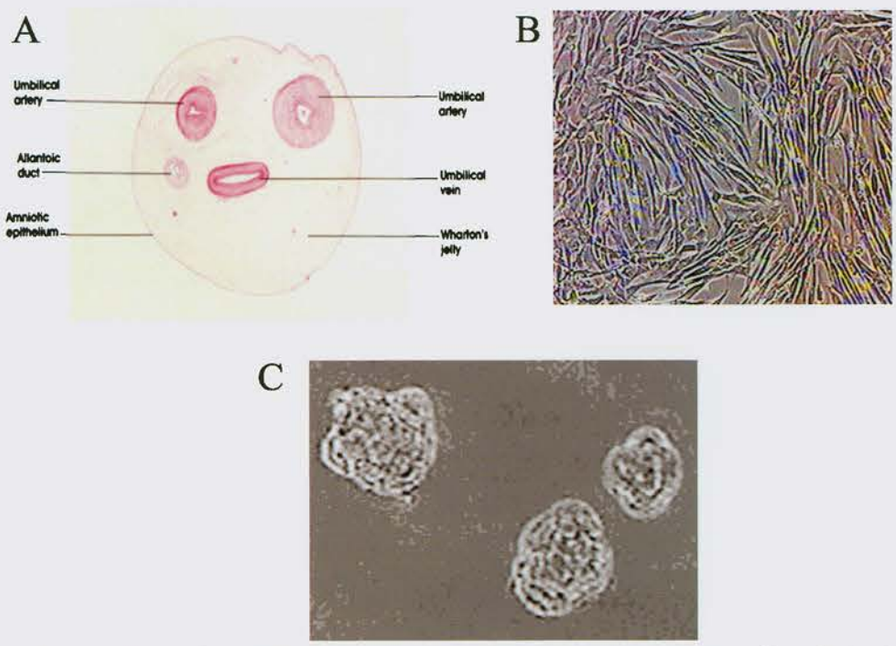


Fig. 2: Islets generated from Umbilical cord Mesenchymal Stem Cells. A: Transverse section of Umbilical cord depicting localization of Wharton's jelly. B: Mesenchymal stem cells (MSCs) isolated from Wharton's Jelly. C: Islets differentiated from these MSCs

their utility in reducing hyperglycemia although in depth studies are needed to claim their normoglycemic potential. These newly generated islets from stellate cells are likely to provide an alternative source of islets for transplantation therapy in diabetes.

Wharton's jelly gels into islets

The Wharton's jelly of the human umbilical cord contains mucoid connective tissue and fibroblast like cells (Fig. 2). It is well known that the umbilical cord is a rich and non-controversial source of stem cells. In present investigation we have isolated and characterized, Messenchymal Stem Cells (MSC) derived from human umbilical cord Wharton's jelly, called human Umbilical Cord Matrix Stem (hUCMS) cells. The isolated hUCMS cells were found to express surface markers of MSC *viz.* CD44, CD90, SMA, Vimentin and were found to be negative for CD33, CD34, CD45 by immunofluorescence. Unlike traditional MSCs derived from adult bone marrow stromal cells a small population of hUCMS cells exhibited expression for endogline marker (CD105) further confirming their MSC phenotype. The hUCMS cells exhibited high proliferating activity as evidenced by a large population of Ki67 positive cells and could be passaged for extended period (above 15 passages) and also cryopreserved. The hUCMS cells showed multilineage potential to differentiate into adipogenic, chondrogenic and osteogenic lineages. We further tested the potential of hUCMS to differentiate into pancreatic lineage. When subjected to a serum free medium containing a cocktail of growth factors and Insulin Transferrin Selenium (ITS), these cells exhibited presence of islet like clusters (Fig. 2C) and acinar like single cells, which were subsequently confirmed as islets by C peptide expression and acinar cells by amylase secretion respectively. This is perhaps the first finding reporting potential of Wharton's jelly derived MSCs to differentiate into islets and acinar cells thus offering yet another novel source for islet neogenesis for cell replacement therapy in diabetes.

Future Work

The functional status of the newly generated islets will be assessed (from stellate as well as human umbilical cord and bone marrow cells) by checking their responsiveness to glucose challenge *in vitro*. These islets will also be transplanted into immuno-competent diabetic mice and parameters like sustained normoglycemia, and period of graft acceptance/rejection will be assessed.

Investigation of mitochondrial dysfunction, oxidative damage and apoptosis in diabetic cardiomyopathy

Sandhya Sitasawad
ssitaswad@nccs.res.in

Participants
Sandeep Kumar, *SRF*
Santosh Kumar, *SRF*
Vasundhara Kain, *JRF*

Background

Diabetic cardiomyopathy affecting the cardiac system, is among the most frequent complications of diabetes. The etiology and the underlying mechanisms are as yet unclear. Recent studies showed that the incidence of apoptosis increases in the heart of patients with diabetes and streptozotocin-induced diabetic animals. Hyperglycemia is the initiating event in the development of cardiomyopathy. Hyperglycemia-induced metabolic disorders are postulated to initiate the sequence of events leading to the development of cardiomyopathy. Many biochemical abnormalities have been identified in the myocardium in diabetes, including significantly increased oxidative stress, as demonstrated by elevation of thiobarbituric acid reactive substances (TBARS) and lipid fluorescent products in left ventricle, elevation in the activity of protein kinase C (PKC), non-enzymatic glycation, polyol pathway, and increased nitric oxide (NO), but which metabolic abnormalities may be critical in the etiology of diabetic cardiomyopathy is unknown.

Diabetes-induced increase in oxidative stress is postulated to play a significant role in the development of complications. The myocardium experiences increased oxidative stress and NO levels in diabetes, and antioxidants inhibit these cardiac abnormalities and the development of cardiomyopathy. Possible sources of oxidative stress in diabetes include increased generation of reactive oxygen species by auto-oxidation of glucose, decreased tissue concentrations of low–molecular weight antioxidants, and impaired activities of antioxidant enzymes.

Recent studies have shown that improved glycemic control is associated with decreased development and progression of cardiomyopathy in diabetes. Clinical studies established a significant correlation between the control of patient's blood glucose level (Glycemic control) and the severity of the damage to the cardiac system. It has been observed that, reinstitution of normal glycemic control after a period of poor glycemic control does not produce immediate benefits on the progression of retinopathy, and the duration of poor glycemic control before initiation of good glycemic control plays a major role in the outcome of good glycemic control. However, the effect of this type of temporal glycemic control in case of diabetic cardiomyopathy has not been studied. Such studies are needed to clarify important questions that remain regarding the time of glycemic control and the relative contributions of the submicroscopic processes present during poor glycemic control on hyperglycemia-induced cardiac cell death.

The purpose of our study is therefore to investigate the role of temporal glycemic control using insulin, insulin-mimetic molecules and anti-diabetic pharmaceutical preparations on hyperglycemia-induced oxidative and nitrosative stress in cardiac muscle cells. For this study, we are using Male Wistar rats and the embryonic rat cardiac muscle cell line, H9c2.

Aims and Objectives

To study the role of oxidative and nitrosative stress in high glucose induced cell death and protective effect of insulin, insulin-mimetic molecules and anti-diabetic pharmaceutical preparations. This study is designed to elucidate the effect of temporal glycemic control on hyperglycemia-induced increased oxidative and nitrosative stress and its correlation with the changes in cardiac function.

1. To investigate the role of temporal glycemic control using insulin, insulin-mimetic molecules and anti-diabetic pharmaceutical preparations on hyperglycemia-induced oxidative and nitrosative in STZ diabetic rats and H9c2 rat cardiac myoblast cells.

2. To investigate the role of mitochondrial ROS and RNS in the induction of hyperglycemia-induced myocardial apoptosis and to clarify which ROS and RNS are required for the cell death response (signaling pathways) including their sources.

3. To characterize the mitochondrial 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.

Work achieved

During the last year we had observed that treatment with high glucose causes apoptotic cell death by inducing mitochondrial generation of ROS and RNS in H9c2 cells and that insulin protects cardiomyocytes apoptosis by inhibiting ROS and RNS generation *in vitro*.

Further to study the effect of temporal glycemic control by insulin on oxidative and nitrative stress in diabetic rat, Wistar rats (male, 200 g) were randomly assigned to normal or diabetic groups. Diabetes was induced with intra-peritoneal injection of streptozotocin (55 mg/kg). Group I was control group, Group II was vehicle control group and Group III was STZ treated (diabetic) group. The diabetic rats were further divided at random into 4 groups according to intended degree and duration of glycemic control, Gr. IV consisted of rats that were allowed to remain in poor glycemic control for 14 months; Gr. V consisted of rats that were allowed to remain in Good Glycemic control for entire 14 months; Gr. VI consisted of rats that were allowed to remain in poor glycemic control for 6 months followed by good glycemic control for 8 months and Gr. VII consisted of rats that were allowed to remain in good glycemic control for 6 months followed by poor glycemic control for 8 months. All diabetic rats received insulin injections. PC (Poor glycemic control) received a single injection of insulin 1-2 units, 3-4 times a week to prevent ketosis and weight loss and the Good glycemic control rats received insulin twice a day 2-4 units total to maintain a steady gain in body weight and to confine the blood glucose levels to <150 mg/dL. Body weight and blood glucose were noted on a monthly basis.

At the end of the experiments, cardiac functions such as rate meter, systolic, diastolic and mean blood pressure were evaluated using a microtip pressure transducer (SPR-320, Millar Instruments) connected to Powerlab chart recorder. Since common intracellular stress signaling pathways contribute to the reversible myocardial dysfunction we tested the stress activated signaling molecules, p38 MAPK and the insulin survival PI3/AKT pathway.

In vivo, in the diabetic rats (Gr. III), there was increase in whole blood glucose and decrease in body weight. There was a significant increase in rate meter, systolic, diastolic and mean blood pressure. Good glycemic control with insulin for 14 months was able to reduce these effects. However, the time of glycemic control did not show much of a difference on the outcome. There was no change in the activity of MAPK but p38 upregulation was increased in STZ treated group (Gr. III) and in Gr. VI and VII. This upregulation was reduced in Gr. V (GC group), although a marginal reduction was also observed in PC group. Upregulation of PI3K was observed in all the three good glycemic control groups. Consistently, we observed an activation of the PI3K/Akt pathway in insulin treated H9c2 cells. This activation was inhibitable by LY 294002.

In conclusion, Insulin protects cardiac cells against high glucose-induced damage by interruption of the mitochondrial apoptotic pathway, reduction of ROS, RNS, reduction of p38 and activation of PI3K/AKT pathway. This latter event might be responsible for the decrease in oxidative stress and myocyte death by insulin.

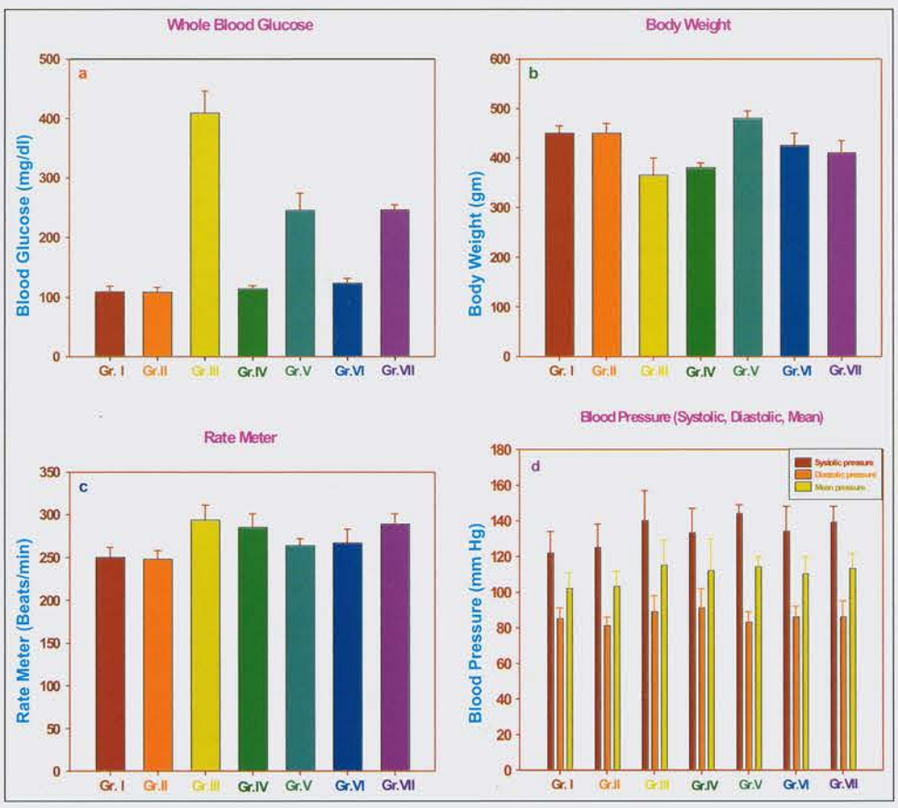


Fig. 1: Protective effect of insulin (glycemic control) on cardiac function *in vivo*. Effect of insulin was studied in male wistar rats for 14 months. *Treatment groups*: Gr. I Control, Gr. II Vehicle Control, Gr. III STZ treated (55mg/kg body weight), Gr. IV STZ treated Followed By Good Glycemic Control (Insulin 2U/day), Gr. V STZ treated Followed By Poor Glycemic Control (Insulin 0.5 to 1 U, 2-3 times a week), Gr. VI STZ treated Followed By Poor Glycemic Control with Insulin 0.5 to 1 U, 2-3 times a week for 8 months) + Good Glycemic Control with Insulin 2U/day for 6 months, Gr. VII STZ treated Followed By Good Glycemic Control (8 months with Insulin 2U/day) + Poor Glycemic Control (6 months with Insulin 0.5 to 1 U, 2-3 times a week). a. Whole Blood Glucose levels $P<0.025$, b. Body weight $p<0.05$, c. Rate Meter $p<0.05$, d. Blood Pressure<0.05

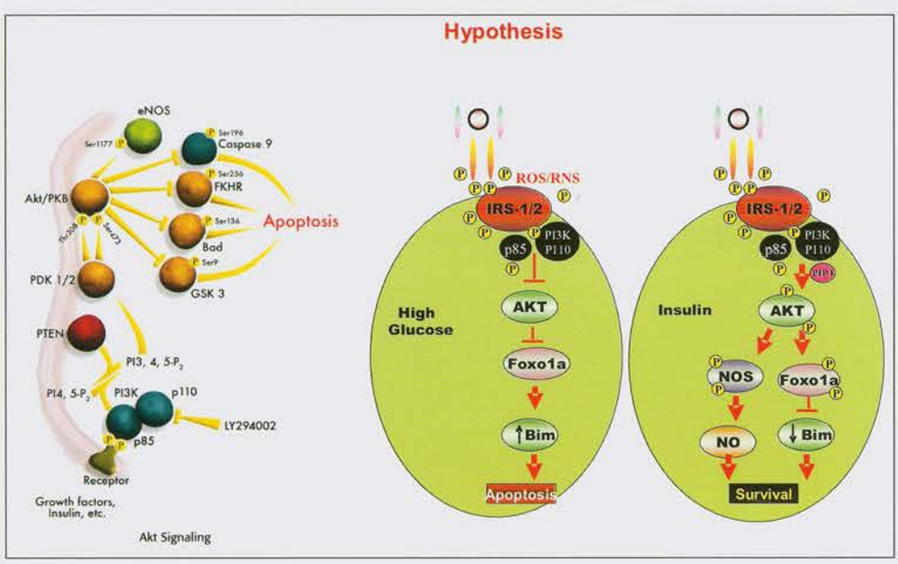


Fig. 2: Schematic representation of the Insulin Signaling Pathways in high glucose treated cells and in cells treated with insulin.

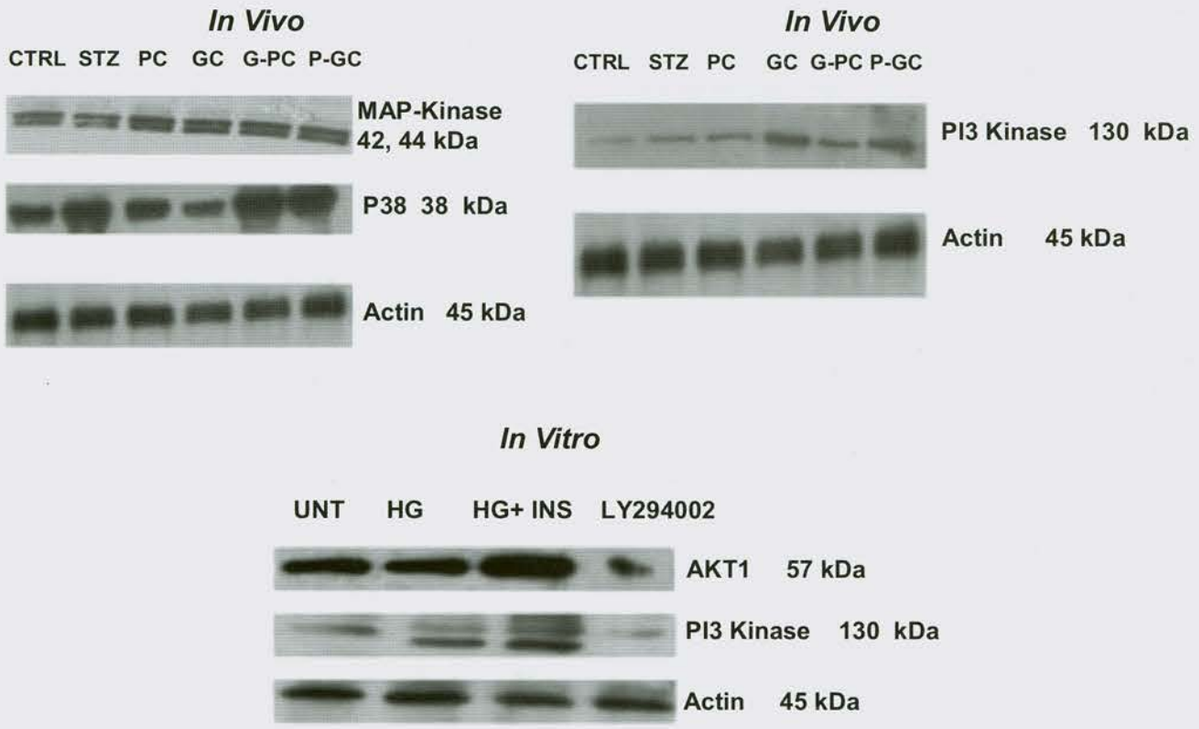


Fig. 3: Influence of insulin on signaling pathways. For in vitro studies, H9c2 cells were pre-treated with insulin (1 μM) for 24h and then with NG (5.5 mM D-Glucose) and HG (33 mM D-Glucose) for 3 h. For *in vivo* studies male wistar rat cardiac tissue from various groups of glycemic control were used. 30 μg of protein from each sample was subjected to SDS-PAGE and Western blot analysis to determine p38 MAPK, PI3/AKT protein levels. β-Actin was used as loading control.

Future work

1. Characterization of high glucose induced mitochondrial ROS, RNS and 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.

Differentiation of human fetal pancreatic progenitor cells for potential use in cell replacement therapy for diabetes

Anandwardhan A. Hardikar
anand@nccs.res.in

Participants
Mugdha V. Joglekar, *SRF*
Amaresh Ranjan, *SRF*
Vishal S. Parekh, *JRF*

Collaborators
Dr. Ramesh Bhonde, NCCS, Pune
Dr. Yogesh Shouche, NCCS, Pune
Dr. Sanjeev Galande, NCCS, Pune

Background

According to the International Diabetes Federation, there are over 246 million people with diabetes worldwide. This figure is projected to rise to 380 million by 2025 and India is believed to be the single country with largest number of diabetics (>60 million). If these predictions are fulfilled, then it would create a major burden on worlds' healthcare budget on diabetes, which is predicted to bounce from 5% to 40%.

Presently, the only successful therapy for treatment of type 1 diabetes is transplantation of human cadaveric insulin-producing cells. Human cadaveric islet transplantation studies carried out in Edmonton, Canada as well as the NIH, Bethesda, MD provide the proof of principle that patients with long-standing diabetes can be successfully transplanted with insulin-producing cells and maintained off any exogenous insulin for at least a year. However, islets isolated and used in such transplantation procedure are from cadaveric donors and the efficiency and efficacy of islet isolation process makes it necessary to transplant islets from at least 2 to 3 donor pancreas into one diabetic individual. Furthermore, the relative scarcity of human cadaveric pancreas donation suggests that neither whole pancreas nor human islets can currently provide a cure for the millions of type 1 diabetics worldwide.

Researchers are therefore trying to look for alternate sources of human pancreatic progenitor cells, which can be expanded (grown) and efficiently differentiated into insulin-producing cells. However, there is a considerable gap in taking such stem/progenitor cells from the laboratory to the transplantation clinics. We therefore need to understand the basic biology of pancreatic progenitor cell differentiation and then identify processes that will lead to efficient differentiation into hormone-producing cells.

Aims and Objectives

1. To understand processes that regulate expansion of human fetal islet-derived progenitor cells (FIPCs)
2. To identify the "gene signatures" of progenitor (FIPCs) and differentiating cell types
3. To assess the differentiation potential of FIPCs

Work Achieved

One of the major goals of diabetes research is to generate large numbers of insulin-producing cells that can be used in cell-replacement therapy for diabetes. Due to the scarcity of human cadaveric pancreas available for transplantation, scientists are investigating the possibility to use tissue specific progenitor cells or embryonic stem cells for cell replacement therapy to treat diabetes. Though human embryonic stem cells (hESCs) are known to demonstrate extensive proliferative potential *in vitro*, directed differentiation of hESCs to insulin-producing cells that are similar to and can replace the function of human cadaveric islet cells post-transplantation has not been reported as yet. Furthermore, since these ES cells are uncommitted/ pluripotent cells, we need to understand several steps that would induce these cells to commit to definitive endoderm and finally to an endocrine pancreatic lineage (Fig. 1A). Bone marrow derived "stem" cells or transdifferentiation of tissue-specific progenitor cells has also been looked at as one of the ways to obtain insulin-producing cells. However, the efficiency of the differentiation process and the levels/ concentration of pro-insulin/c-peptide demonstrated in such cell types are too little to meet therapeutic needs. We therefore believe that human fetal pancreatic islet-derived progenitor cells could serve as better progenitors cell types for the following reasons: i) These cells are in a state of active proliferation during embryonic development (13-26 weeks) and therefore may be easier to expand (proliferate) in vitro and ii) These are lineage committed cells and therefore may be easier to re-differentiate (after expansion) into endocrine pancreatic cell types, as compared to uncommitted pluripotent embryonic stem cells.

Differentiated cell types generally do not proliferate well. However, we found that human fetal pancreatic islet cells

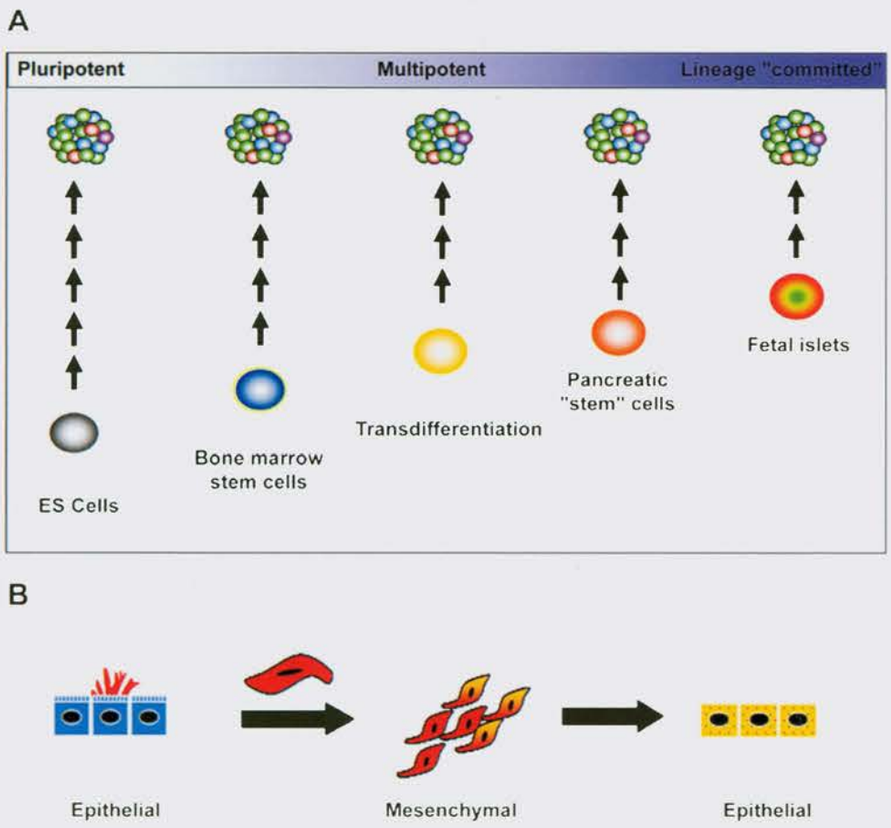
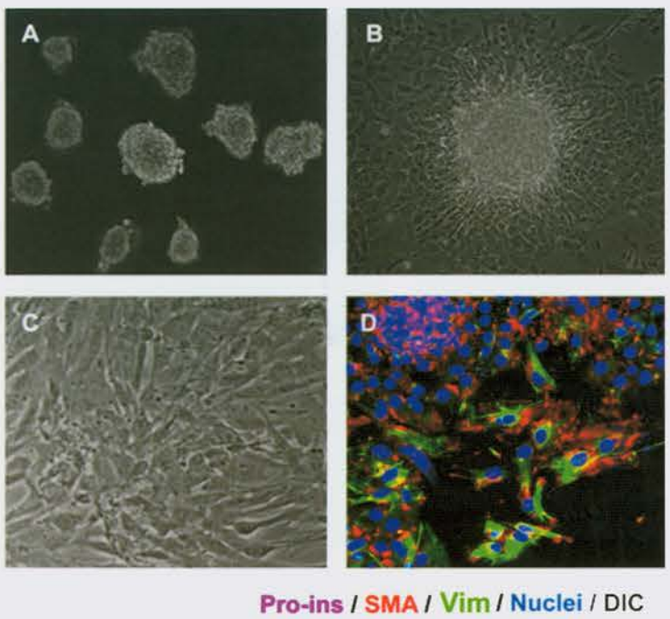


Fig. 1: Generating better islet-progenitor cells: A) Differentiation of ES cells to hormone-producing islet-like cell aggregates involves several steps in induction of these pluripotent cells to definitive endoderm, the pancreatic endoderm and finally to an endocrine pancreatic cell type. We believe that differentiation of fetal islet-derived progenitor cells to hormone-producing cell clusters would involve fewer steps and may be more efficient as compared to ES or other stem cells, as these islet-derived progenitor cells are thought to be committed to differentiate into endocrine pancreatic cells. B) Epithelial-to-mesenchymal transition (EMT) is a phenomenon that is commonly seen during embryonic development as well as during cancer progression. Here, epithelial cells in a tissue transition to a mesenchymal cell type, by virtue of which they can now proliferate better, migrate to another part of the embryo/body and then re-differentiate into a different (or similar) epithelial cell type by reverse-EMT (MET). We believe that lineage committed fetal pancreatic endocrine cells can be induced to generate precursor cell types by EMT.



E	Fetal Islet	FIPCs		
	Day 0	*P4*	*P8*	*p12*
Ins	13	31	36	>39
Gcg	16	28	35	36
Sst	15	31	32	38
Gck	23	37	38	37
Cldn4	19	26	26	27
PECAM1	20	24	25	25
Pdx1	21	35	>39	>39
Ngn3	23	37	37	>39
Pax6	22	29	31	32
Nkx2.2	24	36	>39	>39
Nkx 6.1	21	31	32	32
NeuroD1	22	33	35	36
Isl1	22	26	25	26
Brn4	31	35	38	>39
Vim	14	13	12	12
Snail2	23	20	20	20
P4HA1	24	22	21	20
UPa	25	20	19	19
UPaR	22	19	19	18
Ki67	25	24	23	21

Fig. 2: Generation and characterization of fetal islet derived progenitor cells (FIPCs): Freshly isolated fetal human pancreatic islets (A) attach to tissue culture plates. By day 2 (B), cells within such an islet begin to migrate out as mesenchymal cells. These cells are so proliferative that they form confluent monolayers (C) in around 15 days. These FIPCs can then be maintained in culture for several passages and expanded millions of fold. Fetal islets at 2 days in vitro were assessed for EMT by combined immunocytochemistry and FISH (D). At this time, cells migrating out of a pro-insulin transcript expressing islet (pink) shows co-expression for mesenchymal proteins smooth muscle actin (SMA) and vimentin (Vim). E: Serial analysis of gene-transcripts of different epithelial and mesenchymal markers by taqman based real-time pcr demonstrates that the abundance of epithelial gene transcripts decreases while that of mesenchymal transcripts increases as epithelial cells in islets transition to a mesenchymal cell type. All values are cycle threshold values normalized to 18S ribosomal RNA carried in duplex pcr reactions. Each passage is ~3 days.

can be induced to expand (proliferate) by undergoing epithelial-to-mesenchymal transition (EMT: Fig. 1B). Here, epithelial cells undergo transition to a fibroblast-like (mesenchymal) cell type, which allows the cells to proliferate and migrate in culture dishes. During this process, cells within the islet cell clusters migrate out (Fig. 2) to form monolayers of highly proliferative mesenchymal cells that no longer express pancreatic hormones. The proliferative ability of these cells allows rapid expansion of the cell population when these cells are maintained in a serum-containing/growth-promoting medium. We found that progenitor cells from human fetal pancreatic islets can be expanded for several million-fold after these epithelial and/or hormone-producing cells in islets transition to a mesenchymal cell type.

Such a population of expanding progenitor cells shows diminished/no expression of specialized epithelial markers (insulin, glucagon, somatostatin, pancreatic polypeptide, claudin-3 and -4, connexins and various tight junction proteins) and acquire more of the mesenchymal cell markers (vimentin, nestin, SMA, endoglin, matrix metalloproteinases-2, SNAIL-1, -2 and prolyl-4-hydroxylase). Though we do not have any information on the ability of single cells within the islets to transition into mesenchymal cell types, our studies demonstrate that cells migrating out of fetal pancreatic islets show co-expression of pro-insulin transcript by FISH and the mesenchymal marker Vimentin during EMT (Fig. 2). The FIPCs obtained after 1000-fold expansion do not show immunopositivity to or detectable levels of islet (pro-) hormones. However, they yet express islet-specific transcription factors (nkx6.1, isl1 and pax6). We therefore believe that expansion of cells that do not express pancreatic islet (pro-) hormones but express the islet-specific transcription factors that mark islet progenitor cells during pancreas development, makes FIPCs an interesting cell type to study directed differentiation into hormone-producing cells.

Once we optimized the potential of these cells to proliferate for several million-fold, we focused to find out if these cells would differentiate into hormone-expressing cells. We found that exposure of FIPCs to serum deprived conditions induced aggregation of FIPCs to islet-like cell aggregates (ICAs) that look very similar to human islets. ICAs obtained from 500-fold expanded FIPCs showed expression of islet hormones: insulin/C-peptide, glucagon and somatostatin, indicating that these cells were able to produce and process insulin. Further studies in our laboratory are focused on understanding the mechanisms that may be involved in pancreas development and differentiation.

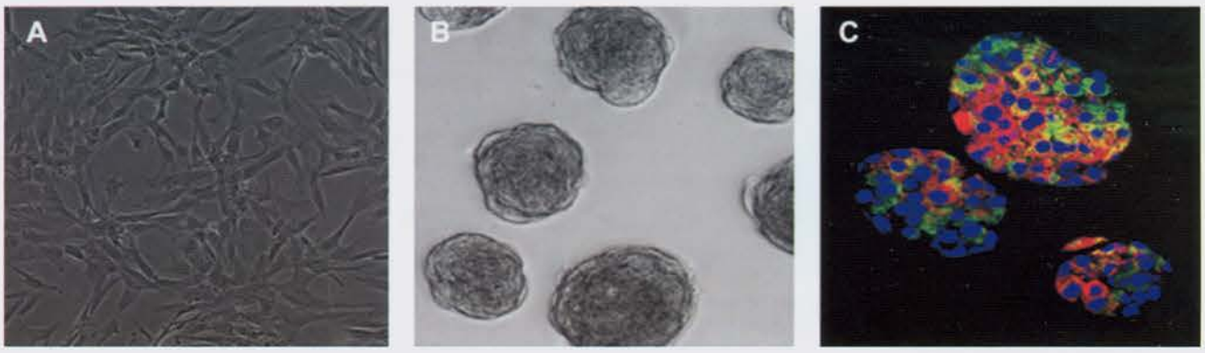
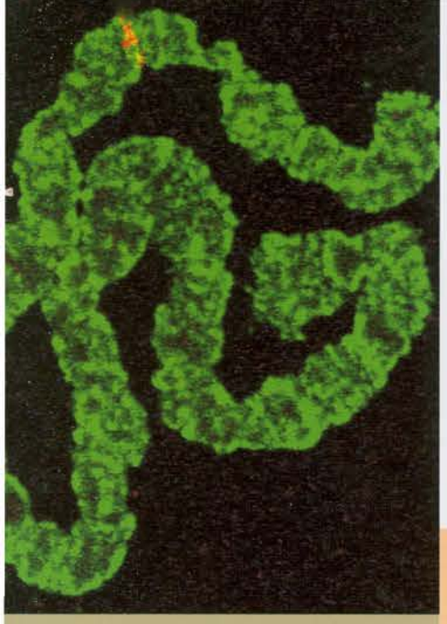


Fig. 3: Differentiation of FIPCs: FIPCs expanded for over 500-fold grow as proliferative populations of mesenchymal cells in growth-promoting media (A). Upon exposure to a defined media, these cells come together to form clusters that look very similar to human islets (B). Such FIPC-derived islet-like cell clusters (ICCs) show expression of islet hormones such as insulin (red), glucagon (green) and somatostatin (pink) at 14 days post induction (C).

Future Work

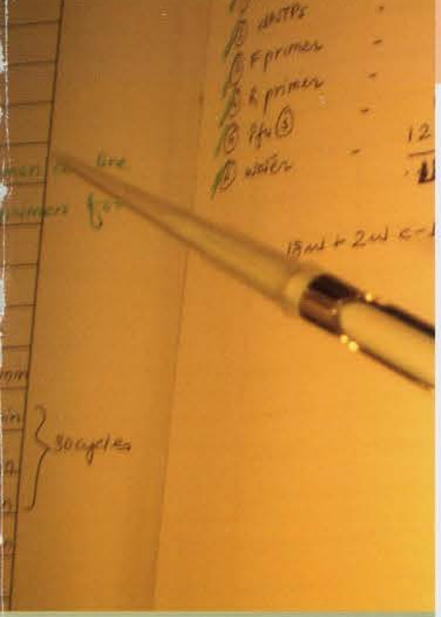
Our present studies demonstrate that human islet-derived mesenchymal cells can proliferate to produce billions of islet progenitor cells. Though early passage cells retain hormone-expression after induction of differentiation, studies to understand directed differentiation of FIPCs generated after million-fold expansion are in progress. We are presently working on:

1. Understanding the mechanisms that regulate mesenchymal transition of FIPCs
2. Understanding the ability of FIPCs to differentiate following transplantation to immune-incompetent mice
3. Assessing the role of microRNAs during development and differentiation



Research Reports

Biodiversity



Insect and Microbial Genomics

Yogesh S. Shouche
yogesh@nccs.res.in

Participants

Parag Vaishampayan, *RA*
Chandresh Thakkar, *RA*
Rajnikant Dixit, *RA*
Bipin Chandra Salunke, *RA*
Gurdeep Rastogi, *RA*
Dayananda KM, *SRF*
Prashant Pandey, *SRF*
Mahesh Dharne, *SRF*
Kaushal Sharma, *JRF*
Jay Siddharth, *SRF*
Aijaz Ahmad Wani, *SRF*
Kaustubh Gokhale, *SRF*
Rasesh Parikh, *SRF*
Pankaj Verma, *SRF*
Ashraf Rangrez, *SRF*
Arvind Gupta, *JRF*
Sajjid Ahengar, *JRF*
Dhiraj Dhotre, *JRF*
Deepak Patil, *SRF*
Shakti Singh, *JRF*
Saif Hasan, *JRF*
Pankaj Ghate, *JRF*
Himanshu Kumar, *JRF*
Rakesh Chandode, *JRF*
Shashikant Sharma, *JRF*
Viduthalai Rasheed Regi, *JRF*
Sandeep Walujkar, *JRF*
Shrikant Pawar, *JRF*
Sandeep Amberkar, *JRF*
Vineet Mahajan, *PA*
Girish Kulkarni, *PA*
Rahul Chaudhary, *PA*
Sarang Satoor, *Technician*

Collaborators

Dr. H.V. Batra, DRDO
Dr. S. Barnabas, NCL
Dr. Shri Prakash, DRDO
Dr. V.K. Batish, NDRI
Dr. D.T. Mourya, MCC
Dr. Sanjay Nene, NCL
Dr. D.R. Ranade, ARI
Dr. Murali Shastry, NCL
Dr. Rajendra Joshi, C-DAC
Dr. B.L.V. Prasad, NCL
Dr. Raghukumar, NIO
Dr. Rakesh Mishra, CCMB
Dr. Ramaiah, NIO
Prof Datta Madamwar, SPU
Dr. A.C. Anil, NIO
Prof S. Biju, DU
Dr. B. Ingole, NIO
Dr. M. S. Patole,NCCS, Pune
Dr. H.V. Ghate, Modern College

Background

Microbial Genomics:

Microbes are omnipresent and are central to health, sustainable cities, agriculture, and most of the planet's geochemical cycles. They are also reservoirs for the discovery of new drugs and metabolic processes. However our understanding of microbiology had been very poor, primarily due to our inability to grow majority of microbes in the laboratory.

The pioneering work of Carl Woese and colleagues on comparative analysis of small-subunit ribosomal RNAs (16S and 18S rRNAs) provided an objective framework for determining evolutionary relationships between organisms and thereby 'quantifying' diversity as sequence divergence on a phylogenetic tree. Further the work by Norman Pace and colleagues outlined a molecular approach that bypassed the need to cultivate a microorganism in order to determine the sequence of its 16S rRNA gene (16S rDNA). Many researchers have applied the rRNA approach to a wide variety of environmental samples over the past decade and, this has added to our understanding of these ecosystems.

In our laboratory we have used these techniques to understand microbial community structure of unique ecosystems like insect mid-gut, human colon and some extreme ecosystems.

Insect Genomics:

Mosquitoes are vectors for diseases like malaria, filaria and many arboviruses. After human genome, worldwide researchers have turned their attention to sequence mosquito genome. The sequence for *Anopheles gambiae,* which is vector for malaria in Africa, is already completed and the one for *Aedes aegypti* is currently under progress. The project aims at complementing this effort by performing extensive EST (Expressed Sequence Tag) analysis of *Anopheles stephensi,* which is vector of malaria in India. Directionally cloned cDNA libraries from infected and uninfected mid-gut of this mosquito are already made and sequencing is also complete from 5' end. Obtained sequences are processed by means of a bioinformatics pipeline of code and are being annotated. We are planning to submit them to international databases as early as possible. These will also be made available on web site designed for this purpose.

Aims and Objectives

1. To understand the "uncultured" microbial diversity with long term aim of utilizing it for the biotechnological purpose.

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 mosquito *Anopheles stephensi*.

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 whole ecosystem processes. We have studied a variety of insects from Western Ghats for their microbial community structure and also to explore the possibility of extracting bioactive compounds from these inhabitants. Insects like *Aspidimorpha miliaris, Aspidimorpha lobata, Coelosterna scabrataor, Poecilocerus pictus*, Ant, *Mylabris sp., Unidentified terrestrial Isopoda* found in leaf filter, *Zygogramma bicolorata, Borer larva Batocera sp.*, Detritus feeding cockroach, Red bug, House Fly, *Yellow Beetle, Noeostylopyga, Protetia, Pycnoscellus surinamensis, Buprestrid beetle, Small dung beetle, Grasshoppers, Flesh fly, Blow fly, Tessartoma sp., Stibara sp.*, Field Cricket, *Elaterid Beetle, Sphingid Moth Larva, Sarcophaga sp., Mole Cricket, Chironomous sp. larva, Chrysocoris purpureus, Batocera rubus, Periplaneta Americana, Blatella sp., Supella longipalpa.* We have also screened these insect species for the presence of endosymbiont *Wolbachia* and further characterized them using Multi-Locus Sequence Typing approach. One of the most interesting outcomes of these studies is the first report of presence of *Wobachia* in cockroaches and their grouping with those from termites. This supports the hypothesis that termites are probably eusocial cockroaches and not a separate clad.

Microorganisms associated with 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 stephensi* and *Aedes aegypti*. We have studied the region and developmental stage associated variation in the flora. In addition, we have also attempted to study the variation, if any, in the microbial community structure of the mosquitoes in the disease endemic and non endemic areas and also in the mosquito strains with varying degrees of disease transmission potential.

Human Gut:

The normal healthy adult human has as many as 10^{23} microbes in the gut, which is about 10 times the total number of cells present in the human body. There is increasing belief that this microbiota should be viewed equivalent to an 'organ', exquisitely tuned to carry out metabolic functions that humans are unable to perform themselves. We have undertaken efforts to understand the questions related to development of this flora and its relation to human health and disease. Currently we have addressed two issues.

The first one is understanding the establishment of this flora after the birth. For this purpose we collected fecal samples of babies borne naturally and through caesarian section immediately, after the birth and different time points afterwards up to the period of one year. The microbial flora is presumed to get stabilized after one year. Total of 7 16S rDNA libraries have been created i.e. of day 0, Day 7, day 30 and day 90 of full vaginally born breast fed infants and day 7 and Day 30 of full term Cesarean section born Infants and day 7 library of full term Cesarean section born infant with initial formula supplementation. Our results clearly indicate that the fecal microflora of the infants born with Cesarean section is completely different from that of normal full term vaginally delivered breast fed babies. The members of enterobacteriaceae family *Escherichia coli* and *Citrobacter* sp. were the most abundant species in the healthy full term Cesarean section born infants together they constituted around 65% of total clones while *Acinetobacter* and *Bifidobacterium* were prevalent in healthy full vaginal born breast fed infant.

It is well known fact that two groups of beneficial bacteria are dominant in the human gut, the Bacteroidetes and the Firmicutes. Very recently, it has been reported that the relative proportion of Bacteroidetes is decreased in obese people by comparison with lean people, and that this proportion increases with weight loss on two types of low-calorie diet. It has been also reported that transplanting the gut microbiota from normal mice into germ-free recipients increases their body fat without any increase in food consumption, raising the possibility that the composition of the microbial community in the gut affects the amount of energy extracted from the diet. These findings indicate that obesity has a microbial component, which might have potential therapeutic implications.

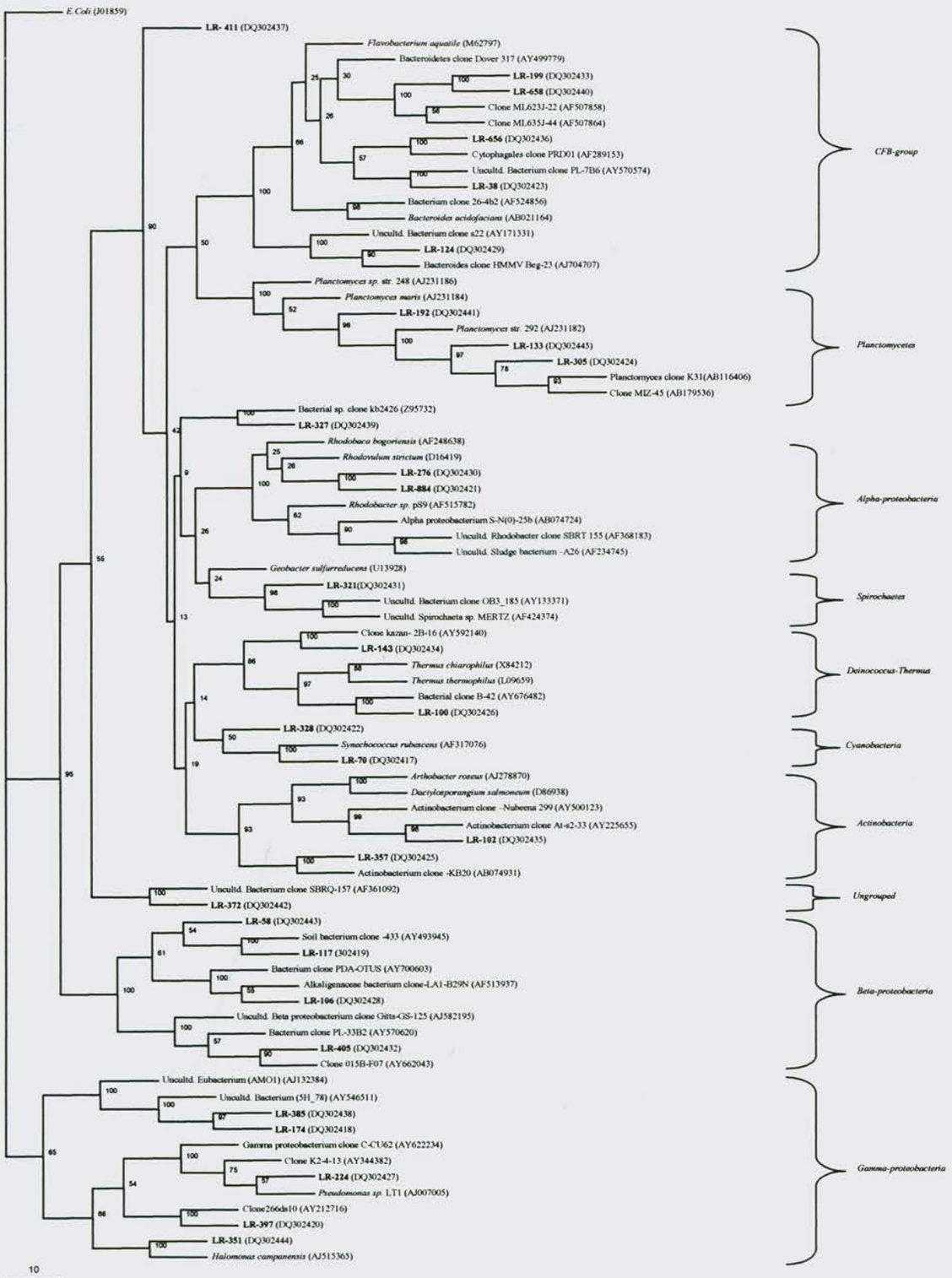


Fig. 1: Phylogenetic tree showing the relationship among bacterial 16S rRNA gene sequences from Lonar Lake with reference sequences obtained through BLAST analysis. The sequence alignment was performed using the CLUSTAL W program and trees were constructed using Neighbor joining with Kimura 2 parameter distances in PHYLIP software version 3.61. (9). Numbers in bold are sequences obtained from the present study. Bootstrap values (1000 replicates) are shown at the nodes. *E. coli* was used as an outgroup taxa.

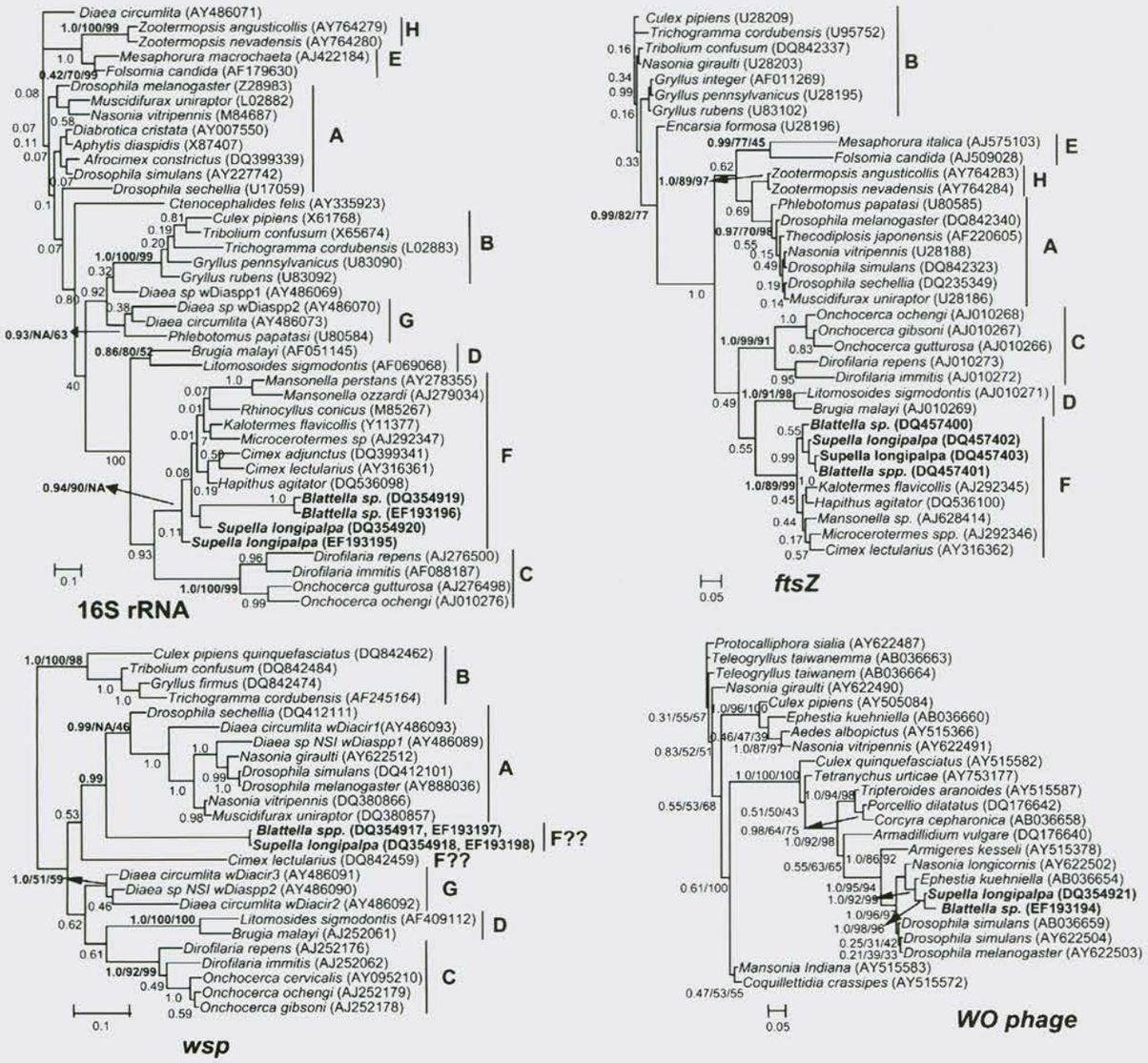


Fig. 2: Unrooted phylogenetic relationships between *Wolbacia* from cockroaches (bold) and those infecting other organisms, based on 16S rRNA, *ftsZ* and *wsp* genes. WO phage refers to *orf7* gene ofWO phage from there specific host. Names are those of the host species. The topology was inferred using the program Mr. Bayes, with following nuleotide substitution models. (GTR+1+G) for *Wolbachia* 16S rRNA, *ftsZ*, *wsp* gene fragments; (HKY+G) for WO phage *orf7* gene fragment. Levels of confidence for each node are shown in the form of posterior probabilities (PP: Bayesian analysis). Trees inferred from maximum likelihood and maximum parsimony using PaUPb10 program were similar though less resolved (data not shown). Bootstrap values obtained from maximum likelihood and maximum parsimony are shown after PP, respectively (only for supergroup claude support). Accession numbers are shown after each species name in parentheses. Supergroups are shown to be right side of the host species names (except for WO phage). Scale bar represents substitutions per site.

With this background in mind, we have initiated the study in collaboration with Obesity Clinic at Ruby Hall Clinic, Pune to explore the gut microbial diversity of Lean and Obese individuals of India.

In the study, we chose following three categories of human beings:

1. Lean individuals (BMI 22-25 kg/m^2),
2. Obese individuals (BMI ≥ 30 kg/m^2), and
3. Individuals undergone obesity surgery and lost significant body weight.

Each category includes ten individuals. For the study, stool sample from each individual is being used for the total DNA isolation which was further used to construct 16S rRNA clone library. Till now, we have constructed one library from treated individual (Post Surgery).

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 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 now undertaken metagenomic analysis of this ecosystem.

Indian west coast:

Ocean, which now covers more than 70% of earth area, acted as the primordial soup and given rise to the origin of life on Earth. The Arabian Sea is one of the most productive seas in the world, characterized by monsoonal up-welling, nutrient enrichment and high biodiversity. It is also a source of the greenhouse gases, carbon dioxide and nitrous oxide in which bacteria play an important role. This study will help in exploring the unexplored diversity of bacteria and their functional role in the marine ecosystem.

To unravel the bacterial diversity, sub-surface sediment samples were collected from Karvar 20m depth, Karvar shore and Mandovi estuary along the central west coast of India. Fifty four representative ribo-groups were present in the library constructed from Mandovi estuary (Fig. 2), Marine Crenoarchaeota Group I was dominant in this estuary. All the sequences obtained were clearly divided into two groups showing two sub-kingdom of Archaea i.e. Crenoarcheota and Euryarcheota. We did not find any member of Koreoarcheota. Out of 179 sequences 144 sequences are form Crenoarcheota and only 35 sequences are from Euryarcheota. Members of sub-kingdom Crenoarcheota were the dominating community of the estuarine sediments.

Ribo-group MES-2 (Most abundant ribo-group in the library, total 48 sequences having >97% similarity were included in this group) show monophyletic origin with recently cultured Archaebacteria *Candidatus Nitosopumilus maritimus*. It is an autotrophic aerobic ammonia oxidizer bacterium. Members related to archaea from hydrate ridges, deep sea sediment, hydrothermal vents and mangrove ecosystem were also present in mandovi estuary.

Thus, the Archaeal communities present at the estuary play an important role in the nitrogen cycle, and production of gases like methane. Also the presence of 54 different ribo-groups suggests the presence of diverse metabolic machinery; which indicates estuary sub surface sediments can be the hotspots for fishing out novel enzymes through metagenomics.

Insect Genomics:

A normalized cDNA library was prepared from the mid-gut of uninfected mosquitoes. A new methodology that depends on cleavage of hybrids representing abundant transcripts by double strand specific nuclease was used for this. More than 20,000 clones were obtained and are completely sequenced. Up till now, 20137 ESTs from the uninfected mid-gut are generated. After filtering low quality sequences, 7721 high quality sequences were obtained and subjected to sequence assembly which resulted in 884 contigs and 2180 singlets. Out of 3064 unique transcripts obtained, 1803 (~60%) show hits with blastx against protein database (nr). Ninety Five percent of them were against Drosophila and Anopheles gambiae. About 1265 (~40%) transcripts haven't shown significant match with any sequence. Some of these clones were mapped to Anopheles stephensi chromosomes confirming that these are genuine transcripts. These results suggest that there are some proteins in *Anopheles stephensi* without any known or novel function. Studying such proteins and their role in vector competence will help in understanding *Anopheles stephensi* and parasite interaction. A similar approach was carried out with *Plasmodium*-infected mid-gut of female *Anopheles stephensi* and the sequencing of over 20,000 clones is also completed. We are further carrying out screening, analysis and annotation of these sequences by means of a bioinformatics code pipeline.

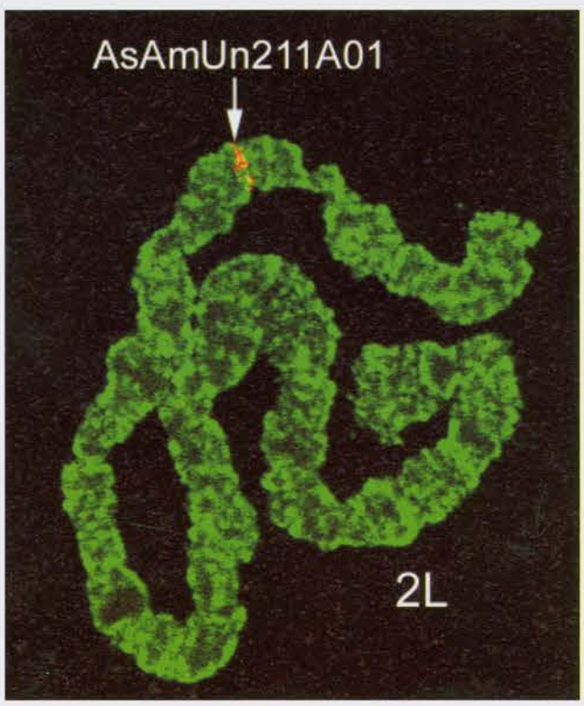
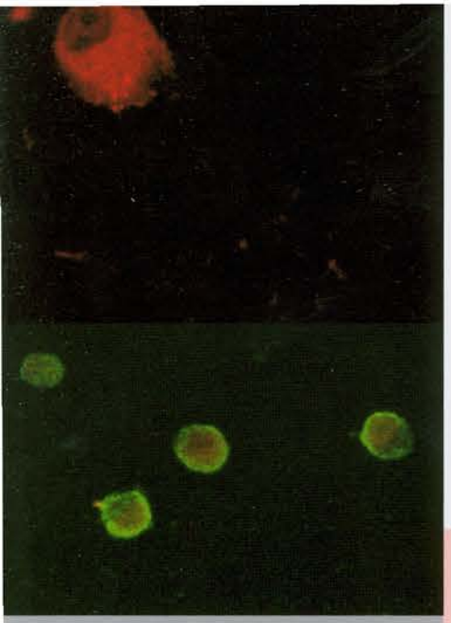


Fig. 3: Fluorescence In Situ Hybridization (FISH) of Expressed Sequence Tag clones on *Anopheles stephensi* chromosomes (Courtesy: Igor Sharakhov, Department of Entomology, Virginia Tech).

In order to understand the architecture of developmentally regulated genes in *Anopheles stephensi* BAC clones carrying parts of the hox genes were identified. Initial characterization of the positive clones with PFGE was also carried out. Till now, subcloning and assembly of genes *sex combs reduced (scr), Ultrabithorax (ubx)* and *abdominal A (abdA)* are completed.

Future Work

1. Understanding of the role of microbial communities in the insect gut and exploration of their biotechnological potential using various approaches using metagenomic analysis
2. Studies on geographical and developmental variation in the gut flora of mosquitoes.
3. Studies on variation of gut flora in the mosquito strains with varying disease carrying capacities.
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 and also to understand the ecology of these ecosystems.
6. Analysis and annotation of EST sequences from *Anopheles stephensi.*



Research Reports

Infection and Immunity

Work Achieved

Studies on Schizogony-merozoites formation: Multiple parasite infection of erythrocyte and merozoite formation. We have observed merozoites formation in single and multiple (2, 3 and 4) parasite infected erythrocytes. The number of merozoites formed by the parasites varies with multiple parasite infection.

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.

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

Gyan Chandra Mishra
gcmishra@nccs.res.in

Participant
Debargh Dutta, *SRF*

Collaborator
Dr. Cecilia D., NIV, Pune

Background

Earlier we reported that Dendritic Cell type 1 (DC1) was stimulatory and DC2 was regulatory in nature for the development of T-cell response. This dichotomy was due to the differential expression of co-stimulatory molecules and the cytokine milieu of the DCs. The expression of co-stimulatory molecules such as CD80, CD86, CD40 and cytokines production viz. IL-12, IL-6, were increased on the surface of DCs activated by anti-CD40 + CPG, LPS and anti-CD40 + LPS with respect to un activated DCs. The expression of co-stimulatory molecules was significantly less on DC2 compared to DC1, whereas the expression of regulatory molecule was significantly more on DC2 compared to DC1. Prominently, anti CD40 + CpG activated DC1 and DC2 showed the same extent of expression of CD80, CD86, CD40 and also IL-12 and IL-6 cytokine synthesis. This study has been extended further by taking dengue infected DC's as antigen presenting cells (APCs). Primarily, we used dengue infected DC1 and DC2 and have analyzed the fate of CD4 and CD8 positive T-cell responses. Dengue Virus (DV) infects both the subsets of DCs, and the infected DCs were used to provide secondary signals to the T-cells. Although reports indicating DV infection mediated immunoregulation of the host are available, nothing is reported on the effects of DV infected DC1 (DV-DC1) and DC2 (DV-DC2) on T cell responses. We made an attempt to evaluate the role of DV-DC1 and DV-DC2 on T–cell priming, activation, proliferation, differentiation and memory recall responses, which may provide important information in understanding the pathogenesis of DV infection.

Aims and Objectives

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

Work achieved

We have been successful in infecting bone marrow derived DC1 and DC2 with DV serotype 2 upto 84% and 72%, respectively, as analyzed by flow cytometry. DV-DC1 and DV-DC2 were tested for their ability to activate and differentiate $CD4^+$ and $CD8^+$ T-cells. Naive murine $CD4^+$ and $CD8^+$ T–cells were cultured in presence of anti-CD3ε as a source of primary signal. DV infected DC1 and DC2 were used as a source of secondary signal. It was found that DV-DC1 and DV-DC2 failed to enhance the proliferation of naïve $CD8^+$ T cells *in vitro*. When the supernatant of the co-culture was estimated for IFN-γ, no significant increase in its level was observed.

DV infected DC1 and DC2 were then examined for their ability to activate naïve $CD4^+$ T–cells. Interestingly, DV-DC1 could enhance proliferation of $CD4^+$ T–cells, while DV-DC2 suppressed the same (Fig. 2A). DV-DC1 co-cultured with $CD4^+$ T-cells showed three fold increases in IFN-γ levels in the culture supernatant as compared to uninfected DC1 co-cultured with $CD4^+$ T–cells. Where as the DV-DC2 co-cultured with $CD4^+$ T–cells, IFN-γ level were approximately 50% lower as compared to those secreted by DC2 with $CD4^+$ T-cells (Fig. 2B). When these co cultured cells were analyzed for IL-10, there was no significant change in the IL-10 secretion (Fig. 2C). However, both DV infected and uninfected DC2 cells stimulated high levels of IL-10 secretion as compared to DC1 cells. Thus DV-DC1 stimulates $CD4^+$ T–cells activation, whereas DV-DC2 suppresses $CD4^+$ T–cell activation and proliferation.

We then looked for the expression of co-stimulatory, regulatory and MHC molecules on the surface of DV-infected and mock infected DCs. The expression of co-stimulatory molecules like CD80, CD86, CD40, CD70 and CD95L was significantly enhanced in DV-DC1 as compared to control mock infected DC1, DC2 and DV-DC2. Similarly the expression of regulatory molecules such as B7H1, B7-DC, B7H3 and B7H4 was also up-regulated in DV-DC1 compared to mock infected DC1. However, the expression of these molecules in DV-DC2 did not show substantial increase. The expression of MHC molecules and TLRs remained unaltered.

Propidium iodide and annexin V staining demonstrated that upon dengue infection, DC1 is more susceptible to apoptosis than DC2. The DCs were subjected to various activation signals and it was found that both infected as well as uninfected DC1 and DC2 respond to anti-CD40 + CpG mediated signaling in relation to proliferation and IFN-γ secretion.

In order to ascertain the effecter function of activated $CD4^+$ and $CD8^+$ T–cells primed with DV-DC1 and DV-DC2, we checked for the expression of granazyme gated on

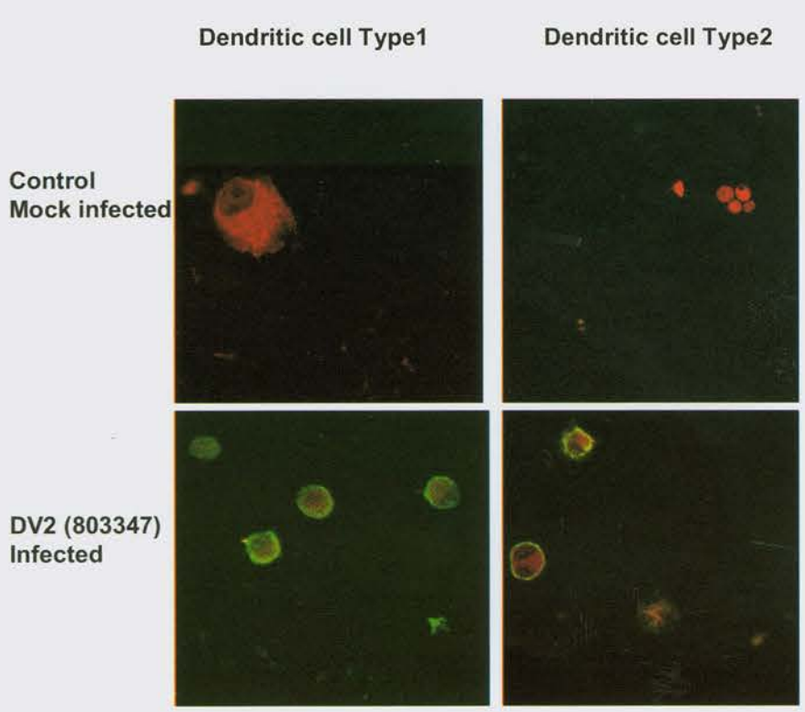


Fig. 1: Confocal pictures showing DV2 infected dendritic cells stained with FITC-anti-DV2 and PE anti-CD11c (DC marker).

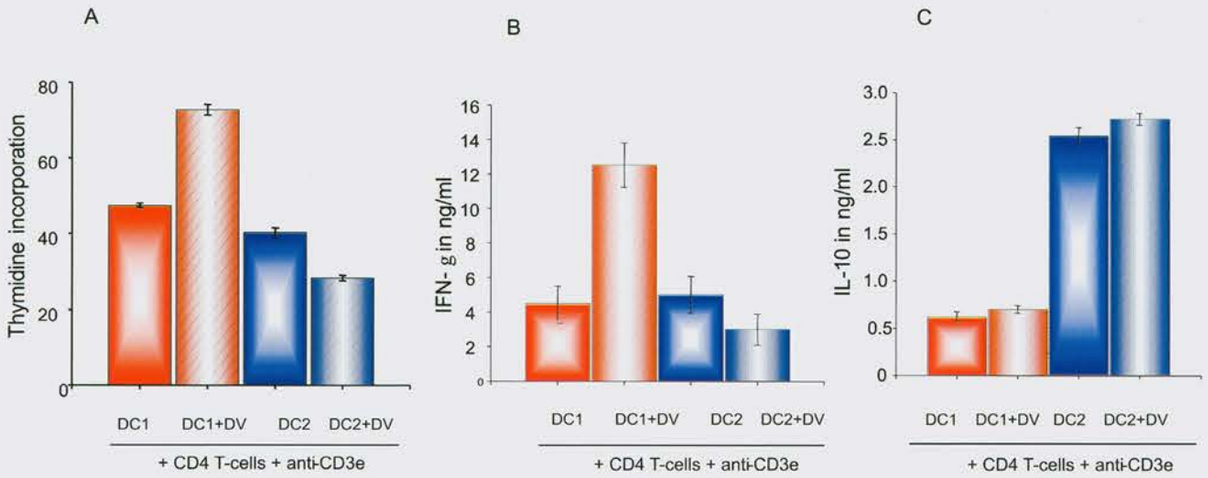


Fig. 2: 5 x 10^5 purified $CD4^+$ T cells were co-cultured with 1 x 10^4, DV infected or mock infected, DC1/DC2. Cells were cultured for 72 hrs and **A**. cell proliferation was assayed by thymidine incorporation for last 12 hrs of the culture, and the supernatant was assessed for **B**. IFN-γ, **C**. IL-10.

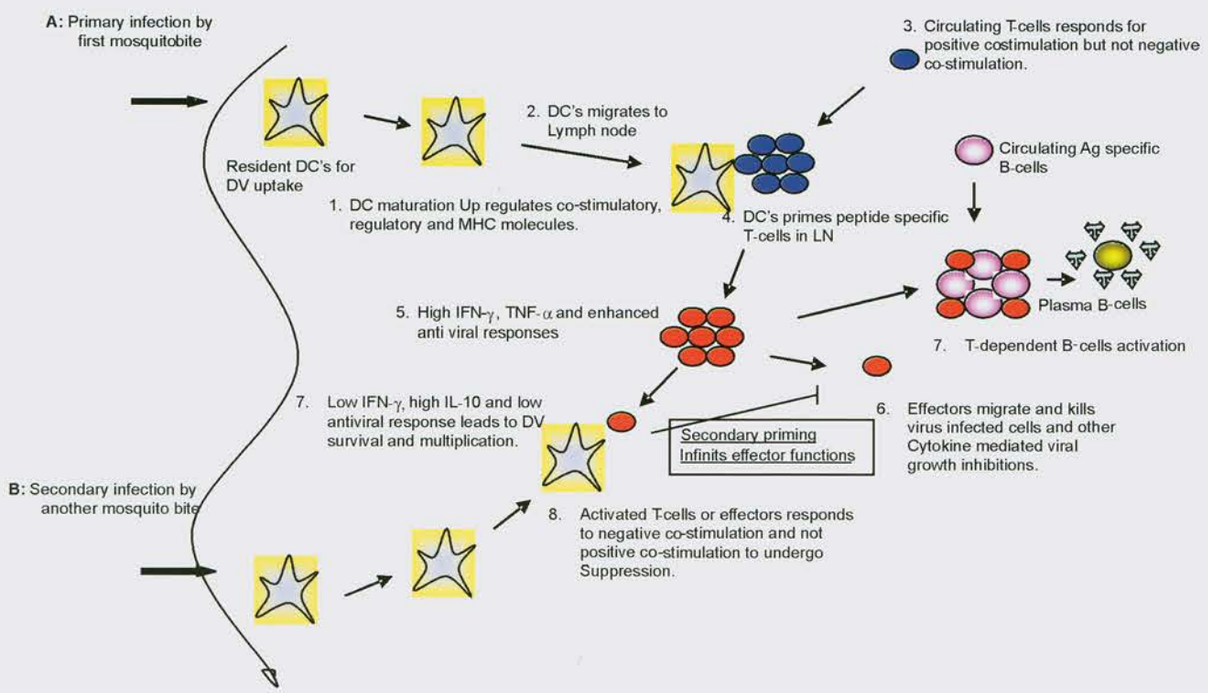


Fig. 3: Schematic representation of possible fates of naïve Ag-specific T-cell development during acute dengue virus infection.

$CD4^+$ and $CD8^+$ T-cells. We observed that the expression of granzyme was not increased in T–cells co-cultured with DV-DV1 and DV-DC2. Prominently, granazyme expression was upregulated in the T–cells co-cultured with anti-CD40 + CpG stimulated DC1 and DC2.

In order to substantiate the *in vitro* findings, we also performed *in vivo* experiments. C57/BL6 mice were injected with DV-DC1 and DV-DC2. Twenty-five days later, spleenocytes were harvested and 10^6 cells were re-challenged with DV2 and DV3. The results showed that priming by DV-DC2 was sensitive to challenge by DV2 compared to priming with infected DC1. There was almost no sensitization by priming with DV-DC2. We further analyzed Th1/Th2 cytokine such as IL-2, TNF-α, IFN-γ, IL-4 and IL-5 in the supernatants of primed mice spleenocytes. It was found that DV-DC1 primed mice spleenocytes showed higher levels of IL-2, IFN-γ, TNF-α, IL-4 and IL-5 as compared to DV-DC2 primed mice spleenocytes. These results suggest that DC1 is stimulatory and DC2 is regulatory in nature. Based on these results we propose a model of immune evasion by Dengue virus (Fig. 3). Resident DCs on primary dengue

infection up-regulates positive and negative co-stimulation signals. DCs upon maturation migrate to localized lymph node to activate Ag-specific T-cells, which bears receptors for positive co stimulation only. Activated T-cells or effectors migrate out of Lymph nodes to kill infected cells and other inhibitions, which now expresses receptor for negative co-stimulation. On secondary infection with heterologous serotype, DV matures DCs to express both +ve and –ve co-stimulation signals. Matured DCs encounter activated T-cells and induce their suppression. Dengue virus might use this strategy to regulate T-cell response for its own survival and multiplication.

Molecular and Cellular Basis of HIV Pathogenesis

Debashis Mitra
dmitra@nccs.res.in

Participants
Shalini Gupta, *SRF*
D.S. Ravi, *SRF*
Manoj Tripathy, *SRF*
Renu Singh, *JRF*
Md. Zulfazal Ahmed, *JRF*
Sohrab Khan, *JRF*
Pratima Rawat, *JRF*
Neeru Dhamija, *JRF*
Sanchita Roy, *RA*
Sudeep Sabde, *Project JRF*
Sujata Bhade Kulkarni, *Technician*

Collaborator(s)
Dr. Inderpal Singh, NIPER, Mohali
Dr. K.K. Bhutani, NIPER, Mohali
Dr. Anil Chatterji, NIO, Goa
Dr. Sekhar Chakrabarti, NICED, Kolkatta
Dr. Bhaskar Saha, NCCS, Pune
Dr. G.C. Mishra, NCCS, Pune

Background

Human immunodeficiency virus (HIV) 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. Molecular basis of CTL dysfunction in HIV infection.

3. Identification of novel molecules with anti-HIV activity.

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 it 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 (Fig. 1). Thus our results clearly indicated that Hsp40 is crucial for Nef-mediated enhancement of viral gene expression and replication. As Hsp40 is normally associated with Hsp70, we have 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 downregulation 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 favors the host by inhibiting the viral replication whereas Hsp40 works in favor of the virus by inducing its gene expression and replication.

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.

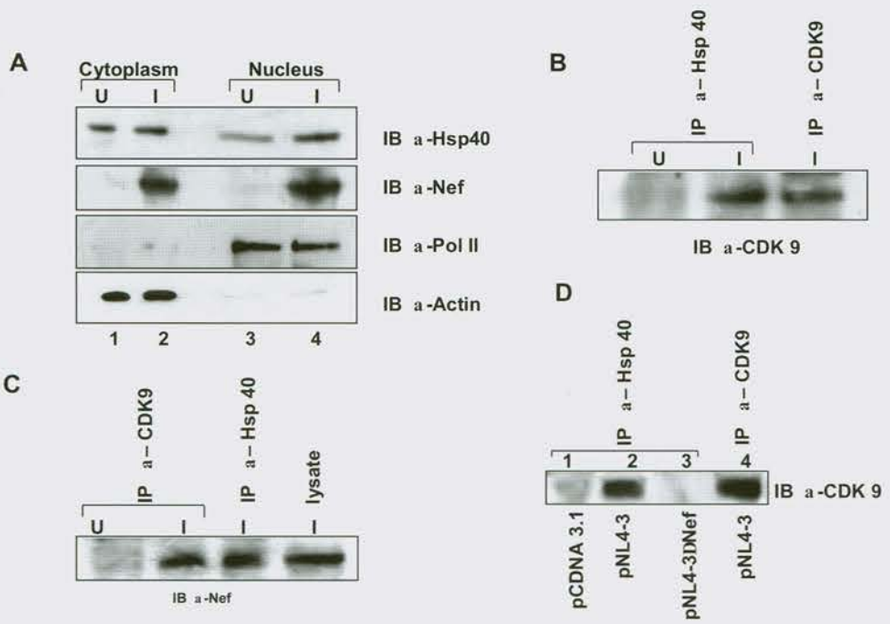


Fig. 1: Hsp40 translocates into the nucleus of HIV-1 infected cells and interacts with cdk9 in a Nef dependent manner. (A) Hsp40 increases in nucleus of HIV-1 infected cells along with Nef. Nucleus and cytoplasm fractions were prepared from uninfected and infected CEM-GFP cells. Equal amount of cytoplasmic and nuclear extracts were used for immunoblotting of Hsp40 and Nef. Same blots were probed with actin and RNA polymerase II antibody as loading controls. (B) Hsp40 interacts with cdk9 in HIV-1 infected cells. CEM-GFP uninfected and infected lysates were used for immunoprecipitation with Hsp40 antibody followed by immunoblotting with anti-cdk9 antibody. Lysate was also subjected to immunoprecipitation with cdk9 antibody as positive control. (C) Nef is a part of Hsp40 and cdk9 complex. CEM-GFP uninfected and infected lysates were immunoprecipitated with either cdk9 antibody (lane 1 and 2) or with Hsp40 antibody (lane 3) followed by immunoblotting with anti-Nef antibody. Lysates from infected cells was used as positive control (lane 4). (D) Hsp40 interacts with cdk9 in Nef dependent manner. Lysates of 293T cells transfected with pCDNA3.1, NL4-3 molecular clone and Nef deleted NL4-3 molecular clone were used for immunoprecipitation with Hsp40 antibody followed by immunoblotting with anti-cdk9 antibody (lane 1, 2 and 3). Immunoprecipitation with cdk9 antibody was used as a positive control (lane 4).

Studies done till date to elucidate the pathways involved in HIV-1 induced T cell depletion has revealed that apoptosis underlie the etiology; however, a clear molecular understanding of HIV-1 induced apoptosis has remained elusive. Although evidences pointing towards the importance of mitochondrial energy generating system in apoptosis exist its exact role remains to be clearly understood. We have previously shown specific down regulation of the complex I subunit NDUFA6 with simultaneous impairment of mitochondrial complex I activity in HIV infection. Our recent results show modulation of complex V during HIV induced T cell infection.

Molecular basis of CTL dysfunction in HIV infection

HIV-1 is a uniquely difficult target to develop immunological intervention against. Despite a high frequency of HIV-specific $CD8^+$ T cells, most HIV-infected patients fail to control viral replication without antiviral drugs suggesting an impairment of the CTLs. Indeed, $CD8^+$ T cells are active in containing acute HIV infection but not in chronic infection. The inability is related to the failure of these cells to mature into fully differentiated effector cells. Maturation into competent CTLs may be blocked during the initial encounter with antigen because of defects in antigen presentation by dendritic cells or HIV-infected macrophages. A large number of multi-component vaccine candidates are currently in clinical evaluation, many of which also include the HIV-1 Tat protein, an important regulatory protein of the virus. However, whether Tat, a known immune effector molecule with well conserved sequence among different HIV subtypes affects the immune response to a co-immunogen is not well understood. Using a bicistronic vector expressing both gp120 and Tat, we have analyzed the role of Tat in elicitation of gp-120 specific immune response. The T cell responses to gp120 were greatly diminished in mice co-immunized with Tat as compared to mice immunized with gp120 alone (Fig. 2). Analysis of cytokine profile suggests that Tat induces IL-10 and since IL-10 has been demonstrated to have appreciable T cell inhibitory activity, it is plausible that IL-10 could be responsible for Tat mediated immunosuppresion.

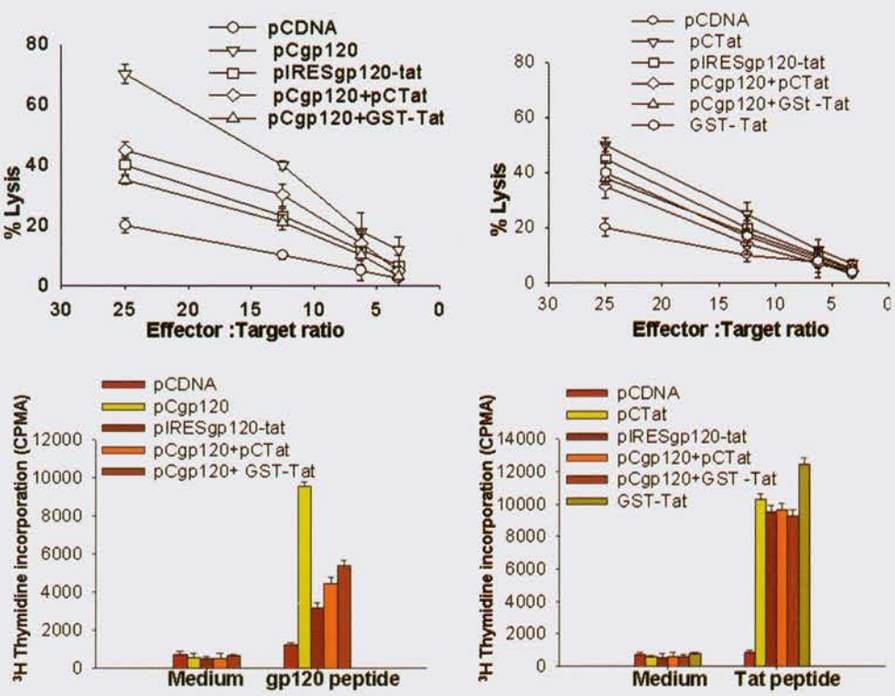


Fig. 2: Tat suppresses T cell responses in mice immunized with bicistronic vector expressing both gp120 and Tat. Splenocytes from pCDNA, pCgp120, pCTat, pIRESgp120-Tat and gp120 and Tat injected mice were plated $2x10^6$ per well in 24 well plate with gp120 or Tat peptide pulsed $1x10^6$ irradiated naïve splenocytes. After 5 days of culture viable cells were harvested and plated against ^{3}(H) thymidine-incorporated gp120 or Tat peptide pulsed EL-4 cells and tested for their cytolytic activity in standard JAM test. The Effector:Target ratio used are shown in the figure. Each data point is the mean of triplicate samples. Also Splenocytes from injected mice were plated $2x10^5$ cells per well in 96 well plates and was pulsed with 10µg of gp120 or Tat peptides or without antigen (medium). Proliferation was assessed by ^{3}(H) thymidine-incorporated assay. The results represent three individual experiments and error bar represent the mean ± SD of a given group. **A**, gp120 specific CTL response in different immunized mice. **B**, Tat specific CTL response in different immunized mice. **C**, gp120 specific proliferation in splenocytes from mice immunized with different vectors mentioned above. **D**, Tat specific proliferation in splenocytes from mice immunized with different vectors mentioned above.

Finally, the immunosuppressive effect of Tat was not observed in IL-10 deficient mice confirming the role of IL-10 in Tat mediated immunosuppression.

Identification of novel molecules with anti-HIV activity

The current therapeutic strategy involving the use of reverse transcriptase and protease inhibitors in combination (HAART) has proven to be useful in controlling the virus but is not sufficient to eradicate the virus from the patients. Extensive work is being done throughout the globe to identify new ant-HIV therapeutic strategies. One of the strategies has been to identify anti-HIV compounds in natural resources. We have performed screening of anti-HIV activity in marine bivalves of Indian coastline. Our studies have revealed the presence of anti-HIV activity in green mussel and clams. The clam lysate have been fractionated and a pure active fraction has been obtained, which has been identified and synthesized now. The synthetic molecule is equally active as the identified molecule. It seems to be a reverse transcriptase inhibitor. We have also initiated a new program along with NIPER under DBT-ICMR joint initiative on HIV microbicides. We are screening compounds being fractionated from plant extracts and new synthetic compounds from NIPER.

Human Immunodeficiency Virus type 1 Long Terminal Repeat (HIV-1 LTR) promoter regulates the expression of viral genes. HIV-1 LTR driven reporter gene expression is used for qualitative and quantitative analysis of viral promoter activity. LTR reporters are widely used in viral transcription studies and in identification of agents that affect viral promoter activity. Although a number of HIV-1 subtype B LTR-reporter gene constructs have been used in HIV research, very little work has been performed with subtype C viruses, which is now the most prevalent subtype in the world. Enhanced Green Fluorescent Protein (EGFP) and Luciferase (Luc) reporter genes are widely used for visual and quantitative analysis of promoter activity respectively. Although subtype C LTR-Luc reporter gene construct has been previously reported, no EGFP based subtype C reporter is currently available. We have constructed a dual reporter vector pLTRC-Luc-EGFP expressing both EGFP and Luciferase under the regulation of HIV-1 subtype C LTR (Fig. 3). This reporter vector will be very useful in HIV-1 subtype C specific research, specifically in studies related to promoter analysis, viral gene expression and transcriptional modulators.

Future Work

Based on our data, it seems that HSPs may play an important role in viral life cycle. We are currently trying to elucidate the role of different heat shock proteins in HIV pathogenesis. The future work also involves characterization of some of the other Nef interacting clones and identifying their functional relevance in HIV lifecycle. We are also looking at the chromatin modulation by Tat protein in HIV infection, both in acute and latently infected cells. Identification of differentially expressed genes and their relevance to HIV induced cell death will be continued, with a focus on mitochondrial oxidative phosphorylation system in infected cells. We are continuing our studies on the role of CD40 in elicitation and maturation of CTL response in HIV infection and have initiated the study of the CD40 mediated signaling in HIV infection. Finally, studies are in progress to identify novel anti-HIV molecules, both from natural resources and synthetic chemistry. Our aim is to identify novel lead molecules with potential for use as anti-HIV microbicides.

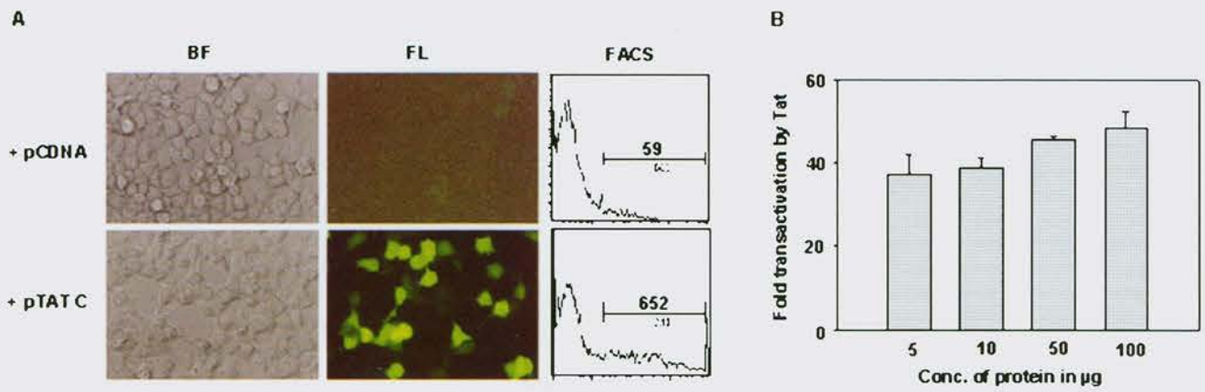
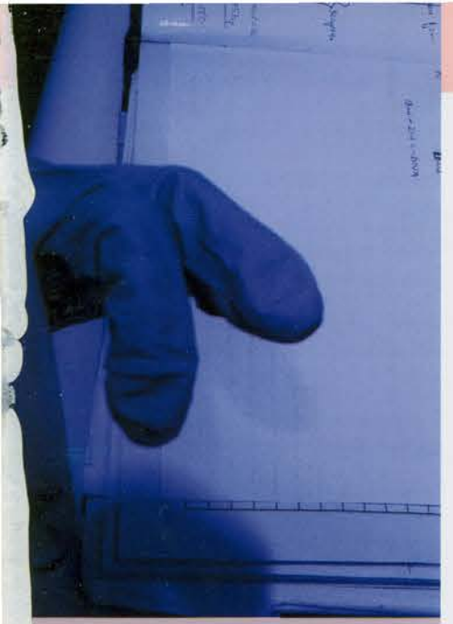


Fig. 3: EGFP and Luciferase reporter gene expression in 293T cells transfected with pLTRC-Luc-EGFP and transactivation by HIV-1 subtype C Tat.

A. Bright Field (BF) and fluorescence (FL) microscopic images; Histograms obtained by FACS analysis (FACS) of 293T cells transfected with pLTRC-Luc-EGFP along with control plasmid (+ pCDNA) or with Tat (+ pTatC).

B. Fold transactivation of Luciferase expression from the pLTRC-Luc-EGFP co-transfected with subtype C Tat in 5-100 µg conc. of 293T cell lysate.



Molecular studies on house keeping genes of Leishmania

Milind S. Patole
patole@nccs.res.in

Participants
Sachin Surve, *SRF*
P.K. Umasankar, *SRF*
Lokesh Sharma, *UGC Teacher Fellow*
Vishal Kalel, *JRF*

Collaborator
Dr. Yogesh Shouche, NCCS
Dr. Armando Jardim, McGill University, Canada

Background

Studies of Hexokinase in Leishmania

Leishmania is an evolutionarily primeval kinetoplastid pathogen that has diverged early from the eukaryotic ancestors. A distinct cellular feature relating evolution with the kinetoplastid parasites is that it contains glycosomes, a microbody organelle that is similar to the peroxisomes in animals and plants. Glycosomes compartmentalize a large number of key metabolic pathways that include purine salvage, pyrimidine and ether-lipid biosynthesis. Energy generation is one of the important functions of glycosomes as it contains enzymes for glycolysis and β-oxidation of fatty acids. Hexokinase occupies important position in glucose metabolism as its product Glucose-6-phosphate is substrate for two important metabolic pathways namely glycolysis, and pentose phosphate shunt. The protein sequence elucidated from hexokinase gene sequence indicated that the enzyme has, at amino terminal end, a sequence which is very similar to the PTS2 type of peroxisomal targeting signal sequence found in enzymes like aldolase in *T. brucei*, thiolase in yeasts and mammals. This signal sequence is believed to be universal and known to help accurate targeting of the proteins to glycosomes in trypanosomes, peroxisomes in mammals and to glyoxysomes in plants. In this study we have used three fusion proteins with green fluorescent protein [GFP] to study targeting of hexokinase to glycosomes in Leishmania. To test whether the targeting signal sequence was capable of directing the GFP to the glycosome, we fused the PTS2 sequence to the amino terminus of GFP. In order to check the specificity of PTS2 signal we transfected the PTS2-GFP construct in mammalian CHO cell line and observed the localization of GFP.

Work Achieved

Immunofluorescence staining of Leishmanial promastigote with anti-recombinant hexokinase antibody demonstrated that the enzyme hexokinase is totally present within microbody organelles. The anti-hexokinase antibody colocalized with anti-Inosine monophosphate dehydrogenase antibody, indicating that hexokinase is compartmentalized exclusively in glycosomal microbodies. In amastigote stage of the parasite, the immunofluorescence staining showed typical punctate pattern similar to that of promastigotes, indicating glycosomal localization of hexokinase is same in both the stages of life cycle. Furthermore, the localization of hexokinase and the role of N-terminal Peroxisomal targeting sequence-2 (PTS2) like domain in the targeting of enzyme to glycosomes was analyzed, using expression of three different GFP fusion constructs in *L. donovani*. GFP fusion protein, having complete Leishmanial hexokinase sequence at its N-terminal end, was targeted to glycosomes as assessed by confocal microscopy and by subcellular fractionation on sucrose density gradient centrifugation. Similar translocation of GFP was observed, when only first 12 amino acids of parasite hexokinase that contains putative PTS2 domain were fused with GFP. Amastigotes stage of the parasite expressing Hexokinase-GFP and PTS2-GFP fusion proteins also showed a punctate pattern of green fluorescence, indicating glycosomal translocation of the GFP in the intracellular stage of the parasite. On the contrary, GFP fusion protein with hexokinase sequence devoid of first 12 amino acids, having PTS2 like domain, was found to be localized in cytoplasm and not translocated to microbody organelles, indicating that PTS2 like domain is necessary and sufficient to target proteins to glycosomes in Leishmania. However, PTS2 sequence from Leishmanial hexokinase was not able to target EGFP efficiently to peroxisomes in mammalian CHO cell line. This Leishmanial specific PTS2 domain and machinery involved in targeting of proteins to glycosomes can be used as a potential drug target.

Studies of Selenophosphate synthetase in Leishmania

Selenium exerts its biological role in the form of selenocysteine. It is incorporated cotranslationally by a specific tRNA and is directed by a UGA codon in the mRNA. Leishmania is a causative agent of visceral, cutaneous and mucosal leishmaniasis. It is a kinetoplastid protozoan and a digenic parasite of blood feeding insects and vertebrates including humans. The promastigote motile form of the parasite from phlebotomine sand fly is injected into vertebrate blood and is engulfed by circulatory macrophages. It is transformed into non-motile amastigote within macrophage phagolysosome. Macrophages produce various ROS such as super oxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radicals, hypochlorus acid, nitric oxide and its peroxynitrile derivatives. The effect of this toxic cocktail is to disable the parasite by oxidizing crucial components of the parasite and thereby killing the parasite. Leishmanial amastigote survives and multiplies within the phagolysosome in the presence of toxic ROS. The key molecules for these capabilities are attributed to enzymes that are involved in metabolism of ROS. As selenoenzymes play a significant role in metabolism of ROS, study of selenoprotein synthesis and activity was necessary to elucidate the pathogenesis of Leishmania. The selenophosphate synthetase catalyses the first step in elemental metabolism. The presence of SPS in Leishmania would also mean presence of Selenium metabolism and presence of selenoproteins in the parasite.

Work achieved

In this regard a homolog of selenophosphate synthetase (SPS), *SelD,* was identified, cloned and sequenced from *L. major*, in our laboratory. Next phase of the work involved characterization of *SelD*, gene with respect to its function, and study the relevance of the selenium biochemistry to Leishmania survival and pathogenesis. Sequencing of the SPS showed us that the gene is highly conserved among Leishmania species. *LmSelD* was cloned from *L. major* and expressed the recombinant LmSPS. The protein expressed was used to raise the antibodies in rabbit against SPS. Western blot analysis, immunostaining and RT-PCR data suggest the constitutive expression of SPS in promastigote and amastigote stages of Leishmania. We are currently in the process of finding out the physiological effects of selenium on Leishmania promastigotes. In order to find out the functionality of the gene we performed the complement assay in a mutant *E.coli* lacking selD. Since the mutant *E.coli* is not an expression host, we had to cotransform two plasmids, one expressing T7 RNA polymerase and other contained cloned *LmSelD*. In order to study the functional importance of the selenocysteine incorporation pathway, we are in process of creating deletion mutants of *SelD* in Leishmania. Deletion mutants would reveal the involvement of the selenium biochemistry pathway in Leishmania survival, virulence and pathogenesis.

Future Work

1. Generation of *SelD* knockout in Leishmania will be carried out.

2. Functional assays will be performed to elucidate the role of Hexokinase UTRs.

CD40 signaling in anti-tumor immune response

Bhaskar Saha
sahab@nccs.res.in

Participants
Sunit Srivastava, *SRF*
Sunil Martin, *SRF*
Archana Sareen, *SRF*
Abdur Rub, *SRF*
Raghu Kumar Penke, *SRF*
Neetu Srivastav, *SRF*
Raki Sudan, *JRF*
Sushmita Pahari, *JRF*
Srijit Khan, *JRF*
Shradha Khurana, *JRF*
Mumtaz Nayyer, *JRF*
Rashmi Pitke, *Project Assistant*
Meenakshi Jadhav, *Technician*

Background

Tumor regression requires 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. CD40-based therapies are also shown to control tumor growth significantly, suggesting that CD40-CD40-L interaction induces anti-tumor T-cell responses and tumor regression. Establishment of host-protective memory T-cells against tumors is the objective of an anti-tumor immunoprophylactic strategy such as reinforcing T-cell co-stimulation via CD40-CD40L interaction. Previous CD40-targeted strategies assumed that T-cell co-stimulation is an all-or-none phenomenon. It was unknown whether different levels of CD40L expression induce quantitatively and qualitatively different effector T-cell responses.

Work Achieved

We demonstrate that the anti-tumor T-cell response can be modulated reciprocally as a function of the levels of CD40 expression. At low expression levels, CD40 promotes tumor growth; at higher expression levels, CD40 induces tumor-regressing T-cell response.

Dendritic cells (DC) sorted into major histocompatibility complex (MHC)-II expression are found to be similar in CD40 and CD80 expression. The MHC-IIhi/CD40hi DC induce interleukin (IL)-12-dominated and T-helper 1 (Th1)-type response, whereas MHC-IIlo/CD40lo DC promote high IL-10 and Th2-type T-cells. The T-cells induced by these DC also differ in terms of regulatory T-cell markers, lymphocyte activation gene-3 (LAG-3) and glucocorticoid-induced tumor necrosis factor (TNF) receptor family-related gene (GITR). Thus, we report for the first time that CD40-induced effector T-cell response depends on CD40 expression levels in vivo.

Using mice expressing different levels of CD40L, we demonstrated that the greater the T-cell CD40L expression the less tumor growth occurred; the anti-tumor T-cell response was host-protective. Lower levels of CD40L expression on T-cells induced IL-10-mediated suppression of tumor-regressing effector $CD8^+$ T-cells and higher productions of IL-4 and IL-10. Using mice expressing different levels of CD40 or by administering different doses of anti-CD40 Ab, similar observations were recorded implying that the induction of pro-tumor or anti-tumor T-cell responses was a function of the extent of CD40 cross-linking. IL-10 neutralization during priming with tumor Ags resulted in a stronger tumor-regressing effector T-cell response. Using IL-10 -/- DC for priming of mice expressing different levels of CD40L and subsequent transfer of the T-cells from the primed mice to nu/nu mice, we demonstrated the pro-tumor role of IL-10 in the induction of tumor-promoting T-cells. Our results demonstrate that a dose-dependent cross-linking of a co-stimulatory molecule dictates the functional phenotype of the elicited effector T-cell response. The T-cell co-stimulation is a continuum of a function that induces not only graded T-cell responses but also two counteracting responses at two extremes.

Activation of T-cells requires signals through Ag-specific TCR and co-stimulatory molecules such as CD40L. Although the use of defined tumor Ags for the induction of protective T-cells met with limited success, the CD40-CD40L interaction that was proposed to induce anti-tumor T-cells did not prevent tumor growth completely. Using a model for prostate tumor, a leading cause of tumor-induced mortality in men, we show that the failure is due to a novel functional dichotomy of CD40 whereby it self-limits its anti-tumor functions by inducing IL-10. IL-10 prevents the CD40-induced CTL and TNF-α and IL-12 production, Th1 skewing, and tumor regression. Priming mice with tumor lysate-pulsed IL-10-deficient dendritic cells (DCs) or wild-type DC plus anti-IL-10 Ab establishes anti-tumor memory T-cells that can transfer the protection into syngenic nude mice. Infusion of Ag-pulsed IL-10-deficient but not wild-type DCs back into syngenic mice results in successful therapeutic auto-vaccination. Thus, we demonstrate the IL-10-sensitive anti-tumor T-cell memory formulating a novel prophylactic and therapeutic principle.

Role of viral complement control proteins in immune evasion

Arvind Sahu
arvindsahu@nccs.res.in

Participants
Malik Reza, *JRF*
Vivekanand Yadav, *JRF*
Kalyani Pyaram, *JRF*
Muzammil Ahmad, *SRF*
Akhilesh Singh, *SRF*
John Bernet, *SRF*
Archana Kadam, *Project Assistant*
Shailendra Salvankar, *Project Assistant*
Yogesh Panse, *Technician*

Collaborators
Jayati Mullick, DST Scientist, NCCS, Pune
Pradeep B. Parab, NCCS, Pune
Debashis Mitra, NCCS, Pune

Background

The complement system is an integral participant in the innate mechanisms of immunity and thus, it must share the burden of surveillance in the host. It achieves this effectively by recognizing and labeling the pathogens as "non-self" by covalently attaching complement protein C3b on to their surface. Unlike other innate immune mechanisms, it recognizes pathogen with as well as without (by "tick over" mechanism) the help of recognition molecules, which provides this system a unique ability to recognize a repertoire of existing as well as newly emerging pathogens. Although viruses are small and relatively simple in structure, they are known to be efficiently recognized and neutralized by the complement system. In addition, the complement system is also capable of attacking the virus infected cells, recruiting inflammatory response at the site of infection and participating in the generation of virus-specific adaptive immune responses. Thus, complement exerts a strong selective pressure against viruses and therefore for successful survival, viruses must co-evolve with the complement system and develop mechanisms to subvert this system. Sequence analyses of viral genomes have shown that large DNA viruses such as pox and herpesviruses encode proteins structurally similar to the human complement control proteins. We, therefore, started the present study with a hypothesis that viruses encode complement regulators (vCCP) to mask themselves against the host's complement attack and are vital for their successful *in vivo* survival and propagation. Currently, we are focusing on the functional characterization of these viral homologs with emphasis on understanding the mechanism by which these homologs inhibit complement, and the molecular basis behind it. In addition, we are also attempting to characterize the role of vCCPs 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

Mechanism of complement inactivation by vCCP

Because vCCPs are found in pox as well as herpesviruses, we focused on the characterization of representative examples of both pox as well as herpesviral complement regulators. We demonstrated that vaccinia virus, Kaposi's sarcoma-associated herpesvirus (HHV-8), and Herpesvirus saimiri (HVS) encode functional homologs of human complement regulators. In our initial functional analysis we observed that these viral homologs inhibit C3b deposition. Based on these data we reasoned that viral homologs must target C3 convertases (C3b, Bb and C4b, 2a), the enzymes responsible for cleaving complement protein C3. Our detailed analysis confirmed this premise and revealed that all the viral homologs inhibit complement through a common mechanism of targeting C3 convertases. The data indicated that they inhibit C3 convertases by two different mechanisms: i) by supporting the irreversible decay of the classical/lectin and alternative pathway C3 convertases (termed decay-accelerating activity), and ii) by inactivating C3b and C4b, the subunits of convertases, by serving as factor I cofactors (termed cofactor activity). These mechanisms are similar to the regulatory mechanisms of the human complement regulators, but there are differences in the bimolecular interaction between vCCPs and the target proteins in comparison to human regulators-target protein interactions. The most notable difference between vCCPs and human complement regulators is enhancement of functional repertoire in vCCPs by encoding various regulatory activities in a single molecule. The only human regulator that possesses all the complement regulatory activities displayed by vCCPs is complement receptor 1, which is 7.5 times larger in size than viral homologs. While studying the vCCP-C3b/C4b interactions we observed that ionic contacts play critical role during these interactions. By performing molecular dynamic simulations and electrostatic calculations we are studying the physiochemical properties of vCCPs and generating charge mutants to address if overall positive electrostatic potential directly correlates with the functional activities.

Mapping of functional domains in vCCPs

Structurally, vCCPs appear like four-beaded string due to the presence of four repeating complement control protein (CCP) domains connected by short flexible linkers. In order to get better insight into the role of individual domains in functioning of these molecules we made efforts to identify complement regulatory domains in vCCPs. Earlier, we identified the functional domains in complement regulators of vaccinia virus (named VCP) and HHV-8 (named Kaposica). Because herpesviral complement regulators are more diverse in structure compared to poxviral regulators we also mapped complement regulatory domains in HVS complement regulator to determine whether these structural variations have led to any differences in domain requirements for functional activities in HVS complement regulator. During this study we came across a unique observation that a single domain in Herpesvirus saimiri complement regulator is sufficient to confer both cofactor activity as well as decay-accelerating activity. According to the current dogma in the field, two CCP domain structure is the smallest stable structural unit and a minimum requirement for imparting a function. Thus, our data disputes the current dogma.

Role of vCCPs in immune evasion

Data from our laboratory and others have clearly shown that virally-encoded complement regulators are functional. These data therefore suggest that vCCPs may serve as immune evasion molecules. In order to address this issue we looked into the role of VCP in vaccinia virus pathogenesis using a rabbit skin lesion model. A series of mAbs were raised against VCP and characterized for inhibition of complement regulatory activities of this protein. Out of twenty one monoclonals produced, eight showed inhibition of cofactor activity as well as decay-accelerating activity of VCP, while three showed inhibition of only decay-accelerating activity. These mAbs were then utilized for in vivo disabling of VCP function. In the rabbit skin lesion model, intradermal inoculation of vaccinia virus results in a characteristic skin lesion formation, which persists for about two weeks. To determine if disabling of VCP would reduce the virus pathogenicity we injected vaccinia virus alone or in combination with monoclonal antibodies (blocking or non-blocking) and measured the lesion size over a period of time. Injection of blocking mAbs, but not the non-blocking mAbs, along with vaccinia virus resulted in significant reduction in lesion size. These results suggested that VCP plays an important role in vaccinia virulence. In order to determine if attenuation of lesion was due to lack of VCP-mediated inhibition of host complement or independent of complement we performed similar experiment in complement depleted animals. The reasoning was, if VCP-complement interaction is important in virulence then disabling of VCP by mAbs should not have any effect on the development of lesions in complement depleted animals. As expected, lesions formed by injecting vaccinia virus along with blocking antibodies were similar in size to that formed by injecting vaccinia virus alone. Together these results indicate that VCP is a virulence determinant of vaccinia virus and effectively protects it from the host complement system.

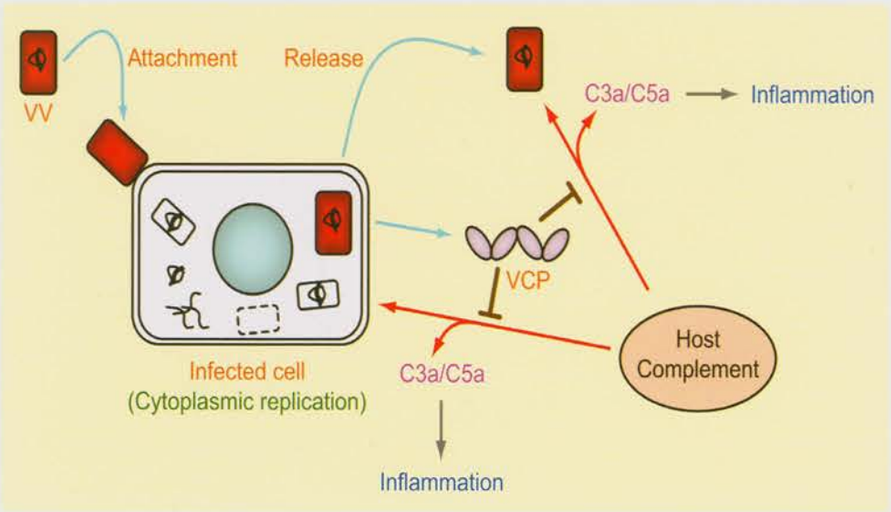
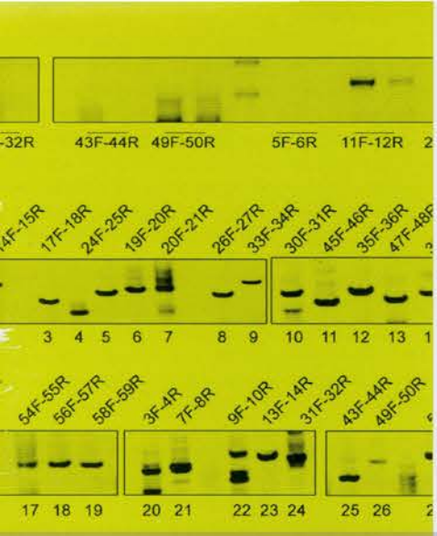


Fig. 1: A model depicting VCP-mediated protection of vaccinia virus (VV) from the host immune responses. Our data suggests that VCP secreted by the VV infected cells protects VV and VV-infected cells from the host complement. Apart from this direct effect, VCP would also inhibit anaphylatoxin-mediated recruitment of inflammatory response at the site of infection.

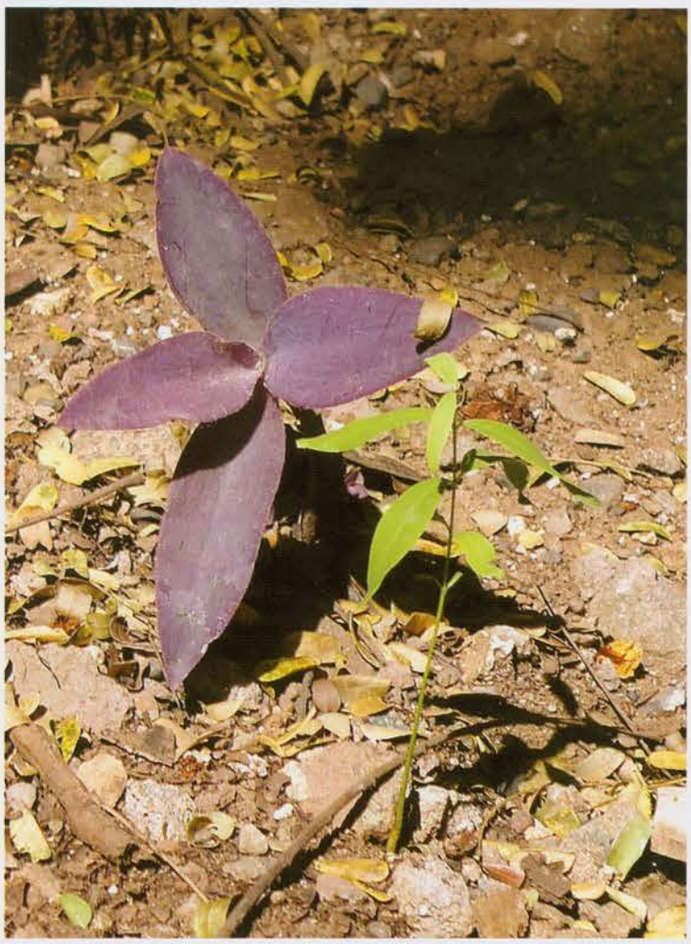
Future Work

1. Fine mapping of functional sites in VCP and Kaposica.
2. Effect of modulation of electrostatic potential on functional activities of vCCPs.
3. *In vivo* role of complement regulating domains of vaccinia virus complement control protein.



Research Reports

Chromatin Architecture and Gene Regulation



Regulation of SMAR1 by anticancer agents and its role in immunomodulation

Samit Chattopadhyay
samit@ncccs.res.in

Participants
Archana Jalota, *SRF*
Kamini Singh, *SRF*
Pavithra L, *SRF*
Surajit Sinha, *SRF*
Sreenath K, *SRF*
Sunil K Malonia, *SRF*
Sandeep Singh, *SRF*
Sulabh Kharbanda, *SRF*
Yogendra Risbud, *SRF*
Kiran K Nakka, *JRF*
Devraj Mogare, *Technician*

Collaborators
Subeer Majumdar, NII, Delhi
Siddhartha Roy, IICB, Kolkata
H.K. Prasad, AIIMS, Delhi
U.D. Gupta, NJIL & OMD, Agra
Olivier Cases, INSERM, France
Debashis Mitra, NCCS, Pune

Background

The tumor suppressor function of MAR binding protein SMAR1 mediated by p53 regulation and Cyclin D1 downregulation has been well documented in several studies from our lab. Consistent with the tumor suppressor role, we have recently reported that a SMAR1 derived p44 peptide can act as a strong cancer therapeutic. Moreover, expression of this protein relies on the expression of p53, establishing an elegant feed back loop that modulates both TGF-β and NFκB signaling pathways involved in metastasis and invasiveness of cancers. In this context, our aim is to identify a mechanism responsible for downregulation of SMAR1 in higher grades of breast cancer and its role in fine tuning the transcription of NFκB target genes. Considering that SMAR1 was identified in the double positive (DP) stage of thymocyte differentiation and that transgenic mice of SMAR1 display a defective T cell V(D)J recombination, it is clear that this protein plays a major role in governing T cell development. The implication of a role in T cell development in the context of immunomodulatory functions would therefore present an interesting case of study and help understanding the role of such proteins in disease manifestations.

Aims and Objectives

1. Stabilization of SMAR1 mRNA by PGA2
2. Regulation of NFκB mediated transcription by SMAR1
3. T helper cell differentiation by SMAR1

Work Achieved

Stabilization of SMAR1 mRNA by PGA2

Prostaglandins are anticancer agents known to inhibit tumor cell proliferation both *in vitro* and *in vivo* by affecting the mRNA stability. We have shown that a MAR binding protein SMAR1 is a target of PGA2 induced growth arrest. We identified a regulatory mechanism leading to stabilization of SMAR1 transcript. Our results show that a minor stem and loop structure present in the 5' UTR of SMAR1 (ϕ1-UTR) is critical for nucleoprotein complex formation that leads to SMAR1 stabilization in response to PGA2. This results in the induction of SMAR1 that in turn represses Cyclin D1 gene expression and governs G1/S phase arrest. We also provide evidence for the presence of a variant 5' UTR SMAR1 (ϕ17-UTR) in breast cancer derived cell lines. This form lacks the minor stem and loop structure required for mRNA stabilization in response to PGA2 treatment. As a consequence of this, there is low levels of endogenous tumor suppressor protein SMAR1 in breast cancer derived cell lines (Fig. 1). Our studies provide a mechanistic insight into the regulation of tumor suppressor protein SMAR1 by a cancer therapeutic PGA2, that leads to repression of Cyclin D1 gene.

Regulation of NFκB transactivation by SMAR1

The pleiotropic roles of NFκB transcription factors under various stimuli make it an important target for several therapies. It is well known that this protein is inhibited by various chemotherapeutic drugs. Recently, the induction of

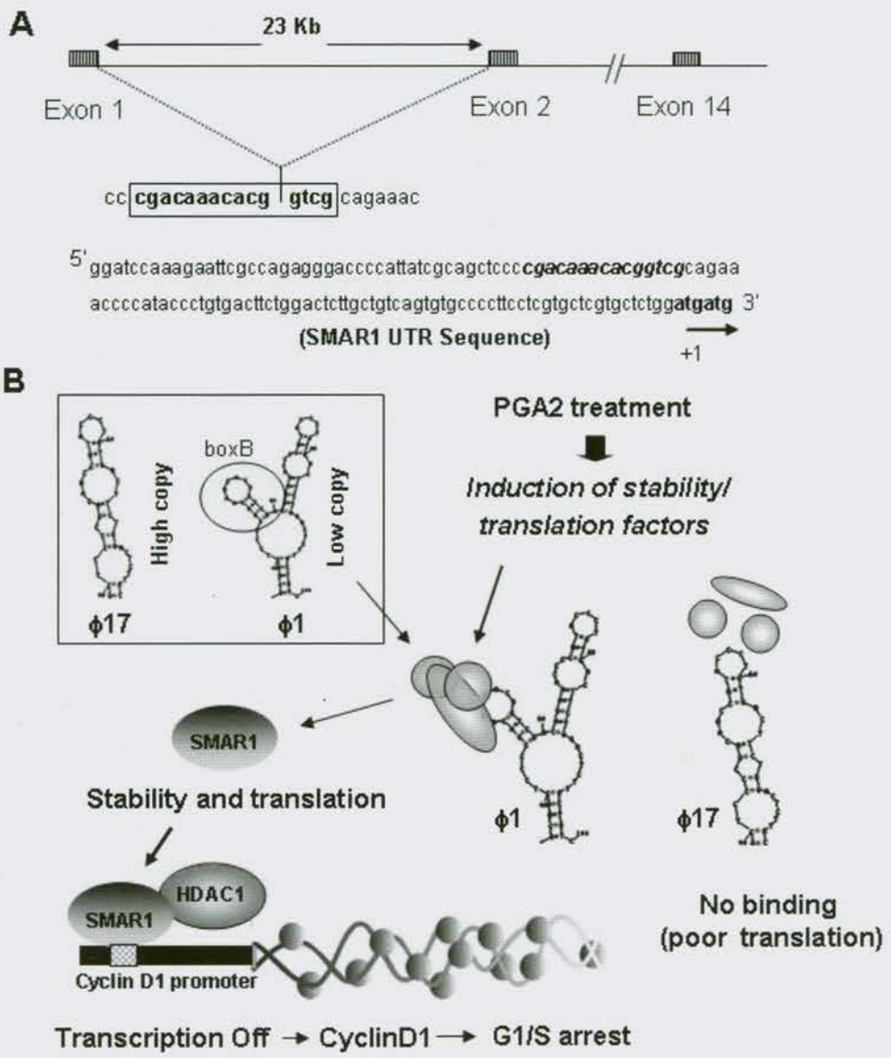


Fig. 1: Diagrammatic representation of SMAR1 regulation by PGA2. (A) The genomic organization of SMAR1 shows that the untranslated exon 1 and a part of exon 2 (23 kb apart) forms the intact 5' UTR of SMAR1. The junction of these exons is marked by the stem and loop structure of 5' UTR. The intact 5'UTR sequence of SMAR1. (B) In MCF-7 cells, ϕ17, a variant 5'UTR of SMAR1 is present in higher amount compared to the ϕ1 form. Factors induced by PGA2 bind to the stem and loop structure of ϕ1, stabilizes the mRNA and facilitates translation of SMAR1. The translated SMAR1 then recruits HDAC1 co-repressor complex to Cyclin D1 promoter, represses transcription and hence causes G1/S phase arrest. ϕ17 form lacks this stem-loop and cannot be translated efficiently.

SMAR1 by Doxorubicin, a DNA damage inducing anticancer drug is established. It is also known to activate NFκB through atypical pathway. This prompted the investigation of SMAR1 in regulating NFκB transcriptional activity. Doxorubicin treatment in breast cancer cells resulted in the induction of SMAR1 protein that in turn lead to the translocation and accumulation of p65/p50 heterodimer and increased DNA binding. IκBα gene promoter was used as a model to evaluate the direct binding of SMAR1 to the MAR region in the promoter, that lead to the recruitment of HDAC1 dependent repressor complex at the locus (Fig. 1). Tumor suppressors such as p53, ARF, ING4 are been shown to modulate NFκB transcriptional activity through different mechanisms (Fig. 2). We observed that SMAR1 can inhibit NFκB transactivation of several genes involved in tumor metastasis, migration and angiogenesis. We hypothesize that gradual loss of SMAR1 expression in high-grade breast cancer consequently enhances the tumor promoting functions of NFκB. Thus, restoration of SMAR1 function is essential for regulating tumor formation and metastasis.

SMAR1 in T helper (Th) cell differentiation

The development of an appropriate subset of T helper cells is critical for determining the outcome of an immune response to various pathogens. Numerous genes have been identified by many research groups that are differentially regulated in TH1/TH2 effector cells and would potentially explain their distinct functions in immunological disorders. Until recently, expression of the canonical TH1 transcription factor, T-bet, encoded by *Tbx21*, was thought to be necessary and sufficient to drive $CD4^+$ TH1 differentiation. However, recent studies suggest that the first 24-48 h of T helper cell differentiation represent a decisive window during which Notch activation is essential for the adoption of a TH1 cell fate by $CD4^+$ cells. Here, our studies using SMAR1 transgenic mice have indicated the existence of an abnormal T helper cell differentiation as demonstrated through a reduced expression of T-bet, IFN-γ and IgG2a, when compared to its wild type littermate. Further we now demonstrate the existence of a fine-tune level of control regulating the expression and localization of SMAR1

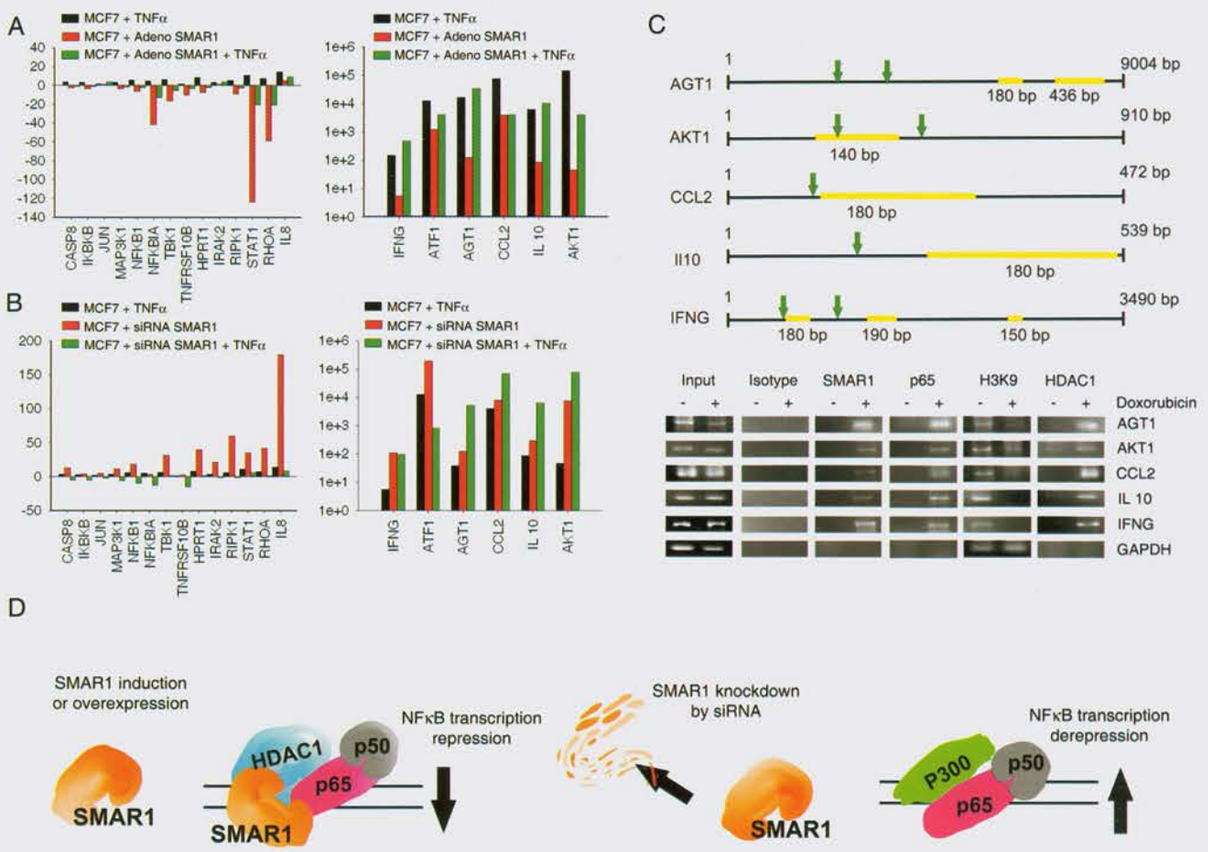


Fig. 2: SMAR1 specifically repress NFκB-mediated transcription. *A*, Real Time PCR array for NFκB target genes in MCF7 cells treated with TNFα (10 ng/ml) with or without transducing recombinant SMAR1 adenovirus. *B*, Real Time PCR array for NFκB target genes in MCF7 cells treated with TNFα (10 ng/ml) with or without siRNA SMAR1 treatment (100 nM). Fold change in transcript levels were compared with control MCF7 cells. *C upper panel*, Schematic representation of promoter region of NFκB target genes. Yellow box indicate the MAR region and green block arrows show NFκB binding sites. *C lower panel*, ChIP assays showing the recruitment of SMAR1, p65 and HDAC1 along with H3K9 status on respective promoters upon Doxorubicin treatment in MCF7 cells. GAPDH promoter is shown as negative control. *D*, Diagrammatic model showing repression of NFκB-mediated transcription upon SMAR1 expression or induction (left panel) and derepression upon SMAR1 siRNA treatment (right panel).

and Notch1 during TH differentiation. We also show that SMAR1-HDAC1 complex is recruited onto Tbx21 promoter forming a corepressor complex with CSL-SMRT and in turn switching off the T-bet transcription under TH2-inducing conditions. Our studies also suggest that GATA-3 mediated activation of SMAR1 may confer $CD4^+$ T cell-intrinsic alterations, including abrogation of Tbx21 induction, which can effectively be used as a protective mechanism to attenuate biological responses characteristic of auto reactive T lymphocytes.

Future Work

1. Identification of post translational modifications that act as switch in regulation of SMAR1
2. Regulation of p53 transcriptional activity by SMAR1
3. Elucidating the structure of various domains of SMAR1 and their role in the protein function

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

Sanjeev Galande
sanjeev@nccs.res.in

Participants
Pavan Kumar P., *SRF*
Prabhat Kumar Purbey, *SRF*
Sameet Mehta, *SRF*
Amita Limaye, *SRF*
Dimple Notani, *SRF*
Ranveer Jayani, *JRF*
Praveena R. L., *JRF*
Kamal G., *JRF*
Sunita Singh, *JRF*
Nitin Sonawane, *Technician*

Collaborators
Dr. Oliver Bischof, Institute Pasteur, Paris, France
Dr. Anne Dejean, Institute Pasteur, Paris, France

Background

Chromatin architecture plays an important role in the regulation of nuclear function. Special AT-rich sequence binding protein 1 (SATB1) participates in the maintenance of chromatin architecture by organizing it into distinct loops via periodic anchoring of matrix attachment regions (MARs) to the nuclear matrix. In thymocyte nuclei SATB1 forms a characteristic 'cage-like' network that demarcates heterochromatin from euchromatin. Furthermore, SATB1 functionally interacts with chromatin modifiers to suppress gene expression through histone deacetylation and nucleosome remodeling at SATB1-bound MARs. While interaction between SATB1 and partner proteins is generally mediated by its N-terminal PDZ-like domain which is also important for SATB1 homodimerization, its MAR-binding- (MD) and homeo- (HD) domains are indispensable for recognition of MARs.

In this study, we set out to define more precisely the role of SATB1 in global gene regulation by identifying its partners and found its interaction with the promyelocytic leukemia (PML) protein. PML is the single-most important constituent of the PML nuclear body (NB) and exists as seven isoforms. PML is a member of a protein family characterized by a RBCC motif consisting of RING finger, B-box, and coiled-coil (CC) domains. It is subject to post-translational modification by small ubiquitin-related modifier (SUMO), and sumoylation of PML was shown to be required for proper formation of the NBs and recruitment of NB-associated proteins. PML NBs were shown to associate with the gene-rich region of the major histocompatibility complex I (MHC-I) on chromosome 6p21-22 and in general, with transcriptionally active loci. Plethora of proteins co-localizing with PML NBs may be good candidates for mediating its attachment with chromatin. However, the dynamic nature and subnuclear localization pattern of PML NBs does not reflect the DNA-binding specificities of any of the proteins it associates with. Thus, the precise mechanism of how PML and the respective NBs associate with chromosomal loci and its implication in transcriptional regulation are far from being clear.

Using modified in vivo chromatin conformation capture (3C) methodology combined with chromatin immunoprecipitation (ChIP), we demonstrate that PML and SATB1 act in unison to organize the MHC-I locus into a distinct higher-order chromatin loop structure by tethering MARs to the nuclear matrix. Interferon γ (IFNγ) treatment as well as RNA interference (RNAi)-mediated knock-down of SATB1 and PML alter higher-order chromatin structure by modulating the physico-functional association between SATB1, PML and MARs, which alters expression

of a subset of MHC-I genes. Our studies support a role for PML-SATB1 complex in governing global gene expression by establishing distinct chromatin loop architecture.

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

SATB1 and PML functionally interact at MARs

In electrophoretic mobility shift assay (EMSA) using radiolabeled IgH MAR probe only SATB1 formed a complex (Fig. 1a, lane 2) but PML failed to do so (lane 5), which is in accordance with the known strong affinity of SATB1 for MARs and the lack of PML's DNA-binding activity. In presence of both proteins we observed a supershift of the original SATB1-DNA complex (lane 4) suggesting that SATB1, PML and MARs form a trimeric complex. Moreover, SATB1-MAR complex appears better defined in presence of PML suggesting that PML may enhance the binding of SATB1 to MARs. EMSA using nuclear extract from Jurkat T cells produced a diffused type of complex (Fig. 1b, lane 2), part of which was supershifted in presence of anti-PML to the well of the gel (lane 3). A monoclonal antibody raised against a region between MD and HD led to formation of a distinct supershifted band (lane 4) while an antibody directed against the MD+HD inhibited formation of the complex itself as evidenced by reduction in the intensity of the complex (lane 5). Interestingly, addition of exogenous PML-I to the nuclear extract shifted the probe in a similar manner as adding anti-PML antibody (lane 6) suggesting that a macromolecular complex has been formed, thus, further emphasizing the role of PML in formation of larger SATB1-MAR complex(es).

To lend further support to a possible functional interaction between SATB1 and PML, we performed MAR-linked luciferase reporter assays. When expressed individually, SATB1 or PML-I repressed the reporter gene about 3-fold (Fig. 1c, bars 2 and 3). Repression by SATB1 was further enhanced by co-expression of PML-I (bar 4). PML-

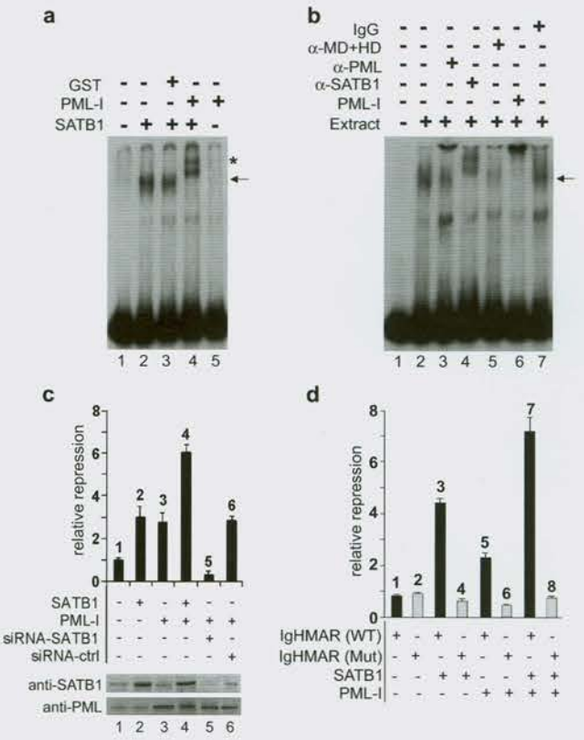


Fig. 1: PML, SATB1 and MARs form a functional complex. (a) EMSA analysis of recombinant SATB1, PML-I, and ^{32}P-end-labeled IgH MAR probe in vitro. The MAR probe was incubated either with 0.1 μg each of SATB1 (lane 2), 0.1 μg of PML-I (lane 5), or 0.1 μg of SATB1 plus 0.1 μg of PML-I (lane 4), with addition of GST serving as negative control (lane 4). DNA-protein complexes were resolved on native polyacrylamide gel and visualized by autoradiography. Arrow depicts the band shift corresponding to SATB1-MAR complex and asterix indicates SATB1-PML-MAR complex. (b) Antibody-mediated supershift of SATB1-PML complex *in vitro*. The MAR probe was incubated with 1.0 μg of Jurkat nuclear extract (lane 2), and additionally with anti-PML (lane 3), anti-SATB1 (lane 4), anti-MD+HD (lane 5), 0.2 μg of PML-I (lane 6), or rabbit IgG (lane 6). DNA-protein complexes were resolved on native polyacrylamide gel and visualized by autoradiography. Arrow depicts the band shift corresponding to SATB1-MAR complex. (c) 293T cells were transiently transfected with the indicated expression constructs or siRNA duplexes together with an IgH MAR-luciferase reporter vector. siRNA-ctrl is a scrambled version of siRNA-SATB1. Luciferase activity is expressed as fold repression. Data from triplicates are plotted and relative luciferase units are represented as fold activity with respect to the reporter alone. The statistical significance of differences between the treatment groups was calculated using 1-way ANOVA and the observed *P* values were always less than 0.001. Shown is also the immunoblot analysis for the expression of SATB1 and PML in the respective transfections. (d) Repression mediated by SATB1 and PML is specific to MARs. 293T cells were transiently transfected with the indicated expression constructs and an IgH MAR-luciferase reporter vector (WT) or its non-MAR mutated version (Mut).

mediated repression was completely obliterated when SATB1 was specifically silenced by RNA-interference (bar 5). Furthermore, the repression mediated both by SATB1 and PML-I was observed only with wild-type MAR and not with the mutated MAR that lacks the base unpairing propensity (Fig. 1d, compare bar 3 with 4 and 5 with 6). The enhanced repression observed with co-expression of PML-I and SATB1 (bar 7) was also not observed when mutated MAR-linked reporter was used (bar 8), highlighting the importance of binding to MARs. Taken together, our results strongly hint

at a role for PML in modulating MAR function via direct interaction with SATB1.

SATB1 and PML are directly associated with the bases of chromatin loops as well as at upstream regulatory sequences of genes within the MHC-I locus

We investigated whether SATB1 and PML could also be detected in the vicinity of transcription units within the MHC-I locus in vivo by performing locus-wide ChIP analysis using primers representing regions at periodic distance within the locus. Anti-SATB1 and -PML ChIPed DNA yielded comparable amplification with certain primer sets suggesting equal distribution of SATB1 and PML in the above mentioned regions of the MHC-I locus (Fig. 2a). Amplifications using primer sets 30F-31R, 16F-17R, 60F-61R, 39F-40R, 54F-55R, 56F-57R, 26F-27R, 41F-42R, 9F-10R, 13F-14R, and 11F-12R showed significant differences between anti-SATB1 and anti-PML immunoprecipitated chromatin indicating that SATB1 is predominantly bound to these regions. Furthermore, primer sets 20F-21R, 37F-38R, 7F-8R and 49F-50R resulted in specific amplifications only with anti-SATB1 but not anti-PML suggesting that SATB1 independently associates with various regions of the MHC-I locus. We failed to observe any region where PML is associated and SATB1 is not, thus, supporting the notion

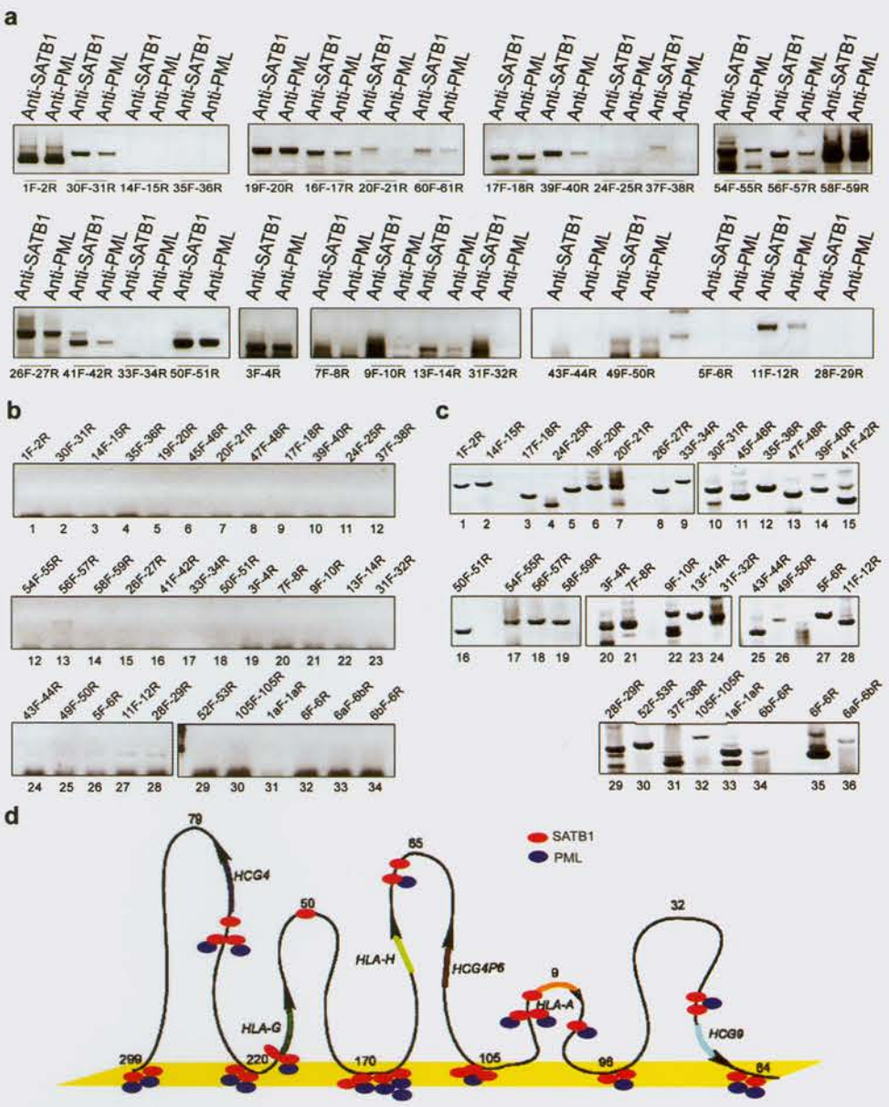


Fig. 2: SATB1 and PML directly associate with specific genomic regions of MHC class I locus in vivo. (a) ChIP assay was performed with Jurkat cells. Applied forward (F) or reverse (R) primer sets are indicated below the panels. Antibodies used for ChIP are anti-SATB1 and anti-PML. Presence of PCR-amplified band indicates occupancy of protein with the corresponding region of MHC-I locus. (b). ChIP assay was performed with rabbit IgG and immunoprecipitated chromatin was amplified with the specific sets of primers as mentioned on top of each panel. (c) DNA purified from input chromatin was used for PCR amplifications using the primer sets as indicated on top of each panel. (d) Schematic representation of occupancy of SATB1 and PML at various locations within the MHC-I locus.

that SATB1 recruits PML to its genomic targets. Moreover, the two proteins were found to bind not only canonical MARs but also upstream regulatory regions of certain genes within the MHC-I locus in a non-random fashion (Fig. 2d). Together, these results imply that SATB1 and PML not merely serve as architectural chromatin components, but also as bone fide transcriptional regulators for certain MHC-I genes.

IFNγ treatment and silencing of SATB1 or PML isoforms alter the expression profile of a distinct set of MHC-I genes

Chromatin (re)organization and transcriptional activity are coupled events and therefore we sought to examine the transcription profile of this locus before and after IFNγ treatment as well as after SATB1 and PML knock-down. IFNγ-treatment resulted in upregulation of most of the MHC-I genes except HCG-9 which was downregulated (Fig. 3a). Moreover, silencing of SATB1 also selectively altered

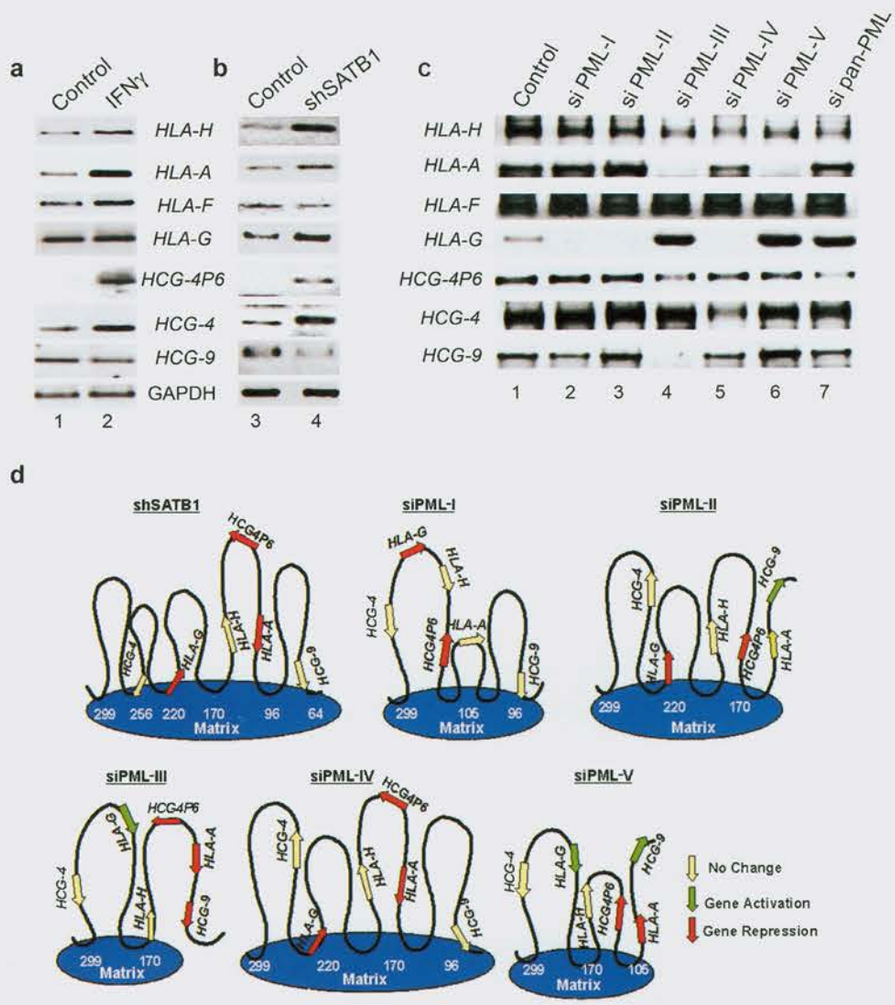


Fig. 3: **PML-SATB1-mediated chromatin-loop architecture regulates transcription of specific MHC class I genes.** (a and b) Silencing of SATB1 mimics the effect of IFNγ treatment. (a) RT-PCR analysis of Jurkat control (lane 1) and IFNγ-treated cells (lane 2) using primers specific for HLA-H, HLA-A, HLA-F, HLA-G, HCG-4P6, HCG-4, HCG-9, and GAPDH. (b) RT-PCR analysis of Jurkat control (lane 3) and pSUPER-shSATB1 transfected cells (lane 4) using primers as in (a). (c) Differential effect of PML isoform-specific in vivo knock-down on the expression of MHC-I genes. RT-PCR analysis of mRNA from Jurkat control cells and cells transfected either with 125 nmoles siPML-I, -II, -III, -IV, -V or pan-PML using gene-specific primers against MHC-I genes as in (a). (d) Differential contribution of PML isoforms I-V and SATB1 towards chromatin loop architecture of the MHC-I locus and the impact on transcription. The alterations of chromatin loop structure were monitored upon specific knock-down of PML nuclear isoforms I-V. The loop structures formed according to the specific nuclear matrix attachments are schematically depicted and the activity status of genes is indicated (color code provided on the far right). Arrows depict individual genes, and arrowheads indicate direction of transcription. Nuclear matrix is depicted in blue oval shape, and numbers at the bases of loops indicate MAR positions in kb.

the transcriptional activity status of the MHC genes in vivo in a manner which mirrored alterations in gene expression obtained after IFNγ treatment (compare Fig. 3a and b). We then studied the influence of each PML isoform on the transcriptional regulation of individual MHC-I genes by performing RT-PCR analysis from cells wherein expression of all or individual PML isoforms were silenced. Expression of HLA-H, HLA-F and HCG-4P6 was only slightly affected after inactivating PML isoforms I-V, either individually (Fig. 3c, lanes 2-6) or altogether (Fig. 3c, lane 7). In contrast, expression of HCG-9, HLA-A, HCG-4 and HLA-G revealed profound differences upon selective silencing of individual PML isoforms (Fig. 3c, lanes 2-6). Expression of HLA-A was abrogated when PML-III or PML-V were knocked down and HCG-4 expression was diminished when PML-IV was silenced. The most dramatic changes were evident in the expression profiles of HLA-G upon PML knock-down in an isoform-dependent manner. HLA-G expression was up-regulated with respect to control when PML-III and PML-V were silenced, whereas the same was completely repressed when PML isoforms -I, -II, and -IV were inactivated. Thus, these results suggest that individual PML isoforms contribute in diverse ways towards the loop domain organization and regulation of MHC-I expression (summarized schematically in Fig. 3d).

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 (2006-2007)

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 providing husbandry, veterinary care and research technical support and 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 and mutant mice-32 lines)

RATS:
WISTAR
LEWIS

RABBITS: NEWZEALAND WHITE

MASTOMYS: MASTOMYS COUCHA

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 7 different gene knock-out/transgenic mice lines to the existing ones, taking the total now to 32 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 *42 (forty two)*. 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 animals bred in the facility and supplied for various R & D activities are given below.

1-4-2006 to 31-03-2007

Sr. No.	Strains/Species	Animals Bred	Animals Supplied
1.	**MICE**		
	BALB/c	7523	6569
	C57BL/6	1888	970
	SWISS#	850	405
	DBA/2	397	7
	DBA/1	79	20
	Nude (nu/nu)	116	236
	BALB/c*	303	34
	FVB/NJ	807	709
	NOD/LtJ	197	50
	Mutant Mice	5725	982
2.	**RATS**		
	WISTAR	373	323
	LEWIS	162	8
3.	**MASTOMYS**	33	----
4.	**RABBIT(NZW)**	14	9

* BALB/c with cataract mutation.
# Outbred

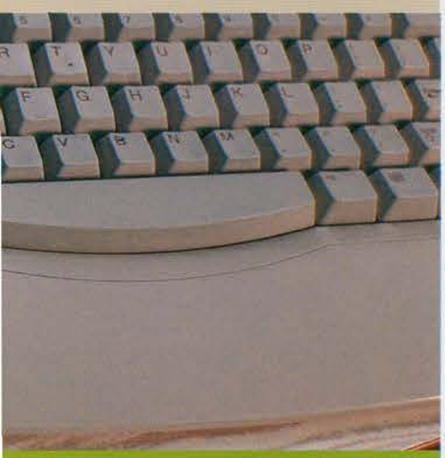
The Team
Rameshwar Nema
Aparna Panse
Madhukar Randive

Library

NCCS Library has a collection of books and journals in the frontier areas of biotechnology having relevance to NCCS research activities. The Library holds approximately seven thousand two hundred bound journals, two thousand seventy books, and subscribe to sixty five scientific journals and twenty five other periodicals. The Library information and important scientific links are available on the library Webpage. Currently, the library is equipped with Linux based SLIM++ LX library software for library housekeeping operations and Web-OPAC for online searching of the library documents. During the reporting period, the library has installed a barcode technology for quick and accurate circulation (Issue & Return) and inventory management of the library documents.

In the development of its collections, the Library's priority is to support NCCS research activities. The Library collection is expanded in consultation with the NCCS scientists. The Library's print collections are growing by approximately seven hundred and fifty volumes per year. During the period of 2006 -2007, the Library has added sixty five books and seven hundred and twenty six volumes of journals to its collection. In addition to journals in print, the library also provides online access to a few key journals and links to these and to all the open access journals have been provided on the library webpage.

Additional documentation facilities include local area network for library activities, PubMed database access and a number of CD-ROM database including, full text and factual database. The Library continues to be a part of the Pune Library Network and Medical Library Association of India.



The Team
R.J. Solanki
S.S. Jadhav
R.C. Patwardhan
K.S. Jadhav

Computer Section

- **Installation and configuration of 4 new Rack mounted servers**
  Four new high end rack mounted servers have been procured and their configuration is in progress. These will be deployed as web/Mail/DNS servers and intranet server for directory services

- **Installation of Wireless Network in Accounts and Board Room**
  A Wireless access point was installed in each department with proper security features configured so as to provide wireless Internet access and local file sharing and printing facilities.

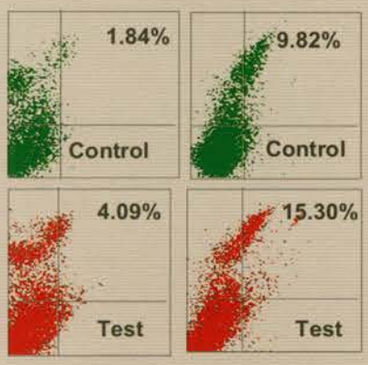
- **Administration and management of existing Servers (mail/Web/DNS Servers)**
  WEB/Mail/DNS server administration and management, which involves user addition/deletion, checking logs, hard disk space management and taking regular backups.

- **Administration, maintenance and updating of NCCS website**
  Routine updating of NCCS website for new contents like Project post advertisement, Project Training/Summer training, Tenders, individual scientists web pages, etc.

- **PC & LAN Management, Maintenance and UpgradationComputer**
  Centre is providing technical support to more than 100 computers and 45 printers connected on LAN which includes installing Operating System, Softwares and Drivers, Anti-Virus package, Installation and configuration of new computers, CDROM/RAM/Network card installation and connecting to LAN, Server operating system upgradation and maintenance.

- **Technical design of upgradation of existing NCCS network to fibre optic network**
  The computer section has prepared a layout and compiled technical specifications for installing a centrally switched Fibre Optic network in the Institute. This network will incorporate centralized network management, monitoring and access control using Single Sign On.

- **Computer Support Services provided**
  To increase the quality of scientific presentation of NCCS staff and students attending national and international conferences/seminars, computer centre is helping in DTP work, CD writing, scanning images and transparency printing on color LaserJet printer.



The Team
Hemangini Shikhare
Swapnil Walke

FACS Facility

We have 4 machines under the facility. All are from Becton Dickinson.

1. FACS Vantage
This is a twelve year old equipment and is extensively used. It has 2 water cooled lasers (488 and UV). It is an analyzer cum sorter. Samples are acquired for analysis on four days in a week and for sorting once in a week.

2. FACS ARIA
We have a FACS Aria high speed cell sorter with UV laser custom made. This was purchased under the DBT funded project "Harnessing the potential of multipotent stem cells". On this machine samples are sorted four days in a week and acquired for analysis once in a week. It has four air cooled solid state lasers. (488, 532, 632 and UV). This equipment is also an analyzer cum sorter.

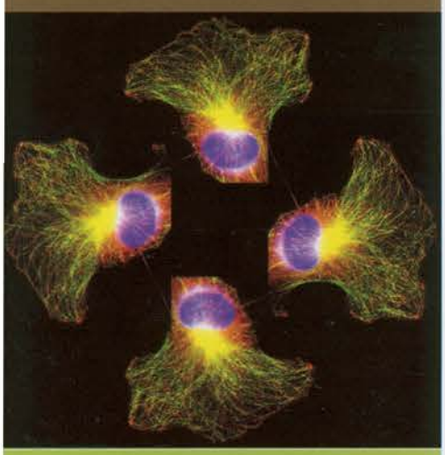
Summary of samples acquired in the period under consideration are shown in the table. We have extended our facility to outsiders like Agharkar research Institute, Pune, Biochemistry Department Pune University and ACTREC, New Mumbai.

3. FACS Canto II
Recently procured and installed and is being made functional. This is a dedicated analyzer.

4. FACS Calibur
Recently procured and installed and is being made functional. This is also a dedicated analyzer.

Yearly Report April 2006 To March 2007

Equipment	Surface Labelling	DNA Cell Cycle	Sorting		Total
FACS Vantage	11,534	1,236	21		**12791**
FACS Aria	794	58	UV Laser 24	Blue, Red Laser 96	**972**



NCCS Facilities

Moflo FACS Facility

A Moflo Flow Cytometer (3 lasers, 9 colours, 11 parameters) has been procured and installed. This is going to be used for high speed sorting and analysis of samples.

Confocal Facility

The confocal facility at NCCS has analyzed about 5,300 samples during the year 2006-2007 and about 85 samples belonging to outside organizations.

Time-Lapse Microscopy - Live Cell Imaging

A Time Lapse Microscope for Live Cell Imaging was procured and established in 2006-2007. During this period about 75 samples were analysed.

Total Internal Reflection Fluorscence (TIRF) microscope

A TIRF microscope has been procured and installed. This has been used for studying surface protein dynamics at single molecule resolution. About 70 samples have been processed since installation.

DNA Sequencer

During the year 2006-2007, 1,00,000 samples were processed on the DNA Sequencer. Out of these 45,000 were related to EST analysis of Anopheles stephensi, 45,000 were related to microbial diversity studies and 10,000 were from the other scientists of NCCS.

Publications and Awards

Publications and Patents

Publications

1. Kumar PP, Mehta S, Purbey PK, Notani D, Jayani RS, Purohit HJ, Raje DV, Ravi DS, Bhonde RR, Mitra D, Galande S. SATB1-Binding Sequences and Alu-Like Motifs Define a Unique Chromatin Context in the Vicinity of Human Immunodeficiency Virus Type 1 Integration Sites. **J. Virol.** 2007; 81: 5617-5627.
2. Ozarkara DA, Prakash D, Deobagkara DN and Deobagkara DD**,** Prediction of B Cell and T Cell Epitopes of DBL*α* Domain in *Plasmodium falciparum* Malaria Vaccine Candidate Var Gene. **Protein and Peptide Letters,** 2007; 14: 528-530**.**
3. Shiras A, Chettiar ST, Shepal V, Rajendran G, Prasad GR, and Shastry P. Spontaneous transformation of human adult nontumorigenic stem cells to cancer stem cells is driven by genomic instability in a human model of glioblastoma. **Stem Cells.** 2007; 25: 1478-89.
4. Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Wani AA, Borgave SB and Sarnaik SS. Cultivable bacterial diversity of alkaline Lonar lake, India. **Microbial Ecol.** (in press)
5. Anand TP, Bhat AW, Shouche YS, Roy U, Siddharth J, and Sarma SP. Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India. **Microbiol. Res**. 2006; 161: 252-262.
6. Rangrez A, Dayananda KM, Atanur S, Joshi R, Patole MS, and Shouche YS. Detection of Conjugation Related Type Four Secretion Machinery in Aeromonas culicicola. **PLoS ONE** (in press)
7. Balasubramaniyan V, Murugaiyan G, Shukla R, Bhonde RR, and Nalini N. Leptin downregulates ethanol-induced secretion of proinflammatory cytokines andgrowth factor. **Cytokine.** 2007; 37: 96-100.
8. Balasubramaniyan V, Shukla R, Murugaiyan G, Bhonde RR, and Nalini N. Mouse recombinant leptin protects human hepatoma HepG2 against apoptosis,TNF-alpha response and oxidative stress induced by the hepatotoxin-ethanol. **Biochim. Biophys. Acta (General Subjects)** (in press)
9. Balch C, Nephew KP, Huang T H-M and Bapat SA. Epigenetic "Bivalently Marked" Process Of Cancer Stem Cell-Driven Tumorigenesis. **BioEssays** (in press)
10. Shravage BV, Dayananda KM, Patole MS and Shouche YS. Uncultured SSU rDNA clone library approach reveals high eubacterial diversity in soil sample from Cape Evans, McMurdo Dry Valley, 2006. Antarctica. **Microbiol. Res** (in press)

11. Chakraborty G, Jain S, Behera R, Ahmed M, Sharma P, Kumar V and Kundu GC. The multifaceted roles of Osteopontin in cell signaling, tumor progression and angiogenesis. **Curr. Mol. Med.** 2006; 6: 819-830.
12. Chhipa, R.R. and Bhat, M.K.: Bystander killing of breast cancer MCF-7 cells by MDA-MB-231 cells exposed to 5-fluorouracil is mediated via Fas. **J. of Cell. Biochem.** 2007; 101: 68-79.
13. Duarte J, Prakash D, Guiyedi V, Mécheri S, Fesel C, Cazenave P-A, Mishra GC, Kombila M and Pied S. Total and functional parasite specific IgE responses in Plasmodium falciparum-infected patients exhibiting different clinical status. **Malaria J.** 2007, 6: 1.
14. Gower DJ, Dharne M, Bhatta G, Giri V, Vyas R, Govindappa V, Oommen OV, George J, Shouche YS, and Wilkinson M. Remarkable genetic homogeneity in unstriped, long-tailed Ichthyophis along 1500 km of the Western Ghats, India. **J. Zool.** (in press)
15. Purbey PK, Jayakumar CP, Patole MS, and Galande S. pC6-2/Caspase 6 system to purify GST-free recombinant fusion proteins expressed in E. coli. **Nature Protocols** 2006; 1: 1820-1827.
16. Datar SP, Suryavanshi DS, Bhonde RR. Chick pancreatic β-islets as an alternative in vitro model for screening insulin secretagogues. **Poultry Sci.** 2006; 85: 2260-2264.
17. Dharne M, Patole M, Shouche YS**.** Microbiology of the insect gut: tales from mosquitoes and bees. **J. Biosci**. 2006; 31: 293-295.
18. Galande S. Purbey PK, Notani D, and Kumar PP. The third dimension of gene regulation: Organization of dynamic chromatin loopscape by SATB1. 2007; **Curr. Opinion Genet. Dev.** (in press)
19. Gurdeep Rastogi, Mahesh S. Dharne, Ashutosh Kumar, Sandeep Walujkar , Milind S. Patole, Yogesh S. Shouche Species identification and authentication of tissues of animal origin using mitochondrial and nuclear markers. **Meat Sci.** (in press)
20. Rastogi G, Ranade DR, Yeole TY, Gupta AK, Patole MS and Shouche YS. Novel methanotroph diversity evidenced by molecular characterization of particulate methane monooxygenase A (pmoA) genes in biogas reactor. **Microbiology Res.** (in press)
21. Hardikar AA, Lees JG, Sidhu KS, Colvin E, Tuch BE. Stem Cell Therapy for Diabetes Cure: How Close Are We? **Curr. Stem Cell Res. Therapy** 2006; 1: 425-436.
22. Jain S, Chakraborty G, Bulbule A, Kaur R and Kundu GC: Osteopontin: an emerging therapeutic target for anticancer therapy. **Expert Opin. Ther. Targets** 2007; 11: 81-90.
23. Jalota-Badhwar, A, Kaul-Ghanekar, R, Mogare, D, Boppana, R, Packnikar, KM and Chattopadhyay, S. SMAR1-derived P44 peptide retains tumor suppressor function through modulation of p53. **J. Biol. Chem.** 2007; 282(13): 9902-9913.
24. Ozarkara AD, Prakash D, Deobagkara DN and Deobagkara DD. Distinct polymorphism of DBLα domain of the var gene in lab cultured Plasmodium falciparum malaria parasites and clinical field isolates. **Curr. Sci.** 2007; 93: 1-5.
25. Kulkarni MJ, Vinod VP, Umasankar PK, Patole MS, Rao M. Intact cell matrix-assisted laser desorption/ionization mass spectrometry as a tool to screen drugs in vivo for regulation of protein expression. **Rapid Commun. Mass Spectrometry**. 2006; 20: 2769-2772.
26. Kumar PS, Shiras A, Das G, Jagtap JC, Prasad V, and Shastry P. Differential expression and role of p21cip/waf1 and p27kip1 in TNF-alpha-induced inhibition of proliferation in human glioma cells. **Mol. Cancer** 2007; 12; 6:42.
27. Kumar PS, Shiras A, Das G, Jagtap JC, Prasad V, and Shastry P. Independent activation of Akt and NF-kappaB pathways and their role in resistance to TNF-alpha mediated cytotoxicity in gliomas. **Mol. Carcinogenesis** (in press)
28. Dharne MS, Misra SP, Misra V, Dwivedi M, Patole MS and Shouche SS. Isolation of urease positive Ochrobactrum intermedium in the stomach of Non-ulcer dyspeptic patient from north India. **J. Microbiol. Immunol. Infection** (in press)
29. Modak MA, Datar SP, Bhonde RR, and Ghaskadbi SS. Differential susceptibility of chick and mouse islets to streptozotocin and its co-relation with islet antioxidant status. **J. Comp. Physiol [B].** 2007; 177(2): 247-257.
30. Murugaiyan G, Agrawal R, Mishra GC, Mitra D, and Saha B. Differential CD40/CD40L expression results in counteracting antitumor immune responses. **J. Immunol.** 2007; 178: 2047-2055.
31. Murugavel P, Pari L, Sitasawad SL, Kumar S, Kumar S. Cadmium induced mitochondrial injury and apoptosis in vero cells: protective effect of diallyl tetrasufide from garlic**. Int. J. Biochem. Cell Biol.** 2007; 39: 161-170.
32. Parag A.Vaishampayan, Rakeshkumar P. Gupta, Pritesh Lalwani , Hemant Ghate, Milind S Patole and Yogesh S. Shouche Molecular evidence and phylogenetic affiliations of Wolbachia infecting Cockroaches. **Mol. Phylogenet. and Evol.** (in press)
33. Parameswaran V, Ishaq Ahmed VP, Shukla R, Bhonde RR, Sahul Hameed AS. Development and Characterization of Two New Cell Lines from Milkfish (Chanos chanos) and Grouper (Epinephelus coioides) for Virus Isolation. **Marine Biotechnol (NY).** 2007; 9: 281-291.

34. Parameswaran V, Shukla R, Bhonde R, Hameed AS. Establishment of embryonic cell line from sea bass (Lates calcarifer) for virus isolation. **J. Virol. Methods.** 2006; 137: 309-316.
35. Pari L, Murugavel P, Sitasawad SL, Kumar KS. Cytoprotective and antioxidant role of diallyl tetrasulfide on cadmium induced renal injury: an in vivo and in vitro study. **Life Sci.** 2007; 80: 650-658.
36. Phadnis SM, Joglekar MV, Venkateshan V, Ghaskadbi SM, Hardikar AA, and Bhonde RR. Human umbilical cord blood serum promotes growth, proliferation, as well as differentiation of human bone marrow-derived progenitor cells. **In Vitro Cell Dev. Biol. Anim.** 2006; 42: 283-286.
37. Muralidharan B, Bakthavachalu B, Pathak A and Seshadri V. A minimal element in 5'UTR of insulin mRNA mediates its translational regulation by glucose. **FEBS Lett.** (in press)
38. Pandey PK, Dhotre DP, Dharne MS, Khadse AN, Hiremath UI, Chaudhari RD, Patole MS and Shouche YS. Conservation and Evolutionary Genetics of Indian Leopard, Panthera pardus fusca as studied by mitochondrial 12S rRNA gene sequence analysis. **Curr. Sci.** (in press).
39. Joshi AA, Kanekar PP, Kelkar AS, Sarnaik SS, Shouche YS and Wani AA. Moderately halophilic, alkalitolerant Halomonas campisalis MCM B-365 from Lonar lake, India. **J. Basic Microbiol.** 2007; 47(3): 213-221.
40. Raut AD, Shashidhar R, Vaishampain P, Shouche YS, Bandekar JR and Kapadnis BP. Non specific PCR amplification of the 16S rRNA gene segment in different bacteria using primers specific for Campylobacter, Arcobacter and Helicobacter **J. Clin. Microbiol** 2007; 45: 1376-7.
41. Ravi DS and Mitra D. HIV-1 long terminal repeat promoter regulated dual reporter: potential use in screening of transcription modulators. **Anal. Biochem.** 2007; 360: 315-317.
42. Rundle NT, Nelson J, Flory MR, Joseph J, Th'ng J, Aebersold R, Dasso M, Andersen RJ and Roberge M. An ent-Kaurene that inhibits mitotic chromosome movement and binds the kinetochore protein Ran-binding protein 2. **ACS Chem. Biol.** 2007; 1: 443–450.
43. Singh S, Upadhyay AK, Ajay AK and Bhat MK. p53 regulates ERK activation in carboplatin induced apoptosis in cervical carcinoma: A novel target of p53 in apoptosis. **FEBS Lett.** 2007; 581: 289–295.
44. Kumar H, Patole MS and Shouche YS. Fungal Farming: A story of four partner evolution. **Curr. Sci.** 2006; 90: 1463-1464.
45. Thacker U, Parikh R, Shouche Y and Madamwar D. Hexavalent chromium reduction by Providentia sp. **Process Biochem**. 2006; 41: 1332-1337.
46. Savita D and Bhonde RR. A simple technique for isolation of functional β-islets from chick pancreas. **J. Cell Tissue Res.** 2006; 6: 605-608.
47. Thacker U, Parikh R, Shouche YS and Madamwar D. Reduction of chromate by cell-free extract of Brucella sp. isolated from Cr(VI)contaminated sites. **Bioresour. Technol.** 2007; 98: 1541-1547.
48. Misra V, Misra SP, Dwivedi M, Shouche YS, Dharne M and Singh PA. Helicobacter pylori in areas of gastric metaplasia in gall bladder and isolation of H. pylori DNA from gallstones. **Pathology** (in press)
49. Surakasi VP, Wani AA, Shouche YS, and Ranade DR. Phylogenetic analysis of methanogenic enrichment cultures obtained from Lonar Lake in India: Isolation of Methanocalculus and Methanoculleus sp. **Microbial Ecol.** (in press)
50. Murugaiyan G, Agrawal R, Mishra GC, Mitra D and Saha B. Functional dichotomy in CD40 reciprocally regulates effector T cell functions. **J. Immunol.** 2006; 177: 6642-6649.
51. Wani AA, Prasad VS, Siddharth J, Raamesh GR, Patole MS, Ranade DR and Shouche YS. Microbial diversity of Lonar Soda Lake, India: An impact crater in a basalt area **Res. Microbiol.** 2007; 157(10): 928-937.
52. Murugaiyan G, Martin S, and Saha B. Levels of CD40 expression on dendritic cells dictate tumour growth or regression. **Clin. Exp. Immunol.** 2007, 149: 194-202.
53. Singh K, Mogare D, Giridharigopalan RO, Gogiraju R. and Chattopadhyay S. p53 Target Gene SMAR1 Is Dysregulated in Breast Cancer: Its Role in Cancer Cell Migration and Invasion. **PLoS ONE**. 2007; 2: e660.
54. Joglekar MV, Parekh VS and Hardikar AA. Generating New Pancreas from Old: Microregulators of Pancreas Development and Regeneration. **Trends in Endocrinology and Metabolism** (in press)
55. Chhipa RR, Kumari R, Upadhyay AK and Bhat MK. Abrogation of p53 by its antisense in MCF-7 breast carcinoma cells increases cyclin D1 via activation of Akt and promoting cell proliferation. **Exp. Cell Res.** (in press)
56. Wani AA, Ahanger SH, Bapat SA, Rangrez AY, Hingankar N, Suresh CG, Barnabas S, Patole MS, Shouche YS. Analysis of mitochondrial DNA sequences in childhood encephalomyopathies reveals new disease associated variants. **PLoS ONE**. 2007; 2: e942.

Book Chapters/Invited Reviews/ Viewpoints

1. Pavithra, L and Chattopadhyay, S. Chromatin and cancer: Reprogramming chaos in the cell. **Natl. Acad. Sci. Lett**., 2007; 30 (3&4): 71-82.
2. Ahmad, M, Pyaram, K, Mullick, J and Sahu, A. Viral complement regulators: the expert mimicking swindlers. **Ind. J. Biochem. Biophy.** 2007 (in press)
3. Banerjee M and Bhonde RR. Autologous bone marrow transplantation/mobilization: a potential regenerative medicine for systemic degenerative disorders and healthy living. **Med Hypotheses.** 2007; 68(6): 1247-51.
4. Bhonde R, Shukla RC, Kanitkar M, Shukla R, Banerjee M and Datar S. Isolated islets in diabetes research. **Indian J. Med. Res.** 2007; 125(3): 425-440.
5. Lenka N, and Mishra GC. 2007. Stem Cells: The Present Status and the Future Prospects. **J. Cell Tissue Res**. (in press)
6. Shukla R, Padhye S, Modak M, Ghaskadbi SS and Bhonde RR. Bis (quercetinato)oxovanadium IV Reverses Metabolic Changes in Streptozotocin-Induced Diabetic Mice. **Rev. Diabet. Stud.** 2007; 4(1): 33-43.

Patents Filed

Samit Chattopadhyay

"Tumor suppressor Activation polypeptide and uses thereof" Indian patent file number: IPR/4.19.20/06083/2006

Memberships/Awards/ Fellowships

Memberships

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)

Anjali Shiras

- Travel Award from ISSCR for participation in the Stem Cell Meeting – Toronto, Canada
- Travel Award from ISSCR for participation in the Stem Cell Meeting – Cairns, Australia

Ramesh R Bhonde

- Member of the State Level Task Force on Research in Biotechnology, Govt of Maharashtra, Mumbai. 2006-2008
- Member of advisory committee for Advanced Diploma in Animal Biotechnology, Department of Zoology, University of Pune, 2006-2007
- Chairman of CPCSEA for Raj Biotech, Pune
- Treasurer and Secretary, Indian Society for Developmental Biologists. 2005-2007
- Task Force Member of the Scientific Advisory Committee meeting of NCLAS, 2005-2008
- Member of executive committee of All India Society of Cell Biology, 2005-2007
- Associate Editor, The Reviews in Diabetic Studies, 2006-2009.

Sharmila Bapat

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

Fellowships

- DBT Overseas Short-Term Fellowship from November 2006–April 2007

Samit Chattopadhyay

- Fellow of National Academy of Science, Allahabad, 2006
- Best oral presentation in the 33rd Immunology Congress, New Delhi, January 28-31, 2007.

Lalita S. Limaye

Life member –

- Indian Society for Cell Biologists
- Indian Society for Biotechnologists
- Indian Women Scientists Association

Musti Krishnasastry

- DBT overseas short-term associateship work at Graduate School of Frontier Bioscience, Osaka University, Osaka, Japan.

Mohan Wani

Awards –

- DBT Overseas Associateship Award 2005-2006.

Memberships –

- Member of the Executive Committee of Indian Society of Cell Biology for the term April 2007–March 2009.
- 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.

Sanjeev Galande

- Member of American Society for Microbiology (ASM), 2005 onwards
- Received the National Bioscience Career Development Award 2006 from the Department of Biotechnology on 12th March 2007.

Nibedita Lenka

- Indian Academy of Neuroscience: Life Member
- International Society for Stem Cell Research (2005 – continuing).

Yogesh S Shouche

- DBT Overseas Short Term fellowship

Extramural Funding

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

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).
- Identification, Development and Characterization of long term Neural Stem cell-lines from adult brain tissue. DBT (2005-2008).

Ramesh R Bhonde

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

Sharmila Bapat

- Mutational analysis of the transcriptional coactivator CBP in the Rubinstein Taybi Syndrome. DBT (2007-2009).
- Protein profiling of human ovarian tumor stem cells. DBT (2007-2009).

Samit Chattopadhyay

- Altered expression of tumor suppressor SMAR1 in breast cancer causes destabilization of Cyclin D1. DBT (2006-2009).
- Indo-foreign Research project "Spatio-temporal expression of SMAR1 and Cux in the CNS". Indo-French ICMR project, (2006-2009).

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

Padma Shastry

- Differentiation of human Mesenchymal stem cells (MSC) towards neuronal lineages. DBT (2005-2008).
- Gene expression profiles during neuronal differentiation and survival induced by lithium. BRNS (2005-2008).
- TF antigen binding unique lectins from Sclerotium rolfsii and Rhizoctonia bataticola for investigating aberrant glycosylation and changes associated with cancer. DST (2005-2008).

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 K.K. Bhutani
- Dissecting the CD40L-CD40 mediated signaling pathway in HIV infection.Funding Agency: DBT-ICMR (2006–2009).
  Co-Investigator: Bhaskar Saha and Sekhar Chakrabarti

Anandwardhan A. Hardikar

- Differentiation of human fetal pancreatic progenitor cells for potential use in cell replacement therapy for type 1diabetes. DBT (2007-2010).

Musti Krishnasastry

- Qantum Dots: New generation Diagnostic Kits against Malaria and HIV, DBT (2006-2008).

Gopal C Kundu

- Role of osteopontin, a chemokine like protein in regulation of vascular endothelial growth factor dependent tumor growth and angiogenesis in breast cancer. DBT (2006-2009).
- Studies on role of Osteopontin in regulation of transcription factor-mediated matrix metalloproteinase-9 activation, cell motility, tumor growth and metastasis. DBT (2006-2009).

Vasudevan Seshadri

- Dual role of insulin in regulating the expression of IGFBP-1. DBT (2005-2008).

Mohan Wani

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

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 (2004-2007).
- 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).

Yogesh S 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.

Sanjeev Galande

- Chromatin organization and signal transduction by SATB1. International senior research fellowship, Wellcome trust, UK, (2005-2010).
- Role of SATB1 in gene regulation upon HIV infection. DBT (2004-2007).
- Study of novel nuclear factors. DBT (2007-2010).
- SATB1 and T cell apoptosis. DBT Bioscience award (2007-2010).

Vaijayanti P Kale

- Harnessing the potential of multipotent adult stem cells. DBT (2005-2008).
  Co-Investigator: Dr. L.S. Limaye
- 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 (2005-2008).
  Co-Investigator: Dr. A. Surolia and Dr. L.S. Limaye
- Identification of biochemical pathways involved in the Hematopoietic Stem Cell (HSC) regulation. DBT (2005-2008).
- Assessment of Caveolin-1 Mutations as Possible Risk Factors in the Development of Human Breast Carcinoma. DST (2004-2007).

Seminars

Seminars delivered at NCCS by visiting Scientists

Dr. Sebastian Joyce

Immunological Functions of the Invariant Natural T (iNKT) Cells
Professor of Microbiology and Immunology
Vanderbilt University
Tennessee, USA
Date: 14th June, 2006

Dr. Ajay Rana

Mixed Lineage Kinase 3: From a Mixed Character to a Drug Target
Associate Professor of Medicine
Texas A & M University-HSC
Cardiovascular and Cancer Res. Institute
Temple, TX 76504 USA
Date: 26th Feb, 2007

Amitabha Chattopadhyay

Interaction of the Serotonin-1A Receptor with Membrane Lipids: Implications in Receptor Function and Organization:
Center for Cellular and Molecular Biology (CCMB), Hyderabad
Date: 1st June, 2007

Prof. Jagat Kanwar

Cancer therapy and targeting of angiogenesis
Deakin University, Australia
Date: 23rd July, 2007

Seminars delivered by NCCS Scientists

Arvind Sahu

1. "Viral evasion of the host complement system" at Department of Bioengineering, University of California at Riverside, CA, USA, November 9, 2006.
2. "Viral mimicry of human complement regulators" at 33rd Indian Immunology Society Conference, All India Institute of Medical Sciences, January 30, 2007.
3. "Viruses avoid complement" at 15th Molecular Immunology Forum, Indian Institute of Science, Bangalore, March 1, 2007.

Anjali Shiras

.1 "Are multipotent brain tumor stem cells (btscs) involved in glioma progression?, National Centre for Biological Sciences", Bangalore, 11th May, 2007

Ramesh R Bhonde

1. "Animal tissue culture and stem cells, Dept. of Biotechnology", CKT college, Panvel, January 5th, 2007
2. "Stem cells and regenerative medicine, Pune ophthalmologists" conference, B.J Medical College, Pune January 6th , 2007
3. "In vitro models in biomedical research" at the workshop on recent trends in life sciences held at Modern College, Pune on January 9th , 2007
4. "Stem cells and Regenerative biology", Department of Zoology, Goa University, under INSA – UGC programme on Frontier Lectures in Animal Sciences on February 5-6, 2007
5. "Stem Cells" at the National Symposium in Zoology with special reference to Biotechnology and Bioinformatics held at Yashwantrao Chavan Institute of Science, Satara, February 10th, 2007
6. "Animal Tissue Culture" at HPT College, Nasik, February 19-20, 2007
7. "Stem cells: hope or hype?" held at Cuttak Medical College, Bhuvaneshwar, April 7th ,2007
8. "Islet tissue engineering", Indo-Australian stem cell and tissue engineering meeting held at SCTIMST, Thiruvanthapurum January 10-12, 2007
9. "Islet neogenesis from pancreatic and extra pancreatic sources of stem cells", First annual meeting of the SCFRI and International Conference on stem cell research, Bangalore, January 29–February 1, 2007

Samit Chattopadhyay

1. "Repression of Cyclin D1 transcription by PGA2 is mediated through stabilization of SMAR1 RNA", Nuclear Architecture: Chromosome-Chromatin Dynamics, JNCASR, Jakkur, Bangalore, Dec 11-13, 2006.
2. "Global gene regulation by MAR binding protein SMAR1" at Society of Biological Chemists, JNU, New Delhi,. December 9th , 2006
3. "Transcription regulation by chromatin remodeling protein SMAR1". Transcription Assembly Meeting, Vedic Village, Kolkata, December 14 to 16, 2006.
4. "In search of p53 mediated global gene transcription: Role of a modulator protein SMAR1". EMBO Workshop, IIT, Kanpur, December 17-19, 2006.
5. International Symposium on Chemical Biology, 7-9th March, 2007.

Lalita S Limaye

1. "stem cell banking". CME on stem cells organized by Department of transfusion Medicine, AFMC, Pune from 23-24 Dec., 2006.
2. "expert level consultation meeting for establishing HLA Registry facility and cord blood bank in Gandhinagar, Gujrat" on 16th May, 2006.

Debashis Mitra

1. "Marine Organisms as a source of anti-HIV activity". World AIDS DAY 2006 conference at Tianjin, China. December 1-3, 2006.
2. "Identification of a novel HIV-1 reverse transtscriptase inhibitor from a marine bivalve". 75th Annual meeting of Society of Biological Chemists at JNU, New Delhi, December 8-11, 2006.
3. "Role of heat shock proteins in regulation of HIV-1 gene expression and replication". International conference on molecular cellular biology and therapeutics of HIV and associated viral infections held in Hyderabad, India from January 12-14, 2007.
4. "Human Immunodeficiency Virus-1 Tat suppresses gp120 specific T-cell response in IL-10 dependent manner". 33rd Annual Indian Immunology Society meeting, AIIMS, New Delhi, January 28-31, 2007.
5. "Cellular heat shock proteins: pro- or anti-viral host factors?" National Science Day symposium, Indian Immunology-Some Selective Trends, in the Department of Biochemistry, Indian Institute of Science, Bangalore on February 28, 2007.
6. "A novel anti-HIV molecule from a marine bivalve that targets its reverse transcriptase". Workshop on Pharmacoinformatics: Tools for Drug Target Identification, NIPER, Mohali, March 14-16, 2007.

Anandwardhan A Hardikar

1. "Pancreatic Stem Cells for Cell Replacement Therapy in Diabetes", at the Indo-Danish workshop organized by the DBT at National Center for Biological Sciences, Bangalore
2. "Human Fetal Pancreatic Islets Undergo Epithelial to Mesenchymal Transition to Generate an Islet Progenitor Cell Population" Invited inaugural speaker at the Pancreas Stem Cells meeting organized by Danish Stem Cell Society and Summer School, Copenhagen, Denmark June 11-16, 2006.
3. "Role of microRNAs in Pancreas Development and Differentiation": Invited speaker at the Annual Conference of Indian Society of Developmental Biologists (ISDB) and International Symposium on Cellular signaling during development' organized in Pune, India November 23-25 2006.
4. "Human Pancreatic Progenitor Cells for Cell Replacement Therapy in Diabetes": Stem Cell meeting organized at the National Center for Biological Sciences, Bangalore, November 29-30, 2006.

Gopal C Kundu

1. "Osteopontin, a Member of SIBLING Gene Family: It's Role in Cell Signaling,Tumor Progression and Angiogenesis". Dr. Reddy's Research Foundation, Hyderabad. 26th June, 2006
2. "The Multifaceted Roles of Osteopontin, a Member of SIBLING Gene Family in Cell Signaling, Tumor Progression and Angiogenesis". Bioinformatics Centre, University of Pune, Pune.18th September, 2006
3. "Osteopontin, a Candidate of SIBLING Gene Family: It's Multifaceted Roles in Cell Signaling, Tumor Progression and Angiogenesis". NICHD, The National Institutes of Health (NIH), Bethesda, MD, USA. 26th October, 2006.
4. "The Multifaceted Roles of Osteopontin, a Candidate of SIBLING Gene Family in Cell Signaling, Tumor Progression and Angiogenesis". Guru Ghasidas University, Bilaspur. 10th November, 2006.
5. "Osteopontin Signaling Network in Cancer: Redefining the Molecular Targets". Society of Biological Chemists, JNU, New Delhi. 11th December, 2006.
6. "Transcriptional Regulation of Target Genes Involved in Tumor Progression and Angiogenesis in Response to Osteopontin". 10th Transcription Assembly, Vedic Village, Kolkata. 14th December, 2006.
7. "Osteopontin, a Chemokine like SIBLING Family of Protein: It's Functional Role in Regulation of Tumor Growth and Angiogenesis". 17th December, 2006. Structure and Dynamics:From micro to macro, University of Calcutta, Kolkata.
8. "The multifaceted roles of Osteopontin, a member of SIBLING gene family in cell signaling, tumor progression and angiogenesis". CCMB, Hyderabad, 28th December, 2006.
9. "The multifaceted functions of Osteopontin, a member of SIBLING gene family in regulation of cell signaling, tumor progression and angiogenesis". Indian Science Congress Association, Annamalai University. 5th January, 2007.
10. "The Cross-talk between Syk and Lck regulates hypoxia/reoxygenation-induced breast tumor progression and angiogenesis". Society of Free Radical Conference, Fariyas Resort, Lonavala, 10th January, 2007.
11. "Osteopontin, a Candidate of SIBLING Gene Family: It's Multifaceted Roles in Cell Signaling, Tumor Progression and Angiogenesis". 26th IACR Conference, Bhubeneswar, 17th January, 2007.
12. "Osteopontin, a Member of SIBLING Gene Family: It's Role in Cell Signaling, Tumor Progression and Angiogenesis", 7th Indo-US Cytometry Workshop, JNU, New Delhi, 13th February, 2007.
13. "Diagnostic and Therapeutic Significance of Osteopontin, a member of SIBLING Family of Protein in Cancers", SSB Conference, Debi Ahilya Conference, Indore, 8th March, 2007.
14. "Diagnostic and Therapeutic Significance of Osteopontin, a Chemokine like protein in Cancers and other Diseases". Advinus Pharmaceuticals, Pune, 14th March, 2007,.
15. "Therapeutic Significance of Osteopontin, a member of SIBLING Family of Protein in Cancers", University of Sydney, Australia, 20th March, 2007.
16. "Osteopontin, a Candidate of SIBLING Gene Family: It's Multifaceted Roles in Cell Signaling, Tumor Progression and Angiogenesis". Deakin University, Australia, 23rd March, 2007.
17. "Osteopontin, a Candidate of SIBLING Gene Family: It's Multifaceted Roles in Cell Signaling, Tumor Progression and Angiogenesis", NIPER, Chandigarh, 29th March, 2007.

Mohan Wani

1. "Commitment of osteoclast precursors towards macrophage lineage by IL-3 through down-regulation of RANK expression" in First Annual Meeting of SCRFI and International Conference on Stem Cell Research held at Bangalore, January 29-February 1, 2007.
2. "Interleukin-4 regulates bone remodeling" on National Science Day held at Department of Biochemistry, Indian Institute of Science, Bangalore, February 28 2007.

Sanjeev Galande

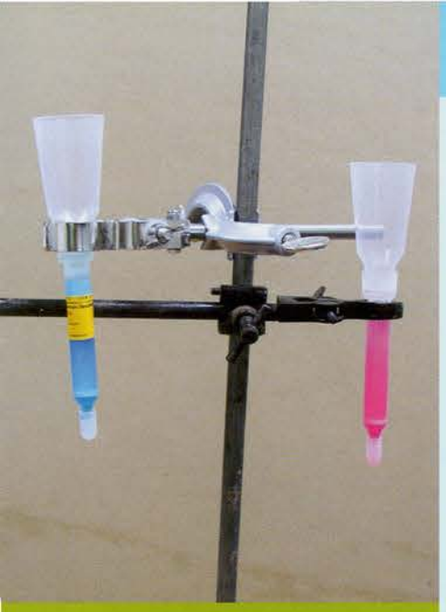
1. "Bridging signal transduction and chromatin architecture: Role of PDZ-like domain of SATB1 in regulation of global gene expression". Invited talk delivered as a part of the Frontiers of Science Seminar series at the University of Turku and Abo Academy of Science, Turku, Finland, September 21, 2006.
2. "The third dimension of gene regulation: Its all in the looping!", Institute Pasteur, Paris, France, October 24, 2006.
3. "Bridging signal transduction and chromatin architecture: Role of PDZ-like domain of SATB1 in regulation of global gene expression", Senior Fellows Meeting, Wellcome Trust, London, UK, October 25, 2006
4. "Systems biology of global regulatory networks: unraveling sequence features in promoters that dictate tissue-specificity of gene expression", 75th meeting of SBC, JNU, New Delhi. December 10, 2006.
5. "Long-range intrachromosomal interactions mediated by SATB1-PML complex regulate gene expression within the MHC class I locus", Nuclear Architecture: Chromosome-Chromatin Dynamics meeting, JNCASR, Bangalore, December 11, 2006.
6. "Mechanisms of regulation of global gene expression by SATB1", Tenth transcription assembly meeting, IICB, Kolkata. December 15, 2006.
7. "Molecular profiling" delivered at the Moving Academy of Medicine and Biomedicine, December 30, 2006.
8. "Large Scale Alignment and Motif Finding in HIV Integration Target Sequences in the Human Genome", Workshop on computational genome analysis, CDAC, February 7, 2007.
9. "Use of Microarray analysis to study the mechanisms of regulation of global gene expression by SATB1", National Microarray Workshop, Sri Ramchandra College, Chennai, February 15, 2007.
10. "The third dimension of gene regulation: It's all in the looping!" Invited talk delivered at the following institutions:
    i. International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, November, 2007
    ii. National Centre for Biological Sciences (NCBS), Bangalore, April 16, 2007.
    iii. National Institute of Immunology (NII), New Delhi, May 10, 2007.

Nibedita Lenka

1. "Restoration of functional activities in Parkinsonian rats by ES cell derived dopaminergic neurons". The Second Bangalore Stem Cell Course and Workshop, National Centre for Biological Sciences, Bangalore, India sponsored by Department of Biotechnology, Ministry of Science and Technology, Government of India.
2. "Stem Cells – The Potentials and Challenges". Invited Speaker, 14th Conference of Association of Medical Biochemists of India (AMBICON)
3. Stem Cells in Development and Therapy: The Present Status and Future Prospects. Pt. J.N.M. Medical College, Raipur.

Yogesh S Shouche

1. "Microbial Diversity of Insect mid-guts" at University of Notre Dame, Notre Dame, Indiana USA, June 2006
2. "Insect Gut Microbes: Diversity and Beyond" at University of Georgia, Athens USA, July 2006
3. "Molecular analysis of the microbial diversity associated with Lonar Soda lake, India: An impact crater in basalt" at Conference on Cosmic Dust and Panspermia held at University of Cardiff, Cardiff UK, September 2006
4. "Biodiversity & Genomics" at Tuniso-Indian Workshop on Biotechnology at Sfax, Tunisia, September 2006
5. "Microbial Diversity of Insect Mid-gut" at Third Convention of BRSI and International Conference on Exploring Horizons in Biotechnology: A Global Venture at Anand, India, November 2006.
6. "Microbial diversity assessment using molecular techniques" at International Conference on Recent Advances in Marine Antifouling Technology at Chennai, India, November 2006.
7. "Gut Microbe association in Insects" at Lecture Workshop on Molecular Ecology at Orange County, March 2006.



Conferences/Workshops

Arvind Sahu

i. XXI International Complement Workshop, Beijing, China, October 22-27, 2006.
ii. 33rd Indian Immunology Society Conference, All India Institute of Medical Sciences, January 28-31, 2007.
iii. 15th Molecular Immunology Forum, Indian Institute of Science, Bangalore, March 1-3, 2007.

Anjali Shiras

i. International Society for Stem Cell Research (ISSCR) Meeting, Toronto, Canada, June 2006.
ii. First Annual Meeting arranged by Stem Cell Research Forum of India (SCRFI) at Bangalore, India, February 2007

Ramesh R Bhonde

i. Workshop on Animal Tissue Culture held at Department of Biochemistry, Shivaji University, Kolhapur, March 8-9, 2007

Sharmila Bapat

i. American Association for Cancer Research's Annual Meeting 2007, held from April 14-18, Los Angeles, USA.

Sanjeev Galande

i. Senior Fellows Meeting, Wellcome Trust, London, UK, October 25-26, 2006.
ii. Guha Research Conference, Leh, December 3-8, 2006.
iii. 75th Annual Meeting of the Society of Biological Chemists, JNU, New Delhi. December 8-11, 2006.
iv. International conference on "Nuclear Architecture: Chromosome-Chromatin Dynamics, JNCASR, Bangalore, December 11-13, 2006.
v. Tenth Transcription assembly meeting, 2006, Kolkata, December 14-16.
vi. Indo-US conference on "HIV/AIDS: Biology, Immunology and Vaccinology", Department of Biotechnology, New Delhi. March 13-14, 2007.
vii. Asia-Pacific Networks: Promoting excellence in research, Meeting organized by 'Nature' international science journal, June 6 2007, Tokyo, Japan.
viii. International conference on Chromosome to Genome, Centre for Cellular and Molecular Biology, Hyderabad, July 3-5, 2007.

Samit Chattopadhyay

i. Guha Research Conference, Leh, Laddak, September 3-8, 2006
ii. Nuclear Architecture: Chromosome-Chromatin Dynamics, JNCASR, Jakkur, Bangalore, December 11-13, 2006.
iii. Presented talk at Society of Biological Chemists, JNU, New Delhi, December 9, 2006.
iv. Transcription Assembly Meeting, Vedic village, Kolkata, December 14-16.
v. In search of p53 mediated global gene transcription: Role of a modulator protein SMAR1. EMBO Workshop, IIT, Kanpur, December 17-19, 2006.
vi. International Symposium on Chemical Biology, Invited speaker, 7-9 March, 2007.
vii. All India Cell Biology Conference, Delhi University, North Campus, Chairing session "Genes and Diseases" February 2-3, 2007.

Padma Shastry

i Indo-US Flowcytometry workshop- Proliferation, Apoptosis and Signal Transduction, Center for Biotechnology, Jawaharlal University, New Delhi, February 10-14, 2007.

Debashis Mitra

i. International conference on molecular cellular biology and therapeutics of HIV and associated viral infections held in Hyderabad, India, 12-14 January 2007.
ii. International conference on molecular cellular biology and therapeutics of HIV and associated viral infections held in Hyderabad, India, 12-14 January 2007.
iii. International conference on molecular cellular biology and therapeutics of HIV and associated viral infections held in Hyderabad, India, 12-14 January 2007.

Anandwardhan A Hardikar

i. Indian Society of Developmental Biologists (ISDB) and International Symposium on 'Cellular signaling during development' organized in Pune, India during November 23-25, 2006

Mohan Wani

i. Molecular Immunology Forum-2007 held at Indian Institute of Science, Bangalore, March 1-2, 2007.

Rajesh J Solanki and Shivaji S Jadhav

i. National Conference on Broadband Communication System (NCBS) organized by VIIT Engg. College, Pune, 1-3 September 2006.

Vaijayanti P Kale

i. Keystone Symposium on "stem cell interactions with their microenvironment" held on at Keystone, Colorado, USA, March 2-7, 2007.
ii. Keystone Symposium on "Stem Cells and Cancer" Keystone, Colorado, USA, March 2-7 2007.

Nibedita Lenka

i. International Society for Stem Cell Research (ISSCR), 5th Annual Meeting, Cairns, Australia.

Yogesh S Shouche

i. Cosmic Dust and Panspermia , University of Cardiff, Cardiff UK (September 2006)
ii. Vector Borne Disease Research: The Road Ahead, at Virginia Tech, Blacksburg, Virginia, USA (October 2006)
iii. Microbial Diversity of Insect Mid-gut, at Third Convention of BRSI and International Conference on Exploring Horizons in Biotechnology: A Global Venture at Anand, India, November 2006.
iv. Microbial diversity assessment using molecular techniques, at International Conference on Recent Advances in Marine Antifouling Technology at Chennai, India, November 2006.
v. Lecture Workshop on Molecular Ecology at Orange County, March 2006

Gopal C Kundu

i. Third International Tumor Progression and Therapeutic Resistance Conference, Baltimore, MD, USA, 23rd October, 2006.
ii. Hunter Cellular Biology Conference, Hunter Valley, Australia, 21st March, 2007

Vasudevan Seshadri

i. Translation Control meeting, Cold Spring Harbor, NY, USA, during September 6-10, 2006.

Conferences/Workshops attended by students

1. Anuradha Vaidya: Poster presentation at the 4th Annual Meeting of the International Society for Stem Cell Research (ISSCR) which was held in Toronto, Canada from June 29th to July 1st 2006

2. Ashwini Hinge: Poster presentation entitled as "*Ex vivo* preservation of hematopoietic stem and progenitor cells using mannose specific plant lectins" at 4th International Society for Stem Cell Research Annual Meeting at Toronto, Canada on June 2006.

3. Mangala Bamane award for best oral presentation by Young Scientist – "ROS triggered caspase-2 activation and feed back amplification loop in beta-carotene induced apoptosis in human leukemic cells" at 26th Annual Convention of IACR, 2007, Bhubaneshwar, India. Presented by Vandna Prasad.

4. M.K. Verma, N. Lenka. 2007. ES cells and Cardiomyogenesis: The Role of Wnt signaling. 1st Annual meeting of Stem Cell Research Forum of India (SCRFI) and International Conference on Stem Cell Research, 29th January-1st February, 2007, Bangalore, India (Received the 2nd best Oral Presentation award).

5. Vivekanand Yadav, JRF and Kalyani Pyaram, JRF attended a workshop on "Fundamentals of Protein Folding" at G.B. Pant University of Agriculture & Technology, Pantnagar, October 18-20, 2006.

6. "Improved ex vivo expansion of cord blood derived CD34+ cells in a serum free medium containing cytokines supplemented with antiapoptotic agents". Sangeetha V.M.*, V.P. Kale and L.S. Limaye. First Annual Meeting of Stem Cell research Forum of India (SCRFI) , and the International Conference on stemcell Research held from 29th January-1st February, 2007 in Bangalore.

7. Effective *ex vivo* generation of Megakaryocytic cells from umbilical cord blood $CD34^+$ cells in serum free medium with Arachidonic Acid as a supplement to growth factor cocktail." NFA Siddiqui, V.P.Kale and LS Limaye. Poster presented by Nikhat Siddiqui in the first annual meeting of SCRFI and International Conference on stem cell research held at Bangalore between 29th January-1st February, 2007.

8. Oral presentation "ROS triggered caspase-2 activation and feedback amplification loop in beta-carotene induced apoptosis in human leukemic cells" at 26th Annual Convention of IACR, 2007, Bhubaneshwar, India. Vandna Prasad, Anmol Chandele, Jayashree C Jagtap, Sudheer Kumar. P, Padma Shastry.

9. 33rd Indian Immunology Society Conference, New Delhi, January 28-31. Transcriptional Regulation of *Tbx21* by SMAR1 dictates TH1-TH2 Lineage Commitment. Poster and oral presentation by Archana Jalota (Received 1st prize in oral presentations).

List of students awarded Ph.D. during 2006-2007

Rishi Raj Chhipa

Thesis Title: Cytotoxic effects of chemotherapeutic agents on breast carcinoma cell lines: Understanding the mechanisms of cell killing, both in vitro and in vivo, and the role of tumor suppressor p53.

Guide: Dr. Manoj Kumar Bhat

Sandeep Singh

Thesis Tile: Studies on the Molecular Mechanism(s) of Apoptosis Induced by Various DNA Damaging 'Chemotherapeutic Drugs' in High-Risk HPV E6 Positive Carcinoma

Guide: Dr. Manoj Kumar Bhat

Lalita Sasnoor

Thesis Title: *In vitro* and in vivo behavioral studies of cryopreservedhematopoietic cells

Guide: Dr. Mrs. Vaijayanti P. Kale

Johnson John Bernet

Thesis Tile: Vaccinia virus Complement control protein (VCP): Role in complement inactivation and structure function analysis.

Guide: Dr. Arvind Sahu

Meenal Banerjee

Thesis Title: Studies on Cell-Cell interaction and/or Cell-Matrix interaction for modulation of differentiation potential of progenitor cells under controlled condition for tissue engineering applications.

Guide: Dr. Ramesh R. Bhonde

Co-Guide: Dr. Anil Kumar

Ruchi Shukla

Thesis Title: Control and Management of Experimental Diabetes through Conventional and Novel Metal-based complexes

Guide: Dr. R.R. Bhonde

Co-Guide: Dr. Subhash. Padhye

Savita Datar

Thesis Title: Evaluation of developing chick as a model for diabetes research in *ex ovo* and *in vitro* studies.

Guide: Dr. Ramesh R. Bhonde

Ms. Vandna Prasad

Thesis Title: Elucidation of Molecular mechanism in apoptosis induced by beta carotene in leukemic cells"

Guide: Dr. Padma Shastry

Sudheerkumar, P.

Thesis Title: Death receptors mediated signaling pathways in Multicellular Spheroids (MCS) and Monolayers derived from Human Gliomas.

Guide: Dr. Padma Shastry

Amit Awasthi

Thesis Title: Characterization of CD40 signaling in Leishamnai-infected and uninfected macrophages

Guide: Dr. Bhaskar Saha

Pallavi Wadhone

Thesis Title: Host cell-dependent anti-leishmanial fucntions of miltefosine

Guide: Bhaskar Saha

G. Murugaiyan

Thesis Title: Role of CD40 in the regulation of anti-tumor immnune response

Guide: Dr. Bhaskar Saha

Manish Bodas

Thesis Title: Inhibition of IL-2 induced IL-10 production as a principle of phase-specific immnunotherapy for visceral leishmaniasis

Guide: Dr. Bhaskar Saha

Manish Kumar

Thesis Title: Identification and Characterization of Human Immunodeficiency Virus-1 Nef Interacting Novel Cellular Factors

Guide: Dr. Debashis Mitra

Anagh Sahasrabuddhe

Thesis Title: Artocarpus integrifolia lectin (jacalin) induced modulation of EGF receptor signaling in human epidermoid carcinoma cell line

Guide: Dr. Musti Krishnasastry

Shruti M. Khapli

Thesis Title: Role of interleukin-3 in receptor activator of NF-κB ligand-induced osteoclast differentiation

Guide: Dr. Mohan R. Wani

Latha S. Mangashetti

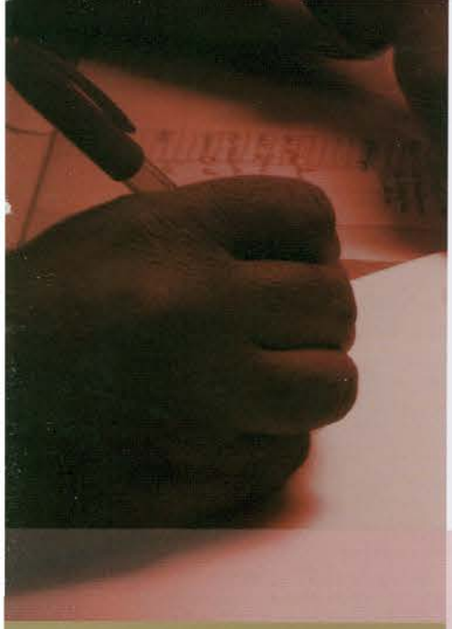
Thesis Title: Role of interleukin-4 in bone remodeling

Guide: Dr. Mohan R. Wani

S.D. Yogesha

Thesis Title: Studies on Regulation of Tumour Necrosis Factor-α-induced Osteoclast Differentiation and Bone Resorption by Interleukin-3 and Granulocyte-macrophage Colony-stimulating Factor

Guide: Dr. Mohan R. Wani



NCCS Organisation

NCCS Organisation

NCCS Committees

Society

1. **Shri Kapil Sibal** — President
   Hon'ble Minister for Science & Technology
   & Earth Sciences,
   Anusandhan Bhawan, Rafi Marg,
   New Delhi 110 001

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

3. **Prof. N.K. Ganguly** — Member
   Director General,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911,
   New Delhi 110 029

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

5. **Prof. Narendra Jadhav** — Member
   Vice Chancellor,
   University of Pune, Ganeshkhind,
   Pune 411 007

6. **Shri. K.P. Pandian** — Member
   Joint Secretary & Financial Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No. 2, 7th Floor, CGO Complex, Lodi Road,
   New Delhi 110 003

7. **Shri Sukhdeb Sinha** — Member
   Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No. 2, 7th Floor, CGO Complex, Lodi Road,
   New Delhi 110 003

8. **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

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

10. **Dr. Vijay Raghavan** — Member
    Director,
    National Centre for Biological Sciences,
    Tata Institute of Fundamental Research,
    GKVK, Bellary Road,
    Bangalore 560 065

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

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

Governing Body

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

2. **Prof. N.K. Ganguly** — Member
   Director General,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911,
   New Delhi110 029

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

4. **Prof. Narendra Jadhav** — Member
   Vice Chancellor
   University of Pune
   Ganeshkhind,
   Pune 411 007.

5. **Shri K.P. Pandian** — Member
   Joint Secretary & Financial Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No. 2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi 110 003

6. **Shri Sukhdeb Sinha** — Member
   Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110 003

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
   Director,
   National Centre for Biological Sciences,
   Tata Institute of 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

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

5. **Shri Sukhdeb Sinha** — Special Invitee
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th-8th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110 003

6. **Dr. G.C. Mishra** — Member Secretary
   Director,
   NCCS
   Pune 411 007

Building Committee

1. **Prof. S.K. Basu** — Chairman
   Professor of Eminence,
   National Institute of Immunology,
   Aruna Asaf Ali Marg,
   New Delhi 110 067

2. **Dr. A.C. Mishra** — Member
   Director,
   National Institute of Virology,
   Dr. Ambedkar Road,
   Pune 411 001

3. **Shri Sukhdeb Sinha** — Member
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110 003

4. **Shri B. Bose** — Member
   Management Consultant,
   DBT Cell and National Institute of Immunology,
   Aruna Asaf Ali Marg,
   New Delhi 110 067

5. **Dr. T. Sahay** — Member
   Registrar,
   Indian Institute of Technology,
   Powai,
   Mumbai 400 076

6. **Chief Engineer** — Member
   Pune (PW) Region,
   New Central Building,
   Pune 411 001

7. **Ms. Maushmi Rudra** — Member
   Director (Finance),
   Department of biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110 003

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

Scientific Advisory Committee

1. **Prof. N.K. Ganguly** — Chairman
   Director General,
   Indian Council for Medical Research,
   Ansari Nagar, Post Box- 4508,
   New Delhi 110 029

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
   Distinguished Biotechnologist,
   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** — Member
   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

11. **Prof. Sandip K. Basu** — Member
    Professor of Eminence,
    National Institute of Immunology
    Aruna Asaf Ali Marg
    New Delhi 110 067

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

13. **Dr. K. Vijay Raghavan** — Member
    Director,
    National Centre for Biological Sciences,
    Tata Institute of Fundamental Research,
    GKVK, Bellary Road,
    Bangalore 560 065

14. **Dr. B. Ravindran** — Member
    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 and Purchase sections. The centre has its Instrumentation and 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 145 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. Dr. G.C. Mishra, Director, has been nominated as Appellate Authority.

Implementation of Official Language

NCCS has constituted a Committee, which meets quarterly 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 Saptah 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.

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.

Ms. Sunita Singh receiving the 1st prize for Hindi Essay writing competition on Hindi Diwas from the Chief Guest Mrs. Seema Deshpande, Asst. Director, Hindi Teaching Scheme, Pune.