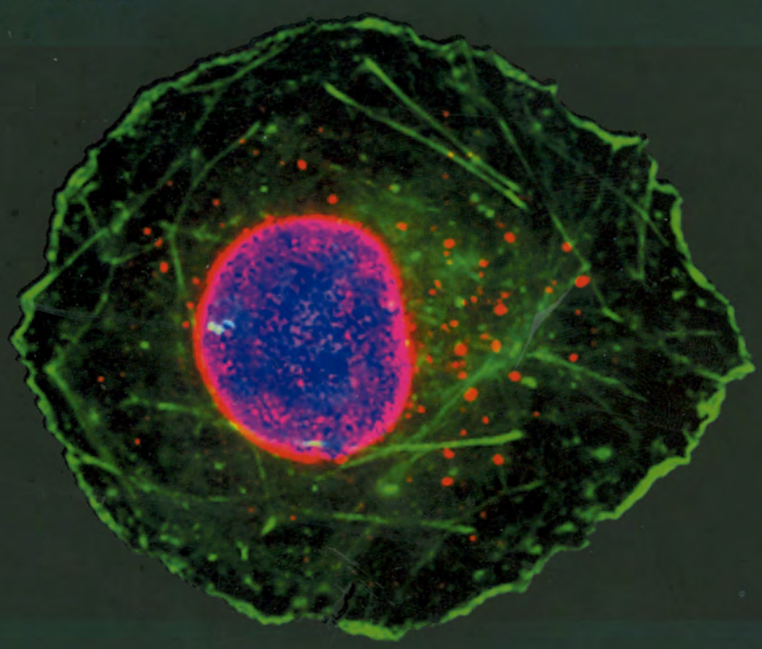
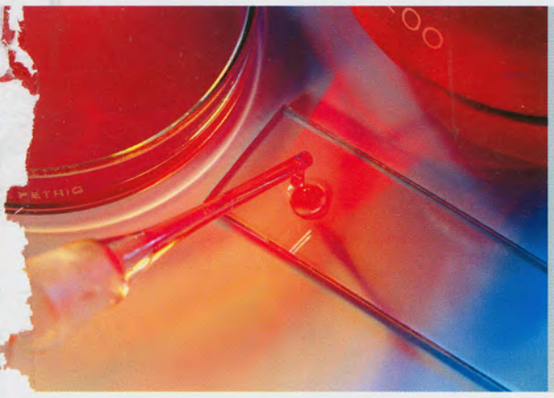
Annual Report 2008-2009

NCCS



National Centre for Cell Science



National Centre for Cell Science

Annual Report - 2008-2009

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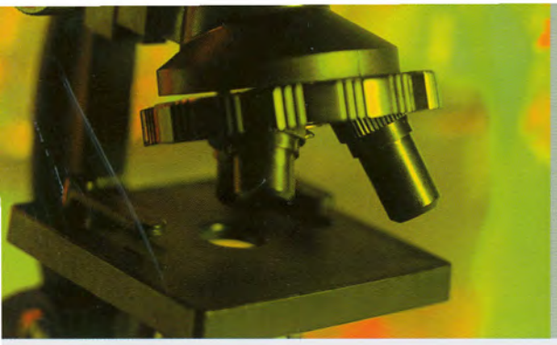

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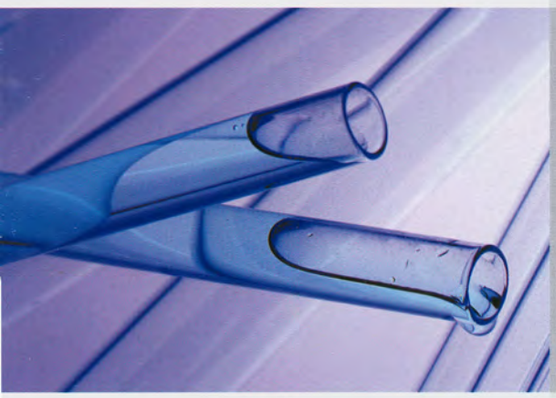


Mandate of NCCS

- To receive, identify, maintain, store, grow and supply:- Animal and human cells/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 and supply culture media and cell products independently or in collaboration with industry and other organizations.

- Research and development.

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

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

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

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

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

From Director's Desk

It is a pleasure as always to present the annual report of National Centre for Cell Science (NCCS) for the year 2008-2009. In addition to serving as the National Cell Repository, NCCS focuses on Human Resource Development, and Research and Development in the areas of cell biology, signal transduction, cancer biology, diabetes, biodiversity, infection and immunity, chromatin architecture and gene regulation, stem cells and regenerative biology. In the reporting period, NCCS has provided more than 1700 cell lines to various research institutions and universities in India. There is a requirement of large number of skilled scientific personnel to utilize the vast information available in the frontier areas of biology for the benefit of the society. Towards this goal, under our teaching and training programme, we have trained over 30 student fellows from all over the country in different aspects of conducting research in modern biology.

As a premier research institution, NCCS focuses on current and emerging public health needs and promising areas of science. Our centre considers many different perspectives in establishing research priorities. One of the areas we are interested in is regenerative and stem cell biology, with special reference to diseases in which a particular cell type is damaged or lost, as in the case of diabetes, osteoporosis and neurodegenerative disorders. We have developed novel methods to preserve and maintain viable stem cells and succeeded in

differentiating these cells to specific lineages, which have potential applications in cell/tissue replacement therapy.

Cell-cell signaling is fundamental to the growth and development of multi-cellular organisms, mis-regulation of which causes various diseases including cancers. We are studying the regulation of critical players, such as p53, eNOS, Osteopontin, TNF-α, involved in some of these cell-cell communication pathways that go awry in cancers. We have demonstrated that deficiency of Osteopontin effectively curbs melanoma metastasis and angiogenesis, thus providing a new target for future cancer therapies. A number of recent reports suggest interesting role for non-coding RNAs in diverse biological processes, and scientists at NCCS have identified a specific non-coding RNA that is important for tumorigenesis. In recent years, cancer has been illustrated as a stem cell disease. Our studies defined a subset of cancer stem cells that contribute to tumour dormancy and resistance to chemotherapy.

Understanding the molecular intricacy of infection and immunity is of great importance, particularly due to the emergence of new infectious agents. Studies at NCCS suggest that successful infection by at least a group of bacteria involves alteration of the host cell membrane and caveoli dynamics by the toxins they produce. Establishment of any infection depends on how effectively the pathogens can counteract the host innate and adaptive immune responses. NCCS investigates how some viruses target the host complement system in order to overcome the innate immune response. Furthermore, a role for CD40-CD40L interaction in regulating the activation of T cells in response to Leishmania and HIV infections is being investigated.

One of the initial steps in gene regulation is at the level of transcription. NCCS scientists recently unraveled the molecular mechanism by which two MAR binding proteins, SMAR1 an SATB1, regulate transcription networks globally. We have initiated systems

biology studies to understand the complex interplay of various regulatory networks during the processes of development and diseases.

The Microbial Culture Collection for the conservation and exploitation of the biodiversity of India is a major project undertaken by NCCS. The microbial distribution in various habitats are also being studied using the metagenomic approach.

NCCS is determined to provide state-of-the-art facilities to its researchers. We have an excellent experimental animal facility that procures and maintains animals, and provide technical support to the scientists. The centre has added new facilities to the existing ones, including an advanced microarray system and an *in vivo* animal imaging set up, to augment the research initiatives in genomics, proteomics, cell biology and translational research. To expand the cell repository facility and to provide accommodation to our research scholars, we have initiated construction of new buildings.

We thrive to excel in our research activities. Our efforts are reflected in the number of publications in several prestigious international journals. In the reporting year we have published 60 scientific papers. Our research is supported by funding from various national and international grant agencies. NCCS also has established Indo-French, Indo-German, Indo-Finland, Indo-US collaborative projects to enhance our scientific activities.

NCCS will continue to focus on important questions relevant to human health, specifically in the area of regenerative medicine, infectious diseases and lifestyle induced health adversities.

G C Mishra
Director

Human Resource Development

During 2008-2009, six students joined the institute for pursuing Ph.D. The total number of Ph.D students as on 31.03.09 was 89.

The project training programme is conducted twice in a year (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 is:

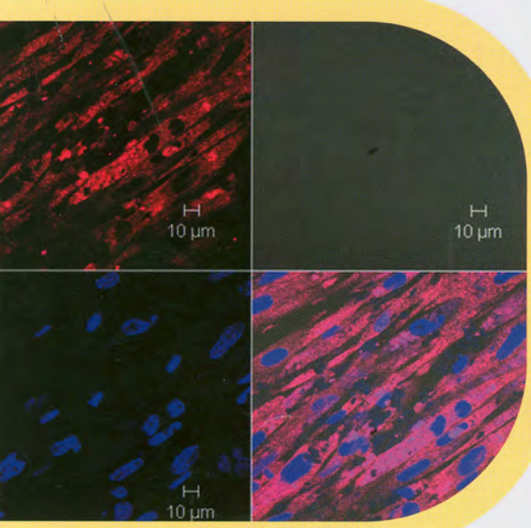
Project Training - 32
Summer Training - 5

During this year, 29 Research Fellows attended seminars/ conferences/ symposium conducted by various reputed organizations in India and 12 Research Fellows attended International seminars/ conferences/ symposium.

Repository

National Centre for Cell Science serves as a National Cell Bank for animal cell lines. The repository manages cell line procurement, expansion, cryopreservation and distribution. In this year, we have procured different cell types from different repositories. The list of cell lines, with details such as media requirement, growth conditions and its use, is available now on demand. In 2008-2009, we have supplied 1770 cell lines to 140 research institutions in the country.

Cell Biology



Stromal cell biology: Identification of stromal cell-mediated signals regulating hematopoietic stem cell fate

Vaijayanti P Kale
vpkale@nccs.res.in

Participants
Ashwini Hinge, *SRF*
Anuradha Vaidya, *SRF*
Roli Misra, *SRF*
Monika Sharma, *SRF*
Manmohan Bajaj, *JRF*
George Fernades, *Technician*

Collaborator(s)
Dr. L.C. Padhy, TIFR, Mumbai
Dr. Avadhesha Surolia, NII, New Delhi
Dr. (Mrs.) Lalita Limaye, NCCS, Pune

Background

The micro-environment created by the marrow mesenchymal cells (MSCs) along with their secreted extra-cellular matrix molecules (ECM) and cytokines forms a niche for the hematopoietic stem cells (HSCs) and is known to regulate their development. However, the molecular mechanisms involved in the process and the nature of signaling mechanisms operative in the niche are not yet clearly understood. We have been carrying out experiments to identify the signaling mechanisms involved in the regulation of HSC proliferation without compromising their stem cell properties. Since the architecture of the marrow tissue is a three dimensional one, we have focused this part of the study on development of three dimensional cultures for the growth of stem cells.

Aims and Objectives

Development of three dimensional cultures of mesenchymal cells for in vitro expansion of HSCs

Work Achieved

We were successful in isolating, culturing and expanding mesenchymal stem cells from full-term human placenta (PMSC). We found that PMSCs show a higher proliferative rate as compared to the MSCs grown from adult bone marrow (Fig. 1). We developed 3D cultures of PMSCs using hydrogels and co-cultured human bone marrow-derived $CD34^+$ hematopoietic stem cells (HSCs) with them. The phenotypic properties of these HSCs were compared with the HSCs grown as co-cultures with PMSCs under 2D conditions (cells grown on plastic surface). We observed that the 3D cultures foster a superior growth of $CD34^+38^-$ primitive stem cells as compared to the 2D culture conditions (Fig.

2). The mechanism behind this superior stem cell support by 3D cultures was related to the hypoxic conditions prevailing in these cultures, as evidenced by the immuno-reactivity of the 3D-PMSC with the hypoxy-probe (Fig. 3). The data show that hypoxic environment created by the 3D culture conditions leads to the expansion of primitive HSCs.

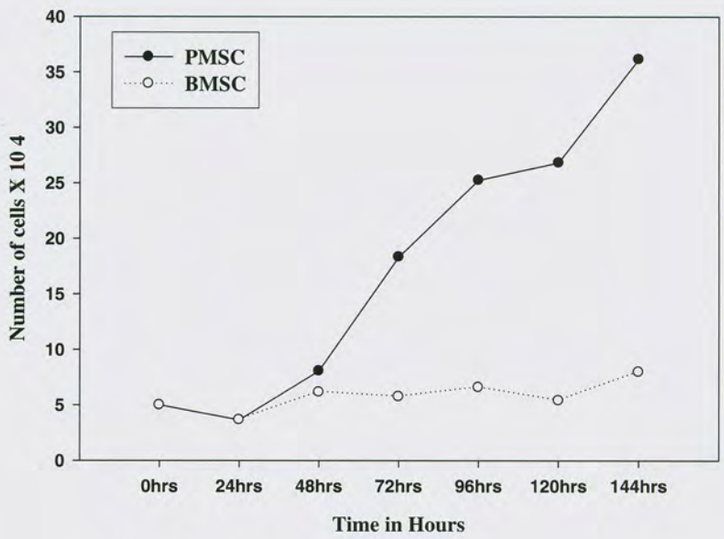


Fig. 1. Comparative growth curve of MSCs derived from placenta and bone marrow shows that PMSC have more proliferative ability as compared to BMSC

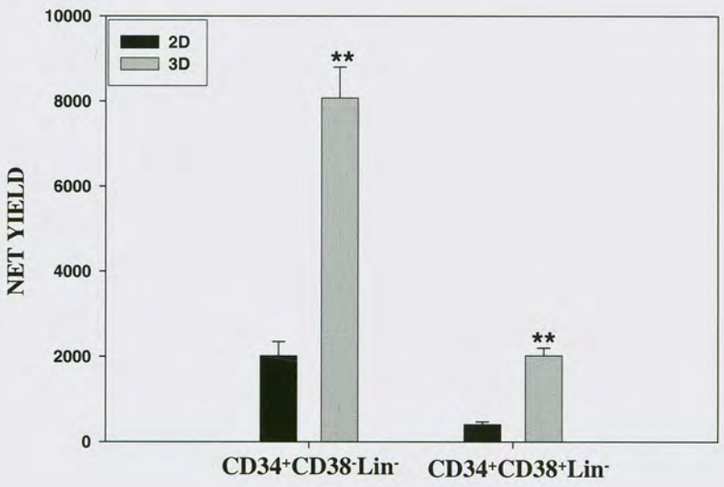


Fig. 2. Net yield of $CD34^{+}38^{-}Lin^{-}$ primitive stem cells and $CD34^{+}38^{+}Lin^{-}$ progenitors under 3D conditions is significantly high. The graph depicts mean S.D. of four independent experiments. ** $p < 0.01$

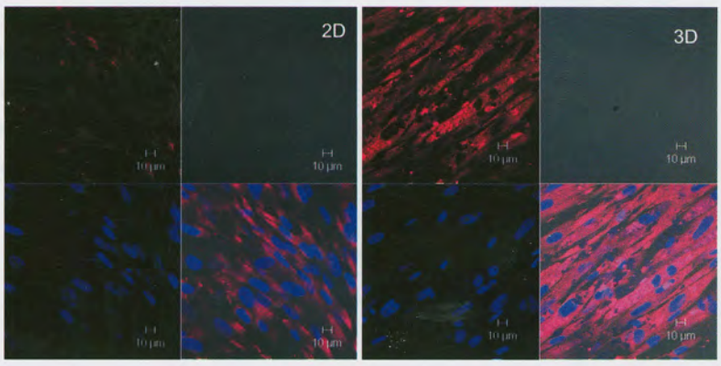
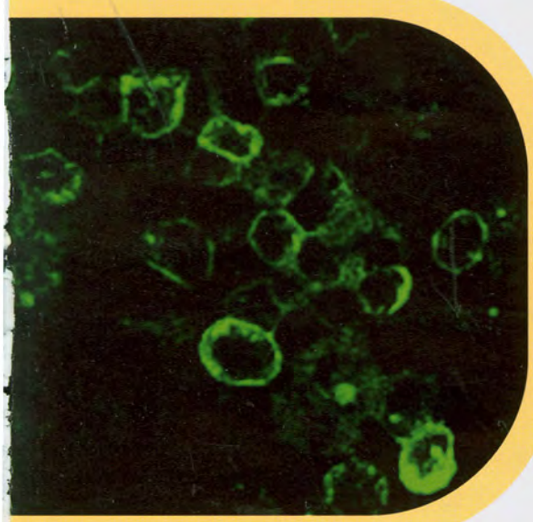


Fig. 3. PMSC grown under 3D conditions show immuno-reactivity with hypoxy probe indicating they are hypoxic

Future Work

1. Characterization of the HSCs grown under 3D conditions in terms of stem cell-specific phenotypic and functional characters
2. Comparative genomic and proteomic analysis of MSCs grown under 3D vs. 2D culture conditions to identify the players involved in the expansion of primitive HSCs
3. Use of MSCs possessing specific biochemical properties to examine the signaling pathway-specific effect on the HSC fate



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*

Collaborator(s)
Dr. R.L. Marathe, Jehangir hospital, Pune
Dr. (Mrs.) Vaijayanti P. Kale, NCCS, Pune

Background

In vitro expansion and cryopreservation of hematopoietic stem cells

Ex vivo expansion of Umbilical Cord Blood (UCB) derived $CD34^+$ cells is one strategy to increase the number of cells available for transplantation of stem cells. We have consistently observed an increase in apoptosis after cytokine culture of $CD34^+$ cells compared to the freshly isolated cells resulting in impaired stem cell functions. The present study was done to assess whether prevention of apoptosis improves the outcome of the expansion of UCB $CD34^+$ cells. Our strategy was to use two anti-apoptotic agents zVAD-FMK and calpain inhibitor in the expansion media.

Megakaryocyte generation

In vitro generation of megakaryocytes (MKs) from hematopoietic stem cells (HSCs) is important both for therapeutic applications as well as for basic research. The clinical applications include infusion of ex vivo expanded megakaryocyte progenitors in patients undergoing HSC transplantation. Many investigators have attempted a combined infusion of $CD34^+$ cells and ex-vivo expanded megakaryocytes in patients to improve post transplant platelet recovery with a variable degree of success. Platelets may also be used for wound healing and regeneration of tissue. However, most medical facilities often suffer from a shortage of platelet products since platelet concentration for transfusion supplied by blood centers is derived from donors .Thus a new source of platelets is urgently required. The transition from megakaryocyte to platelets is a complex process. Although the basic mechanisms of platelet production have been investigated, elucidating the specific molecular controls and cellular events involved in platelet formation and release is an unfinished task. Ex vivo cultures to generate platelets provides a good model to carry out such fundamental studies.

Taken together, optimization of *ex vivo* generation of megakaryocytes and platelets from stem cells is a topic of current interest. We have used two nutraceuticals as supplements in the expansion media and show that they enhance megakaryocyte and platelet generation from cord blood derived $CD34^+$ cells.

Dendritic cell generation

In vitro generated dendritic cells (DCs) are widely used as adjuvants in cancer immunotherapy. The major sources for DC generation are monocytes and $CD34^+$ cells. $CD34^+$ derived DCs are less frequently used in clinical applications because it requires complex generation methods. Here we describe a simple method for the large scale generation of mature functional DCs from umbilical cord blood derived $CD34^+$ cells. $CD34^+$ cells were first expanded with a combination of early acting growth factors in a medium containing autologous plasma. In the second step the DC precursors were further enriched either by plastic adherence or sorted on a cell sorter and differentiated as DCs. DCs generated by both the methods were compared for their morphology, phenotype and different functional parameters.

Aims and Objectives

1. *In vitro* expansion and cryopreservation of $CD34^+$ haematopoietic stem cells
2. *In vitro* generation of megakaryocytes and dendritic cells

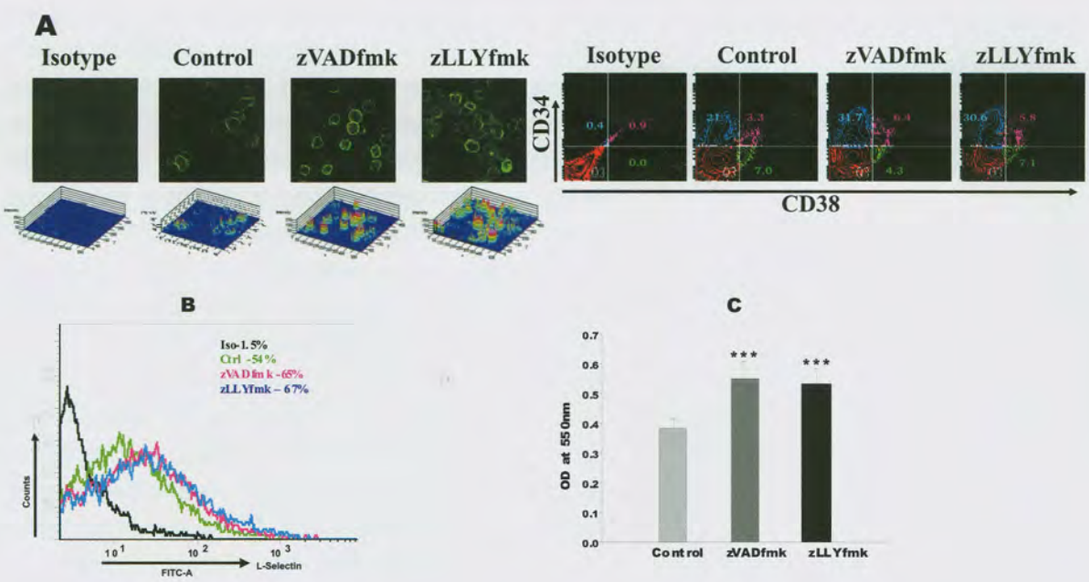


Fig. 1. Preferential expansion and higher adhesion of UCB $CD34^+$ cells upon apoptotic protease inhibition. **(A)** The left panel is a representative image of the cultured HSPCs, showing a significantly higher CD34 expression compared to their control counterpart. The right panel is a representative flowcytometry profile, showing a higher number of cells expressing the primitive $CD34^+CD38^-$ phenotype (upper left quadrant) within the test population indicating a higher self renewal. **(B)** Representative flow cytometry profile showing a significantly higher number of cells expressing L-selectin in the test sets after the ex vivo culture. **(C)** The expanded HSPCs were assessed for their adhesion potential to fibronectin.The cells cultured in the presence of anti apoptotic compounds showed a significantly higher adhesion correlating well with the higher L selectin /CD62L content in them

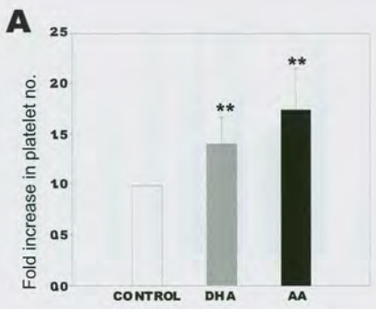
Work Achieved

Expansion of haematopoietic stem cells

Supplementation of anti apoptotic agents in the expansion media improved the expansion in terms of total cell yield and $CD34^+$ $CD38^-$ cell content (Fig.1A). L-selectin (CD62L) is one of the major adhesion molecules for HSCs. Protease inhibition during ex vivo expansion increased the surface expression of CD62L. Flow cytometry profile of a representative sample is shown in Fig. 1B. Adhesion of the hematopoietic stem and progenitor cells to the extracellular matrix is one of the important events after transplantation. Since there was an increase in the CD62L positive cells in the test sets, it was tempting to see the effect of the same on adhesion properties also. As shown in Fig. 1C, the number of cells adhered to fibronectin was significantly higher in the test sets (***$p \leq 0.001$). The study shows that the prevention of apoptosis cascade during the ex vivo culture, protects stem cell pool and committed progenitors, up regulates the adhesion/homing receptors in them and improves their engraftment potential. This observation may have a significant impact in UCB transplantation settings.

Megakaryocyte generation

Cells cultured in presence of Docosahexanoic acid or Arachidonic acid showed better platelet generation as compared to control cultures. Platelets were isolated and enumerated by counting in Neubaur's chamber. As seen in Fig. 2A, test sets showed significantly higher platelet numbers as compared to control



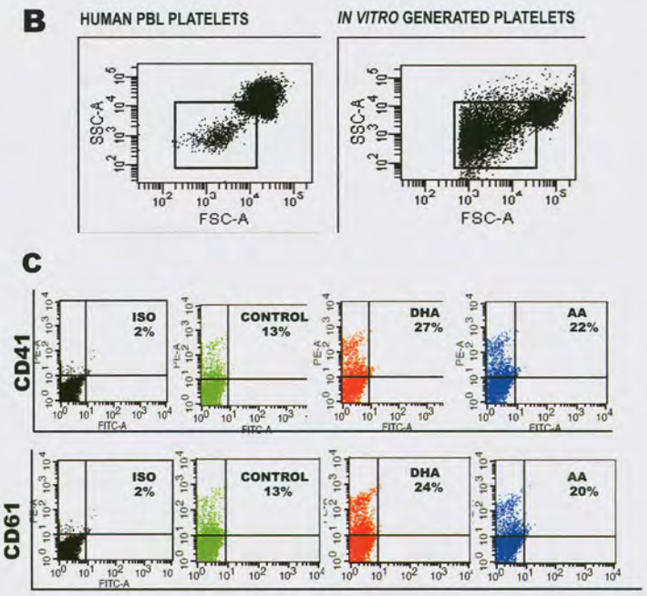


Fig. 2. Platelet generation from UCB $CD34^+$ cells. **(A)** Enumeration of platelets shows that significantly higher numbers are generated in nutraceutical containing sets as compared to control cultures. **(B)** Gating of normal human peripheral blood platelets applied to culture generated platelets. **(C)** % CD41/CD61 platelets are higher in test sets as compared to controls

sets. Data is depicted as fold increase in platelet number (**p≤0.01 n=6). Platelets were stained with CD41 and CD61 or appropriate isotype control antibody and analysed by flow cytometry. Human peripheral blood platelets were acquired to set the gate using a log scale on FSC and SSC for culture generated platelets (Fig. 2B). Fig. 2C shows dot plot profile of one representative experiment where % CD41 and CD61 positive platelets were higher in test sets as compared to control. Taken together, our data suggest that AA and DHA promote megakaryocyte platelet generation in conjunction with TPO and SCF, due to their antiapoptotic and antioxidant properties. Thus, their inclusion in culture media may be a promising method for megakaryocyte and platelets generation from UCB CD34$^+$ cells for both cell therapy and basic studies in the future.

DC generation

In continuation of our previous studies we wanted to check whether our method of DC generation works equally well if the starting population is frozen CD34$^+$ cells. As seen in Fig. 3A the phenotypic profile of DCs generated from frozen CD34$^+$ was similar to those generated from fresh CD34$^+$ cells. It was also possible to freeze the in vitro generated DCs and the phenotype of frozen DCs (Fig. 3B) and their migration properties were unaffected by cryopreservation. (Fig. 3C)

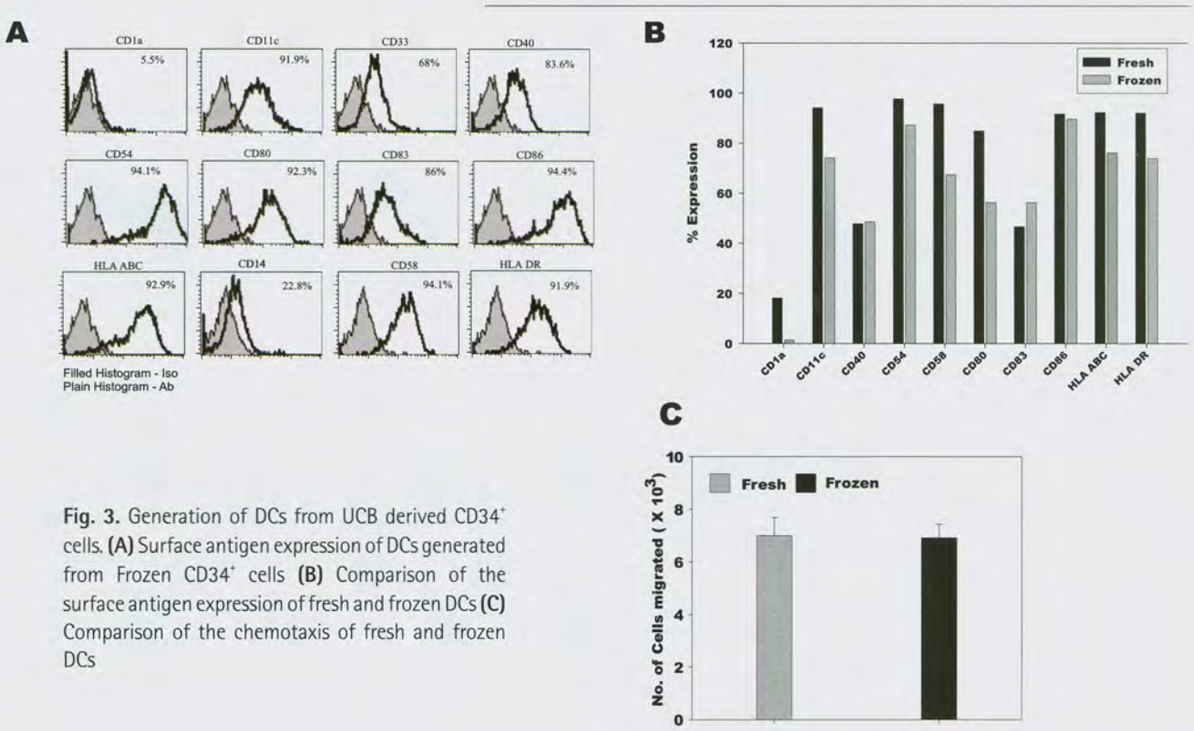
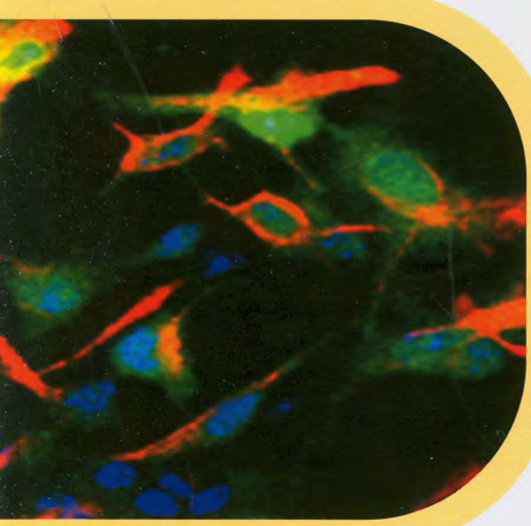


Fig. 3. Generation of DCs from UCB derived CD34$^+$ cells. **(A)** Surface antigen expression of DCs generated from Frozen CD34$^+$ cells **(B)** Comparison of the surface antigen expression of fresh and frozen DCs **(C)** Comparison of the chemotaxis of fresh and frozen DCs



Delineation and functional analysis of midbrain precursors during neural differentiation from ES cells

Nibedita Lenka
nibedita@nccs.res.in

Participant
Saravana Kumar Ramasamy, *SRF*

Background

Parkinson's Disease (PD) is one of the dreaded neuronal disorders caused by the progressive degeneration of dopaminergic (DA) motor neurons present in the substantia nigral region of midbrain. Although a number of treatments are available, there is no cure for PD. One of the most plausible therapeutic approaches is to replace the dead cells with the healthy ones in order to re-innervate the region and restore the functional ability subsequently. In this context, embryonic stem cells (ES) cells are advantageous in having unlimited proliferation potential and can be manipulated to differentiate into cells of interest. However the available neural differentiation protocols produce asynchronous population as well as non-neural cells. Therefore, proper selection of cells is mandatory for the safety and efficacy of therapeutic implementations. Our earlier studies using TH EGFP stable ES cell clones indicated one week differentiated cells to be ideal for functional restoration in hemi-Parkinsonian rodent models, while nestin-EGFP clone deduced the one week time window to be the effective one for optimum neural progenitor generation. Accordingly, we intended to identify and isolate the overall neural progenitors as well as midbrain specific neural progenitors and use them in transplantation studies using hemi-Parkinsonian animal models with a view to compare their efficacy. The present study deliberates the generation of several stable ES cell clones by incorporating mid-brain specific enhancers from nestin intron II fused with live reporter and their subsequent characterization.

Aims and Objectives

1. The maintenance of ES cells in undifferentiated state
2. The differentiation of ES cells into various lineages such as neural, cardiac

etc. and understanding the underlying molecular basis of lineage commitment and specification

3. Establishment of stable transgenic ES cell clones and promoter/enhancer mediated cell trapping to demarcate the cells of interest during differentiation of ES cells and their subsequent characterization.
4. Manipulation of extrinsic factors for the efficient generation of functional cardiomyocytes, proliferative neural progenitors and differentiated neurons with special reference to the dopaminergic neuronal subtypes from ES cells *in vitro*
5. Exploration of the efficacy of *in vitro* generated cells in cell replacement therapy using animal models

Work Achieved

Nestin, an intermediate filament protein is a common neuroepithelial stem cell marker. Its expression is localized mainly in the progenitor population that gets down-regulated upon maturation and reappears upon injury. Nestin gene consists of four exons and three introns, where the intron II includes CNS specific enhancer elements. In fact, existence of two individual enhancer elements has been reported within the 1.8 kb intron II region; (i) a midbrain specific enhancer and (ii) a pan-CNS enhancer element. The presence of such region-specific elements and binding proteins suggest the presence of heterogeneity in the neural precursor pools, though all of them express the same protein, nestin. In the present investigation, we have exploited this special property to demarcate the midbrain specific neural progenitors, isolate those, and understand their developmental progression and functional behavior using murine ES cell model.

Three EGFP reporter constructs were designed that included 442-, 366- and 227-bp long regions from nestin intron II along with thymidine kinase basal promoter, to understand the minimal region showing midbrain specific EGFP expression (Fig. 1). While the 442bp region encompassed binding elements for

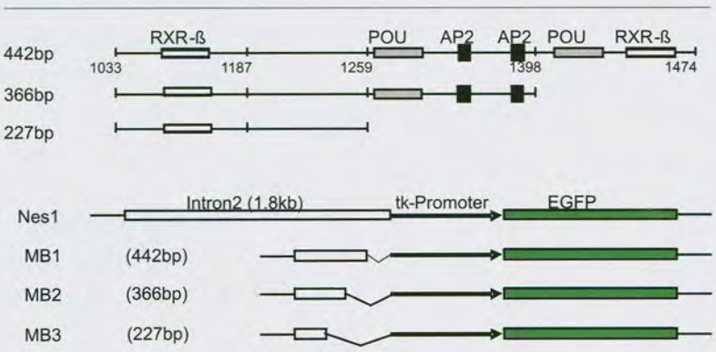


Fig.1. The different regulatory regions present in the 3' region of nestin intron II has been shown. The schematic representation displays the basis for sub-cloning of different regions of intron II and the regulatory regions present thereof. The bottom panel displays the constructs used for transfection of ES cells.

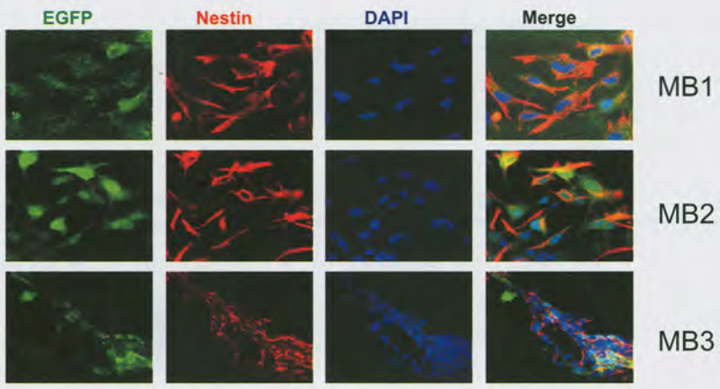
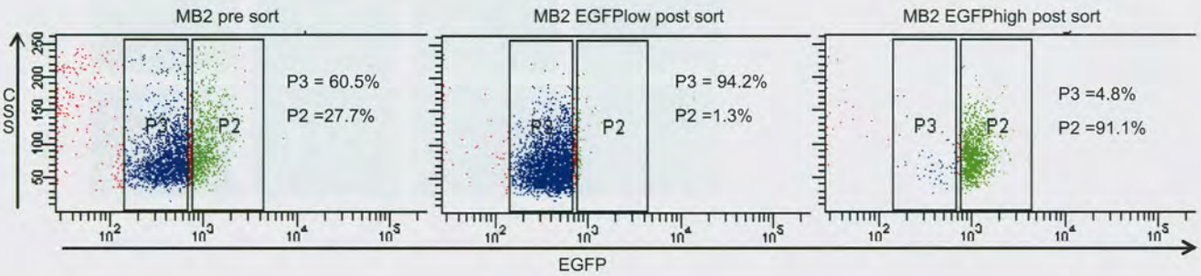


Fig. 2. The EGFP expression driven by the respective enhancers present in the clones were seen co-localized with the endogenous nestin expression in the cells after 7days of neural differentiation.

POU, AP2 and RXRβ, two sites each, the 366bp region had the distal rostral-specific POU and RXRβ binding sites removed, and the smallest 227bp region carried only the proximal supposedly midbrain specific RXRβ binding site. A number of stable clones were generated by transfection of ES cells with these constructs and selecting the G418 resistant clones. ES cell clones were named MB1, MB2 and Mb3 for 442-, 366- and 227-bp constructs respectively. All the clones displayed a weak basal EGFP expression even at the undifferentiated state similar to that seen with nestin clones, with MB3 displaying the least expression among all. Neural differentiation pattern was studied in these clones by analyzing the expression of various neural markers by immuno-cytochemistry and simultaneously monitoring for EGFP expression. Interestingly, no difference was registered among the clones on their differentiation potency. All the clones illustrated the presence of various early and mature neural markers. The EGFP expression in the stated clones matched with endogenous nestin expression during differentiation (Fig. 2). However, MB3 clones demonstrated some non-specific/ectopic EGFP expression as well. The EGFP expression pattern was further studied in all the clones comparing them with nestin clones. There was progressive increase in EGFP expression till first week that decreased subsequently with the appearance of mature neural populations in culture.

Flow cytometry quantification of $EGFP^+$ populations displayed maximum expression during 6-9 days of differentiation. The fold induction in the number of $EGFP^+$ population during differentiation was similar in all the clones, except in MB2 that displayed the highest fold induction (35.8 ± 4.2). The EGFP intensity was also brightest in MB2, and least in MB3 clones and MB1 clones showed similar EGFP intensity as that of nestin clones. Therefore, to understand the nature of these cells with varying EGFP intensities, the

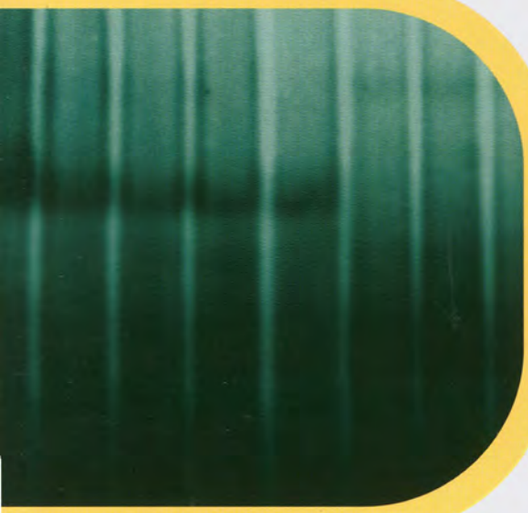
Fig. 3: 7day differentiated MB2 cells were sorted for the EGFP^{low} and EGFP^{high} populations. P3(blue) represents EGFP^{low} populations with 94.2% purity after sorting and P2 (green) represents EGFP^{high} with 91.1% purity after sorting.



differentiating cells were sorted on the basis of EGFP expression (Fig. 3). EGFPlow (10^2 to $0.5x10^3$), EGFPhigh ($>0.6x10^3$) and EGFPneg ($<10^2$) populations were purified by FACS and maintained in culture. While the purity of both EGFP^{low} and EGFP^{high} populations was from 91-94%, the viability ranged from 60-80% post-sorting. Interestingly, the EGFPlow and EGFPhigh populations showed different characteristics in post-sorted culture. While the morphology of most of the EGFP^{low} population was round shaped, EGFP^{high} populations displayed bipolar and multipolar structures. Both these populations had the ability to form neurosphere like clusters (NLC) that showed expression of various markers like Nestin, Sox1, Sox2, Nucleostemin, GFAP, Map2 etc. However, EGFP^{low} cells displayed larger clusters (>200μM) compared to EGFP^{high} ones. Moreover, most of the EGFP^{high} cells showed better adhesion to the culture dishes, while EGFP^{low} formed mostly NLCs with few attached cells. These clusters could also form secondary and tertiary spheres. In fact, both the populations seemed to be hierarchically at different stages of neurogenesis, even though both differentiated into neuronal and glial lineages. The EGFP^{high} populations were deduced to be the late neural progenitors and the EGFP^{low} ones as early, based on the CD133 expression and appearance of neuronal network in these cells during differentiation. Among the three clones analyzed, EGFP^{high} population in MB2 clone illustrated a better differentiation towards midbrain than the other clones. Hence, with the help of 366bp region of nestin intron II we could demarcate the midbrain specific progenitors in vitro. Further, all the three clones along with nestin clone were used for transplantation studies to investigate their efficacy *in vivo*.

Future Work

We have been successful in establishing 6-hydroxy dopamine based hemi-Parkinsonian animal models. These animals have been transplanted with neural progenitors from the stated clones and the investigation is ongoing to study their functionality in restoring the motor neuronal activities in them.



Translational regulation of insulin mRNA

Vasudevan Seshadri
seshadriv@nccs.res.in

Participants
Bhavana M, *SRF*
Baskar B, *SRF*
Amaresh C P, *SRF*
Shardul K, *JRF*
Arti B, *Technician*

Background

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

Glucose induced translation of insulin in pancreatic beta cells is mediated by the 5'UTR of insulin mRNA. We have previously reported the minimal sequence/structure in the 5'UTR of rat insulin gene1 required for this regulation. We had shown that specific factors in the pancreatic islets bind to the 5'UTR of the insulin mRNA upon glucose stimulation. A minimal 29-nucleotide element in the 5'UTR was shown to be sufficient for the glucose mediated translation activation of insulin mRNA. Conserved residues in the predicted stem loop region of the UTR seem to be important for the complex formation and translational regulation. Using RNA affinity pull down method, we isolated and identified the 5'UTR binding protein, as protein disulfide isomerise (PDI). We verified the presence of this protein in the activation complex through super shift RNA EMSAs (electrophoretic mobility shift assays) using specific antibodies. The Ins-5'UTR binding activity of PDI was confirmed further using a yeast three hybrid assay as well as RNA immunuprecipitation assay. We have previously shown that glucose stimulation increased association of PDI with the Ins-5'UTR in insulin

producing cells resulting in increased translation. We have now characterized the regulation in greater detail. We show that glucose stimulates specific protein kinase that can then phosphorylate PDI and regulate insulin translation. Further characterization of the interaction of PDI with basal translation machinery is in progress.

Aims and Objectives

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

Work Achieved

Major regulation of insulin biosynthesis occurs at the secretion and the translational level in β-islet cells. Glucose stimulates the beta cells to increase the translation of insulin mRNA but the mechanism is not completely understood. The UTR of the insulin mRNA is thought to be essential for this regulation. Rat insulin mRNA has a 57 base 5'-UTR. We synthesized radio-labeled wild type rat insulin 5' UTR and performed the RNA gel shift assay using RIN cell extracts to identify specific RNA-protein complexes. A similar specific complex was also formed with extracts from rat pancreatic islets. The complex formation was induced by high glucose treatment of the islets. We identified a minimal 29 nucleotide element that is necessary and sufficient for the complex formation. Our analysis also revealed that the predicted stem loop structure is important for the complex formation. We synthesized biotinylated insulin 5'UTR and purified the binding factor(s) associated with the RNA. The insulin 5'UTR mRNP was isolated by RNA-affinity chromatography using biotinylated 5'UTR element as ligand. Protein Disulfide Isomerase (PDI) was identified as one of the proteins and was confirmed by Western analysis of the eluates. We performed RNA-EMSA in the presence of specific PDI antibodies to show that the complex formed is specific for PDI. We also showed the insulin 5'UTR specific RNA binding activity of PDI in yeast three hybrid assay and RNA immunoprecipiotation. The functional role of PDI as insulin 5'UTR specific translation activator was confirmed by the inhibition of the glucose stimulated translation activation by anti PDI antibody. We further showed that recombinant PDI was able to neutralize the anti-PDI antibody, indicating the specificity of the antibody for PDI. However bacterially expressed recombinant PDI was unable to bind to insulin 5'UTR and cause translation regulation suggesting a role for post translational modification.

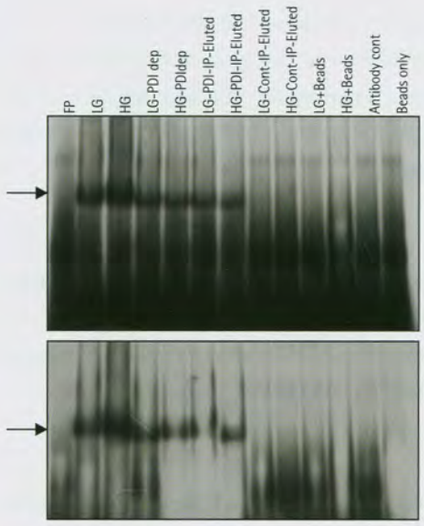


Fig. 1. RNA-EMSA performed with various immunoprecipites from *β*tc6 (upper) and MIN6 (lower) cell lines. The extract used for the RNA EMSA is indicated above each lane with LG and HG indicating low or high glucose extracts respectively. Arrow indicates the position of the RNA-Protein complex.

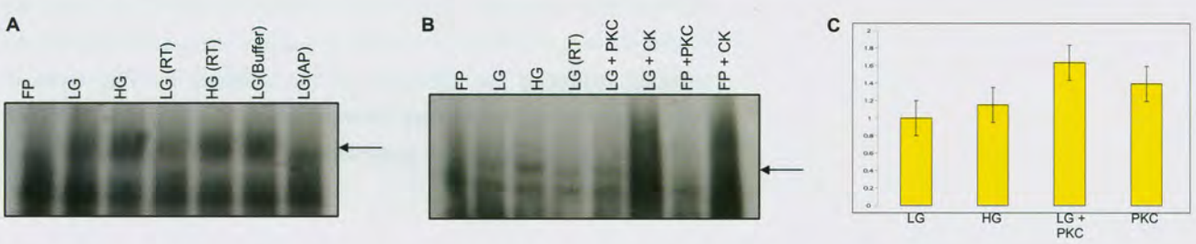
Our previous experiments have shown that PDI is one of the UTR binding proteins in rat pancreatic extract. To check if the same complex exists in pancreatic β-cell lines, we immunoprecipitated PDI from βtc6 and MIN6 cells treated with either low (LG) or high (HG) glucose conditions. Immunoprecipitation of PDI inhibited the complex formation, indicating that PDI is required for complex formation. To further confirm the presence of PDI in the complex, we performed RNA-EMSA using Ins-5'UTR probe with the immunoprecipitated PDI. The Immunoprecipitated proteins formed a complex with the UTR. The binding was specific to anti PDI-IP and not with IgG-IP (Fig. 1). This experiment demonstrated that PDI is indeed present in the UTR binding complex.

Phosphorylation regulates the RNA binding and Translation activity

Since bacterially expressed recombinant PDI was unable to bind to the RNA we investigated a possible role for post translational modification in RNA binding and translation regulation. The HG and the LG extracts were treated with phosphatase and the RNA-EMSA was performed. Phosphatase treatment of the extracts resulted in complete loss of the binding activity (Fig. 2A). We then assessed the role phosphorylation directly by phosphorylating the extract with protein kinase C (PKC). We detected increased binding activity in the LG extract and only a marginal change in the binding activity of the HG extract (Fig. 2B). These results clearly show that phosphorylation is an important step in the insulin 5'UTR-protein complex formation. We performed in vitro translation experiments using luciferase reporter construct and show that increase in binding activity of the extract correlated with increased translation in case of PKC treatment (Fig. 2C), while the phosphatase treatment resulted in complete inhibition of general translation.

We then assessed the ability of the PDI to be phosphorylated by PKC. Incubation of recombinant PDI with PKC and labeled gamma-ATP resulted in specific phosphorylation of PDI (Fig. 3A). We also show that the PDI specific phosphorylating activity is induced by the high glucose (Fig. 3B). Taken

Fig. 2. Phosphorylation regulates the RNA binding and translation activity. **(A)** RNA-EMSA performed with various extracts treated with alkaline phosphatase. The extract used is indicated above each lane. Arrow indicates the position of the RNA-Protein complex. **(B)** RNA-EMSA performed with various extracts treated with PKC and Caseine Kinase CK. The extract used is indicated above each lane. Arrow indicates the position of the RNA-Protein complex. **(C)** In vitro translation of reporter luciferase containing the insulin 5'UTR in the presence of various extracts.



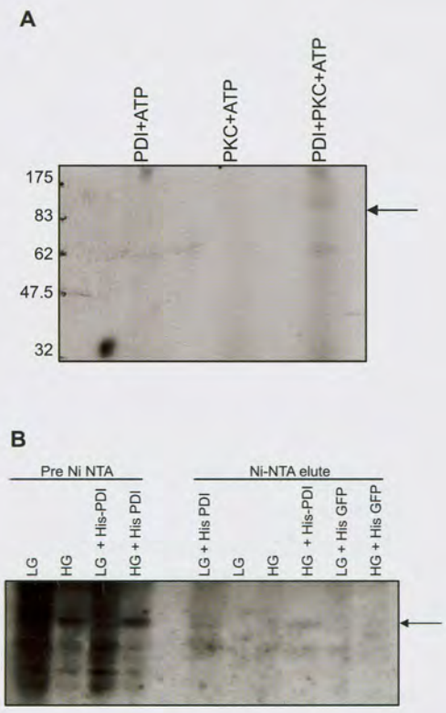
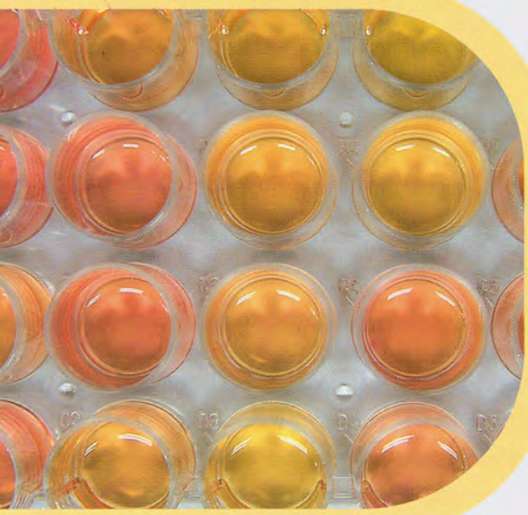


Fig. 3. PDI can be phosphorylated in vitro by **(A)** PKC lanes indicate the reagents added along with recombinant PDI. **(B)** PDI specific kinase activity is induced by high glucose. His tag recombinant PDI was incubated with high or low glucose treated extracts in the presence of the radioactive ATP. The recombinant PDI was then purified using NiNTA coloumn and the purified protein was resolved on 10% SDS PAGE. The arrow indicates the position of PDI.

together, these results suggest that upon glucose stimulation specific PKC is activated. The PKC phosphorylate the PDI and this phosphorylated PDI then associates with insulin mRNA and regulate its translation.

Future Work

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



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

Mohan Wani
mohanwani@nccs.res.in

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

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

Background

The co-ordination between bone forming osteoblasts and bone resorbing osteoclasts is very crucial in maintaining the bone homeostasis and structural integrity of the skeleton. Increased bone resorption by osteoclasts is a major pathological factor in osteoporosis, rheumatoid arthritis, periodontitis, and most adult skeletal diseases. Osteoclasts, the multinucleated cells, differentiate from hemopoietic precursors of monocyte/macrophage lineage. Differentiation of osteoclasts depends primarily on two critical cytokines, macrophage-colony stimulating factor (M-CSF) and receptor activator of NF-κB (RANK) ligand (RANKL), which are produced by stromal cells/osteoblasts. This is evident by the osteopetrotic phenotype of the M-CSF-deficient op/op mouse, and the RANKL-deficient mouse that lacks osteoclasts. The differentiation and activation of osteoclasts are under the aegis of a variety of cytokines. IL-3, a cytokine secreted by activated T lymphocytes is known to regulate the proliferation, differentiation and growth of hemopoietic cells. Although osteoclasts differentiate from hemopoietic stem cells, the role of IL-3 in human osteoclast differentiation and bone resorption is not delineated. In previous studies we have shown that IL-3 significantly inhibits RANKL-induced multinuclear osteoclasts formation in human peripheral blood monocytes (PBMCs). IL-3 treatment reduced the number of tartrate-resistant acid phosphatase (TRAP)- and human $\alpha_v\beta3$ integrin 23c6-positive osteoclasts. IL-3 also inhibited bone resorption through inhibition of human osteoclast differentiation. The quantitative assay of bone resorption confirmed that IL-3 inhibits collagen type I degradation product, the C-terminal telopeptide fragment of collagen type I (CTX-I). In further studies we investigated the mechanism(s) of IL-3 action on differentiation of human osteoclasts.

Aims and Objectives

1. To investigate the role of IL-3 in regulation of human osteoclast differentiation and activation.
2. To understand the mechanism(s) of IL-3 action on human osteoclast differentiation and bone resorption.

Work Achieved

IL-3 irreversibly inhibits human osteoclast differentiation

To investigate at which stage of osteoclast differentiation IL-3 inhibits bone resorption, we incubated PBMCs with M-CSF (25 ng/ml) and RANKL (40 ng/ml), and IL-3 (100 ng/ml) was added for 7, 14, and 21 days, and bone resorption was quantified on day 21. IL-3 treatment for 7 days was sufficient for significant inhibition of bone resorption, and complete inhibition of bone resorption was seen when cells were incubated with IL-3 for 14 and 21 days (Fig. 1A). Osteoclast precursors were then incubated with M-CSF and RANKL, and IL-3 was added to the cultures on days 0, 7, and 14. IL-3 completely inhibited bone resorption when added on day 0, and the inhibitory effect was decreased when addition of IL-3 was delayed. These results suggest that IL-3 does not act on mature osteoclasts (Fig. 1B). We also observed that osteoclasts precursors preincubated with IL-3 were resistant to RANKL action. These results suggest the irreversible inhibitory effect of IL-3 on bone resorption.

IL-3 inhibits human osteoclast differentiation by inhibiting expression of c-Fms on osteoclast precursors

RANKL mediates osteoclastogenesis through binding to its receptor on Osteoclast precurosors, and M-CSF, binding to its receptor c-Fms on Osteoclast precurosors, and function as survival and proliferation factors. Osteoclast precursors sequentially express c-Fms, followed by RANK, and RANK expression in early stage precursor cells is stimulated by M-CSF. To

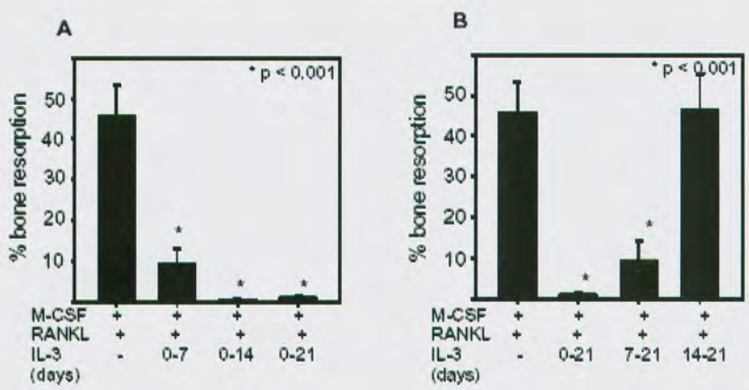


Fig. 1. IL-3 treatment of human osteoclast precursors for 7 days is sufficient for significant inhibition of bone resorption induced by RANKL

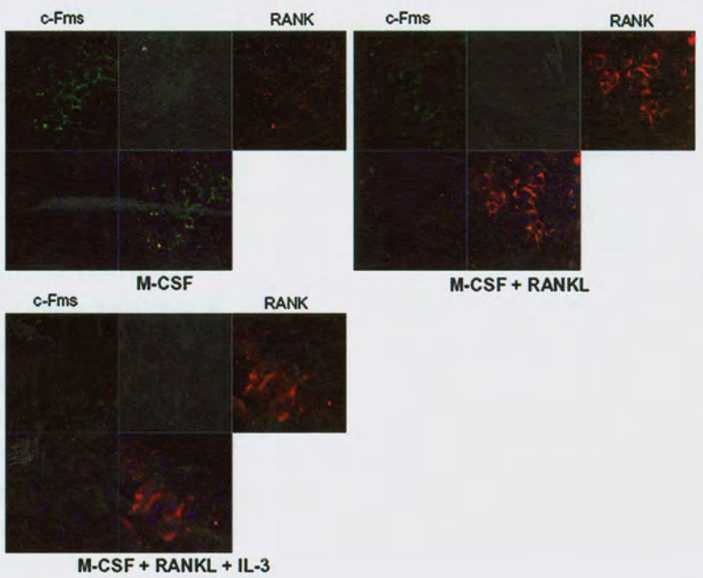
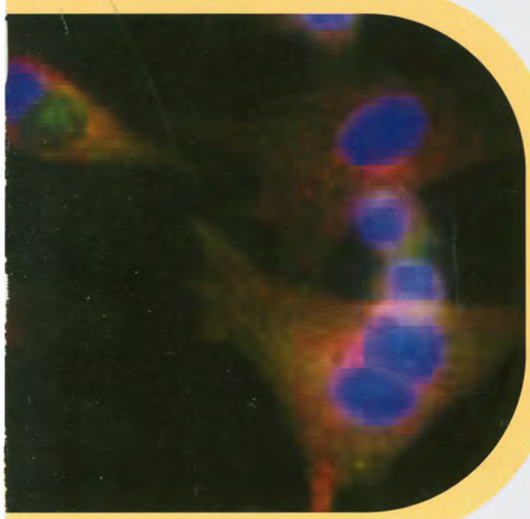


Fig. 2. IL-3 inhibits expression of c-Fms on human osteoclast precursors

investigate the action of IL-3 on proximal signaling we evaluated the effect of IL-3 on c-Fms and RANK expression on day 3 and 7 by flow cytometry. We found that IL-3 significantly down-regulated c-Fms expression on days 3 and 7. RANK expression was slightly down-regulated by IL-3 on days 3 and 7. By immunofluorescence staining we confirmed that IL-3 significantly inhibit c-Fms expression (Fig. 2). Thus, our results suggest that IL-3 inhibits human osteoclast differentiation and bone resorption by inhibiting c-Fms expression.

Future Work

Investigate the role of IL-3 on human osteoblast differentiation, mineralization and bone formation



Nup358 is a negative regulator of Wnt/β-catenin signaling

Jomon Joseph
josephj@nccs.res.in

Participants

Prayag Murawala, *SRF*
Mukesh Mani Tripathi, *SRF*
Pankhuri Vyas, *SRF*
Pabitra Kumar Sahoo, *JRF*
Aparna Salunke, *Technician*

Background

Wnt signaling is a highly conserved pathway that regulates a variety of processes during embryonic development including cell proliferation, cell fate decision, axis formation and mesoderm induction. The key player of Wnt signaling pathway is β-catenin, a protein that has a dual role; acting as a cell-cell adhesion molecule in the cytoplasm and as a transcriptional activator in the nucleus. Wnt is an extracellular protein ligand that binds to specific receptors on the target cells, such as Frizzled (Fz) and Low-density lipoprotein (LDL) receptor-related protein (LRP), and transduces the signal further. In the absence of Wnt a lower cellular level of β-catenin is maintained. Excess β-catenin is phosphorylated by a degradation complex containing Axin, Adenomatous Polyposis Coli (APC), Casein Kinase 1α (CK1α) and Glycogen synthase kinase 3β (GSK3β), and subsequently targeted for ubiquitination followed by proteosomal degradation. Binding of Wnt to its cognate receptor(s) causes inhibition of the degradation complex by a mechanism that is not fully understood, and results in accumulation of β-catenin in the cytoplasm and nucleus. In the nucleus, β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1 (LEF-1)/T cell-specific transcription factor (TCF) to transactivate the expression of a subset of genes. In case of most colorectal cancers (CRCs), the APC gene is mutated leading to the production of a C-terminally truncated protein product. The resultant APC protein fragment lacks the regions required for the degradation of β-catenin and therefore, results in the accumulation of β-catenin in a Wnt-independent manner. The mis-regulation of Wnt signaling is believed to contribute significantly to the development of CRCs.

The transport of β-catenin across the nuclear membrane is critical for the control of Wnt signaling, nevertheless, little is known about the mechanism involved in the nucleo-cytoplasmic transport of β-catenin. The nucleus is surrounded by a double layered membrane and nuclear transport occurs through the nuclear pore complexes (NPCs), the molecular gates present on the nuclear envelope. NPC is a very large protein assembly with an estimated mass of 60-125 MDa in vertebrates. Each NPC contains at least 30 to 50 distinct proteins, generally termed as nucleoporins (Nups).

Although the mechanism of nucleo-cytoplasmic transport of β-catenin is unclear, it is generally believed that the transport would involve interactions between β-catenin and at least some of the nucleoporins. We found that the nucleoporin Nup358 interacts with β-catenin by immunoprecipitation assays using Nup358-specific antibodies in different colon and non-colon cancer cell lines. Preliminary results from our laboratory also indicate that Nup358 associates with other players of Wnt signaling such as APC and GSK 3β. Based on these observations, we hypothesize that Nup358 is involved in the transport of β-catenin across the nuclear envelope and thereby playing a role in the Wnt signaling pathway.

Aims and objectives

1. Characterizing the regions involved in and the nature of interaction between β-catenin and Nup358
2. Investigating the functional significance of Nup358-β-catenin interaction in Wnt signaling in normal and disease conditions

Work Achieved

Nup358 (also called RanBP2) is a large nucleoporin present on the cytoplasmic side of the NPC (Fig. 1). Previous results from our lab indicated that Nup358 interacts with APC and this interaction is important to regulate APC's function in cell polarity during migration. We reasoned that Nup358 being a nucleoporin could also be involved in the nucleo-cytoplasmic

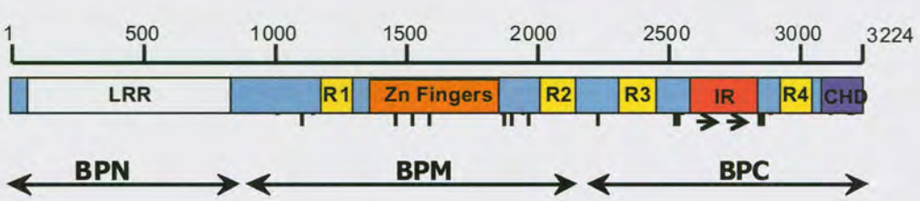


Fig. 1. Schematic representation of Nup358 showing different domains. It is a large nucleoporin (3224 amino acids) present at the cytoplasmic side of the nuclear pore complex. LRR, Leucine Rich Repeat; R1, R2, R3 and R4, RanGTP binding domains; IR, internal repeats; CHD, Cyclophilin Homology Domain. BPN, BPM and BPC represent the N-terminal (900 amino acids), middle (1320 amino acids) and C-terminal (1006 amino acids) used for expression in mammalian cells as GFP-fusion proteins.

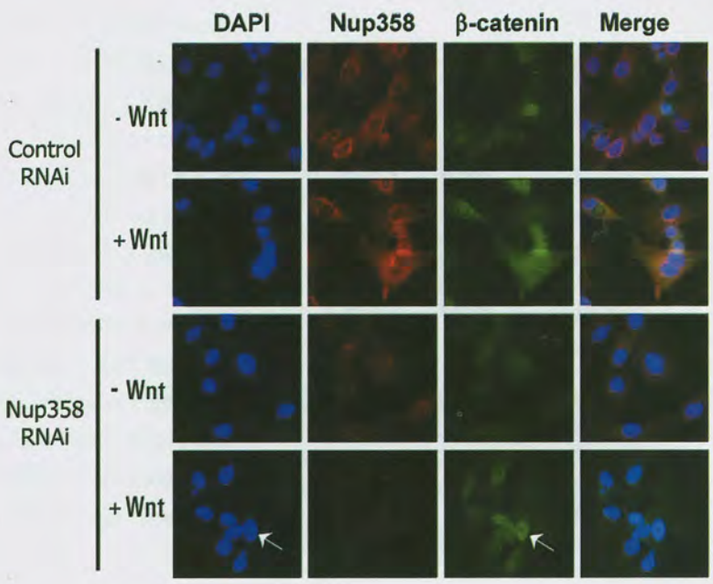


Fig. 2. Nup358 depletion results in increased accumulation of β-catenin in the nucleus. CHO-K1 cells were subjected to Nup358 RNAi and treated with control (-Wnt) or Wnt3A (+Wnt) conditioned medium. Cells were fixed and analyzed for Nup358 depletion (red) and β-catenin localization (green). Arrows indicate β-catenin accumulation in the nucleus of Wnt3A-treated, Nup358-depleted cells.

transport of β-catenin. As mentioned earlier, Nup358 physically interacts with β-catenin in colon and non-colon cancer cell lines.

Nup358 siRNA impairs the nucleo-cytoplasmic localization of β-catenin during Wnt signaling

To explore the possibility that Nup358 plays a role in the localization of β-catenin, CHO-K1 cells were treated with control or Nup358-sepcific siRNAs and analyzed for the localization of β-catenin after incubation with Wnt3A- or control-conditioned medium for three hours. In the control-siRNA treated cells, Wnt3A-induced β-catenin was found to be more-or-less uniformly distributed in the nucleus and cytoplasm, whereas in Nup358 depleted cells, β-catenin was relatively enriched in the nucleus as compared to the cytoplasm (Fig. 2). Previous studies showed that β-catenin shuttles between nucleus and cytoplasm, and our result suggests that in the absence of Nup358, either the active export of β-catenin out of the nucleus or its cytoplasmic retention is impaired. A recent study, however, demonstrated that β-catenin localization is regulated by a retention mechanism mediated through interaction with the β-catenin-binding proteins. Hence, the results reported here, points to a role for Nup358 in β-catenin localization, particularly by retaining it in the cytoplasm.

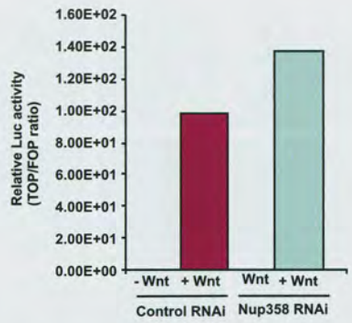


Fig. 3 Nup358 RNAi results in enhanced Wnt/β-catenin signaling. CHO-K1 cells that were transfected with initially control or Nup358-specific siRNA oligos and later with TOP or FOP constructs, were treated with control (-Wnt) or Wnt3A (+Wnt) conditioned medium and assayed for TCF/β-catenin dependent translational activation of the luciferase reporter gene.

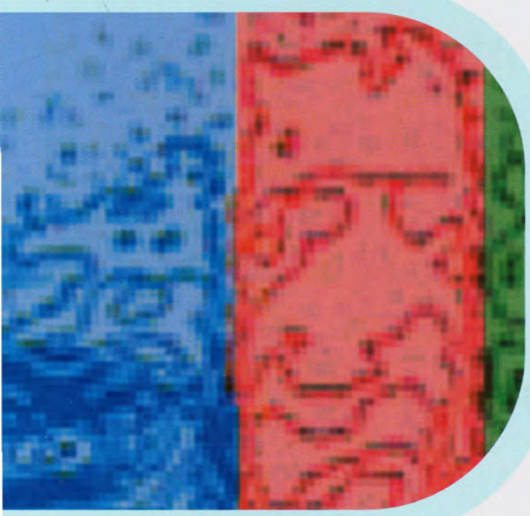
Nup358 negatively regulates Wnt/β-catenin signaling

Accumulation of β-catenin in the nucleus when Nup358 was depleted prompted us to check if it resulted in increased β-catenin signaling. For this we used a luciferase based assay, which involves luciferase expression driven by a TCF/β-catenin based promoter (TOP) or a mutant promoter (FOP). CHO-K1 cells were initially transfected with control or Nup358-sepcific siRNAs for 48 hours and later transfected with TOP or FOP constructs for 12 hours. Control or Wnt3A conditioned medium was added to the cells for 18 hours and the luciferase activity was measured. Such a TOP/FOP based assay indicated that in Nup358-depleted cells, upon Wnt3A addition, β-catenin signaling was significantly enhanced as compared to control siRNA-treated cells (Fig. 3). These results are consistent with increased nuclear accumulation of β-catenin in the absence of Nup358, and suggest that this nucleoporin negatively regulates Wnt signaling, possibly by retaining β-catenin in the cytoplasm.

Future Work

1. Define the regions involved in and the mode of interaction between Nup358 and β-catenin
2. Understand the molecular mechanism by which Nup358 regulates β-catenin localization

Cancer Biology



Label Retention Based Identification of Quiescent Cancer Stem Cells

Sharmila Bapat
sabapat@nccs.res.in

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

Background

Tumor dormancy is an operational term that presents a conceptual framework for elucidating a prolonged quiescent state in which tumor cells exist, but clinical disease progression is not evident. Tumor dormancy thus reflects the persistence of certain quiescent residual tumor cells within the host, occasionally for time periods extending up to decades after treatment, however eventually followed by relapse. The exact identity and nature of cells that contribute to tumor dormancy remains poorly elucidated. In recent years, cancer has been illustrated as a stem cell disease. This hypothesis entails the notion that a rare fraction of self-renewing cells termed as cancer stem cells (CSCs) are responsible for the tumor development, maintenance and recurrence. Derivation of the CSC terminology for this rare cellular fraction is an outcome of their similarities with adult stem cells. Analogous to normal stem cells that are protected and nurtured in a quiescent state within the tissue niche, it has been speculated that the CSCs retain this property. Correspondingly, cancer dormancy has been conjectured to be determined by CSC quiescence. However experimental proof for the capacity of CSCs to undergo quiescence is still lacking. Identifying the quiescent cell type/s that contributes to the tumor dormancy is imperative with the perspective of developing effective therapeutic strategies adept to target this resistant cellular fraction.

Aims and objectives

1. To isolate quiescent/dormant cells from tumor tissues based on label retention
2. To evaluate whether these label retaining quiescent cells posses stem cell-like characteristics and functionality

3. To identify the effects of short- and long-term drug treatment on enrichment of cell label retaining quiescent cells as a reflection of tumor dormancy

Work Achieved

Identification of a proliferation hierarchy marked by long-term label retaining cells within the primary tumors and metastases

Long-term label retention is a broadly used criterion for the identification and isolation of the relatively quiescent stem cells from several adult organs. A standard label retaining cell (LRC) assay exploits the relatively quiescent nature of the stem cells due to which these cells retain the label while more rapidly dividing, transit-amplifying cells generated through the intermittent divisions of the stem cells undergo dilution of the label. LRCs thus represent experimentally marked, *in vivo* quiescent stem cells residing in the stem cell niche within each tissue. PKH labels consist of a fluorophore attached to an aliphatic carbon backbone that irreversibly binds to the lipid bilayer on the cell membrane. Consequently, following each cell division, the PKH label gets equally partitioned among the daughter cells, resulting in a gradual reduction of fluorescence intensity. We exploited the classical LRC assay to decipher whether the CSCs existed as a relatively quiescent fraction within the tumors that would consequently correlate with tumor dormancy. Towards this, human ovarian cancer cells (A4 cells) were PKH labeled and injected subcutaneously into NOD/SCID mice for tumor development. Post 3 weeks of injections, tumors were harvested; collagenase digested and analyzed for the distribution of PKH intensity. Such analysis demonstrated arrangement of the tumor cellular content into a continuous gradient ranging from high PKH intensity (equivalent to pre-injected cells) to cells that underwent complete quenching of the PKH dye. Such a characteristic PKH intensity profile observed here indicated the subsistence of a distinctive proliferation heterogeneity during tumor development. Apart from the identification of the PKH^{hi} fraction, arrangement of the tumor cellular content along the PKH intensity gradient enabled the identification of two additional cellular subsets; namely the PKH^{lo} subset with dim PKH fluorescence and the PKH^{neg} subset that had completely quenched the label. The PKH based proliferation hierarchy thus identified was concluded to consist of three subsets (Fig.1A), namely: 1) PKH^{hi} subset forming the pinnacle of this hierarchy due to their no-to slow-cycling nature; 2) PKH^{lo} subset with limited proliferation potential and hence retains dim fluorescence and 3) PKH^{neg} subset that had undergone rapid consequent divisions required for complete label quenching. In addition to primary tumors LRCs were detected within the tumor ascites and secondary

tumors generated by A4 cells in mouse models. In conclusion, this data provided the evidence for the persistence of the long-term label retaining cells during the development of primary tumors and also during metastasis and associated disease progression.

Long-term label retaining subset marked the relatively quiescent CSC fraction

Prompted by the above findings we next investigated whether the LRCs identified as PKH^{hi} subset represented the CSC fraction within tumors. Towards this we analyzed the clonogenic and tumorigenic potentials (functional parameters to identify CSCs) of the PKH^{hi} subset as compared to the PKH^{lo} and PKH^{neg} subsets. PKH^{hi} subset demonstrated an elevation of both of these potentials as compared to the PKH^{lo} subset while the PKH^{neg} subset almost lacked these capacities. Thus these functional assays demonstrated that albeit the fact PKH^{hi} cells stayed quiescent within the tumor niche, they had high proliferation and clonogenic capacities, a proof of their CSC identity. This strongly supports our notion that CSCs, due to their relatively quiescent nature, can be isolated from experimental tumors as LRCs. At the same time lack of these potentials by the PKH^{neg} subset reflected their differentiated nature. Analysis of the stem cell markers Nestin, Oct4, Nanog and Bmi provided a further support to our above findings. While the Nestin and the Oct4 were exclusively expressed by the PKH^{hi} subset, the markers Nanog and Bmi showed an up regulation in PKH^{hi} subset (Fig. 1B-i). Recently, the c-kit+/CD44+ cell surface phenotype had been shown to define ovarian CSCs.

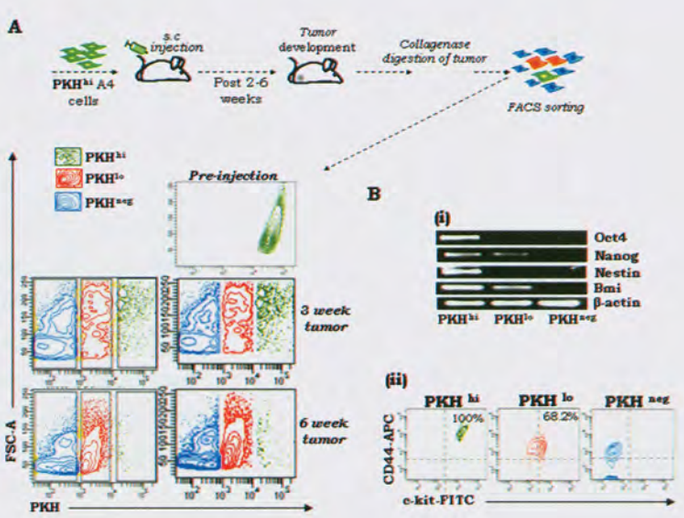


Fig. 1. Identification of long-term label retaining CSCs within primary tumors. **(A)** Schematic representation of the experimental design adapted for the PKH label based in vivo detection of LRCs / quiescent cells within tumors. Representative PKH intensity profiles of the pre-injected cells, three week and six week A4 tumors. **(B)** (i) Top panel represents the semi-quantitative PCR analyses of Bmi1, Nestin, Oct4 and Nanog expressions that were performed with tumor derived PKH^{hi}, PKH^{lo} and PKH^{neg} cell populations. β-actin mRNA expression was used as an internal control. (ii) Representative contour plots of the flow cytometric analysis for the expression of the ovarian CSC markers *viz.* c-kit and CD44 in the three PKH subsets.

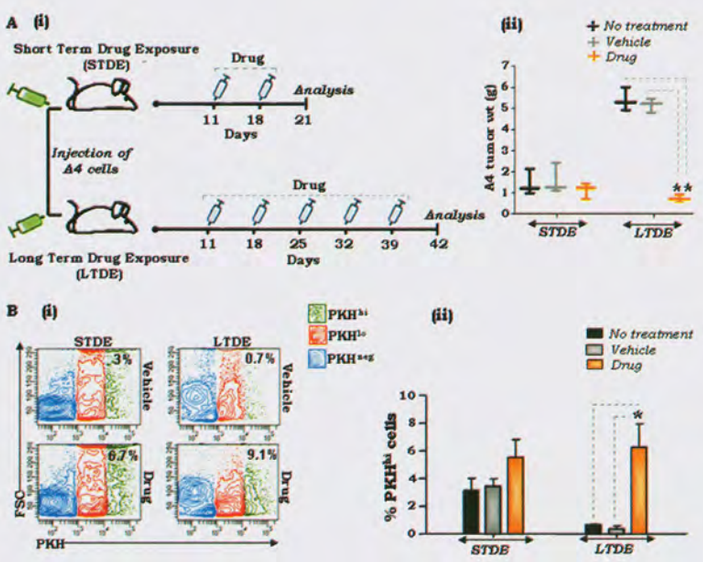


Fig. 2. Chemotherapy leads to an enrichment of quiescent CSCs within tumors. **(A)** (i) Schematic representation illustrates the experimental design adapted for the short-term drug exposure (STDE) and the long-term drug exposure (LTDE) regimes. (ii) Line plots with their median values represent the weights of tumors generated after subcutaneous injections of A4 cells in the NOD/SCID mice either unexposed to the drug treatment i.e. no treatment; the vehicle controls that were exposed to DMSO or the mice receiving drug (paclitaxel) treatment. The mice receiving the drug treatment were under two groups namely STDE and LTDE. (**p<0.01). **(B)** Representative dot plots (i) and the error bar graphs (ii) indicate the percentage of PKH^{hi} cells within A4 tumors harvested post-STDE, LTDE and their respective vehicle controls (*p<0.05).

We thus further determined whether the PKH^{hi} subset identified in our study also conformed to these phenotypic expressions. This analysis demonstrated that the PKH^{hi} subset was double positive for these ovarian CSC markers (Fig. 1B-ii). Thus, the label retaining PKH^{hi} subset conformed to the functional and the phenotypic standards adopted for the identification of CSCs from tumor tissues. Collectively, the above results firmly establish that the long-term LRCs identified in our study were indeed the relatively quiescent CSCs.

Chemotherapeutic exposures lead to an enrichment of quiescent CSCs

The identification and isolation of CSCs as LRCs achieved in our study established the ability of CSCs to stay in a state of quiescence. To validate the further derivation from these properties viz. that CSCs contribute to cancer dormancy, we investigated the outcome of the chemotherapeutic exposure on the quiescent CSC/PKH^{hi} subset. PKH labeled cells were s.c. injected into NOD/SCID mice; subsequently mice were exposed to 25 mg/kg paclitaxel, while the vehicle control groups were treated with DMSO. The two regimes followed for the treatment included short-term drug or long-term drug exposure (STDE and LTDE respectively - Fig. 2A). Analysis of tumor weights in both treatment groups as compared to their respective control groups demonstrated that while enrichment of PKH^{hi} cells was evident in both groups, only the LTDE was effective in inducing a significant reduction in the tumor weight (Fig. 2B). If as speculated the quiescence of CSCs operates like a safeguard mechanism to shield treatment effects consequently leading to

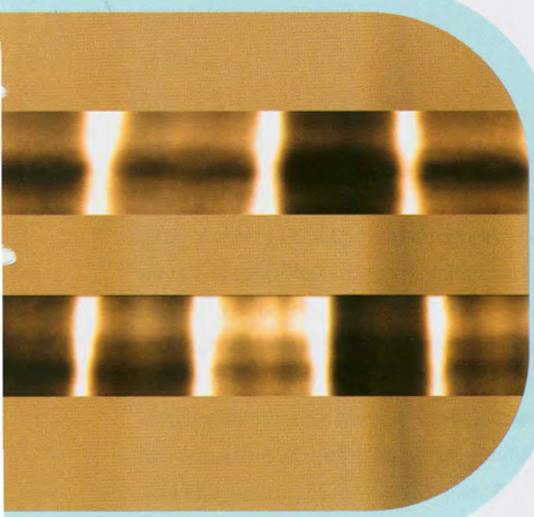
ineffective treatment in targeting these CSCs; the exposure to the paclitaxel should lead to an enrichment of the quiescent CSCs within the tumors followed by disease recurrence on withdrawal of treatment. While enrichment of the PKH^{hi} CSCs following chemotherapeutic exposures is evident within tumors the further effects on drug withdrawal are being evaluated.

In summary our study demonstrates-

- The persistence of a distinctly quiescent long-term label retaining fraction of cells within tumors. Subsequent analysis of functional and phenotypic characteristics revealed the CSC identity of this LRC fraction. Correspondingly, along with expression of stem cell associated markers, this subset also exhibited elevated clonogenicity and tumorigenicity.
- Chemotherapeutic regimes results in a significant enrichment of quiescent CSCs within the tumors thereby reflecting the therapeutic escape potential of this fraction and suggesting their contribution to the tumor dormancy.

Future Work

To decipher interactions and systemic signals that governs the maintenance of the quiescent state and the switch between quiescence and proliferation in these CSCs.



Chemo-sensitivity of cancer cells to drugs: elucidation of mechanism of cell death and resistance

Manoj Kumar Bhat
manojkbhat@nccs.res.in

Participants

Mr. Amrendra Kumar Ajay, *SRF*
Mrs. Ratna Kumari, *SRF*
Miss. Vimal Pandey, *SRF*
Miss. Aanchal Sharma, *SRF*
Mr. Avtar Singh Meena, *JRF*
Mr. Vijayakumar M.V., *Technical officer A*

Background

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

Cell cycle plays a central role in normal growth and development. Deregulation of cell cycle control leads to uncontrolled cell division which is one of the initial events in the development of all types of cancers. Cyclin dependent kinases (Cdks) are the one which decide the fate of cell cycle and their discovery has always fascinated the cell biologist involved in drug-discovery. These kinases are positively regulated by cyclins (A, B, D and E) and are negatively regulated by cyclin dependent kinase inhibitors (CdkIs). Interestingly, neuron-specific cyclin dependent-kinase, Cdk5, is a member of small serine/threonine kinases family that is ubiquitously distributed in most tissues, including proliferating cells. Cdk5 is primarily functional in post mitotic neuronal tissues. These cells specifically express the non-cyclin Cdk5 activator proteins, p35, p39 and a proteolytic fragment of p35 i.e. p25. Till now, little is known about its functional role in cell cycle regulation or in cancer though recently in few reports, the expression and activity of Cdk5 has been reported in prostate, breast and medullary thyroid carcinoma cells.

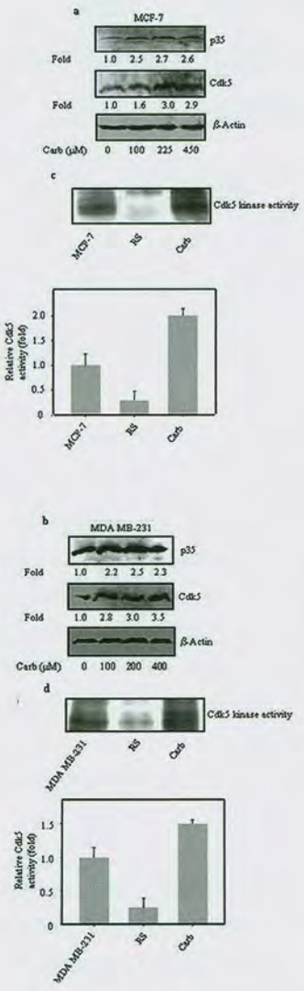


Fig. 1. Expression of p35, Cdk5 and activity of Cdk5 increases following treatment with carboplatin. MCF-7 **(a)** and MDA MB-231 **(b)** cells were treated with increasing concentration of carboplatin for 36 h. In all the panels, cells were harvested and equal amount of protein was processed for western blot analysis to detect p35 and Cdk5 protein expression. Same membranes were stripped and reprobed for β-Actin to ensure equal protein loading. Densities of the bands were quantified by image analyzer and fold expressions are with reference to control cells. In kinase assay (**c** and **d**), cell lysates were immunoprecipitated with anti-Cdk5 antibody which were subjected to in vitro (^{32}P) ATP kinase assay using Histone H1 as the substrate. Densities of the bands were quantified by image analyzer.

We analyzed the possible involvement of neuronal cell-specific molecule Cdk5 in the proliferation as well as chemosensitivity of breast cancer cells by utilizing Cdk5 specific inhibitor(s). Our findings suggest that Cdk5 positively regulates the proliferation of MCF-7, and hyper-activation of Cdk5 after carboplatin treatment is involved in death of MCF-7 and MDA MB-231 cells. Earlier, we and others have reported that carboplatin treatment also activates ERK, which is known to play a central role in cell death in different cancer cell-types. Collectively, based on our findings, we propose a novel mechanism of ERK mediated cell death in MCF-7 and MDA MB-231 cells following carboplatin treatment, which involves activation of its new downstream target, Cdk5. Overall, the data presented here not only has important implications in understanding of the molecular mechanisms underlying carboplatin induced cell death in breast cancer cells but also emphasize the significance of Cdk5 activation in cancer cell death.

Aims and objectives

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

Work achieved

Carboplatin induces the expression and activation of Cdk5 and its regulatory protein (p35) in MCF-7 and MDA MB-231 cells

To examine the effect of carboplatin treatment on Cdk5 and p35 expression, MCF-7 and MDA MB-231 cells were treated with indicated doses of carboplatin for 36 h and assessed by immunoblot analysis. As shown in Fig. (1), carboplatin treatment resulted in increased Cdk5 protein expression in MCF-7 (Fig. 1a) and MDA MB-231 (Fig. 1b) cells. Simultaneously, increased expression of p35 protein was detected in both the cells following carboplatin treatment (Fig. 1a, b). We also investigated the changes in Cdk5 activity following carboplatin treatment by *in vitro* Cdk5 kinase assay in MCF-7 and MDA MB-231 cell-lysates and observed almost 1.6 fold increase in Cdk5 activity (as indicated by increased phosphorylation of Histone H1) in carboplatin treated cells (Fig. 1 c, d). In all kinase assays roscovitine (RS) treated cells were used as negative control to ascertain specificity. These data clearly suggest that carboplatin not only increases Cdk5/p35 protein expression, but also enhances Cdk5 activity in both these cell lines.

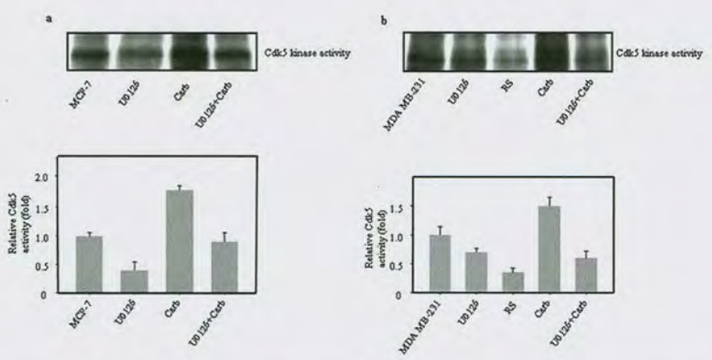


Fig. 2. Blocking of basal as well as carboplatin induced ERK activation inactivates Cdk5 in cells. MCF-7 **(a)** and MDA MB-231 **(b)** cells were treated with indicated concentration of U0126 (20 μM), carboplatin (450 μM in MCF-7; 400 μM in MDA MB-231) or RS (20 μM) as per experimental requirement. In all panel inhibitors or drug treatment were given for 36 h either alone or in combination. Cells were treated with U0126 1 h prior to addition to carboplatin and further exposed in presence of inhibitor for 36 h. Cdk5 kinase activity was examined by autoradiography using Histone H1 as a substrate as given in material and method section.

Carboplatin induced activation of Cdk5 is directly associated with ERK mediated cell death

Many conflicting reports in neuronal cells suggest that ERK activation may be either upstream or downstream of Cdk5, which probably depends on the normal or stressed condition of the cells. We investigated whether there is a direct link between ERK and Cdk5 in breast cancer MCF-7 and MDA-MB 231 cells. Inhibition of ERK activity by U0126 significantly blocked Cdk5 kinase activity in MCF-7 and MDA MB-231 cells (Fig. 2 a, b). Importantly, treatment with ERK inhibitor not only decreased basal Cdk5 activity, but also diminished carboplatin induced Cdk5 activation. This result provides a strong evidence for ERK being an upstream activator of Cdk5 in MCF-7 and MDA MB-231 cells. Collectively our data suggest that ERK and Cdk5 kinases coordinate at basal level, which is important for cell survival. Interestingly, in response to carboplatin treatment, their activation results in cell death in MCF-7 and MDA MB-231 cells.

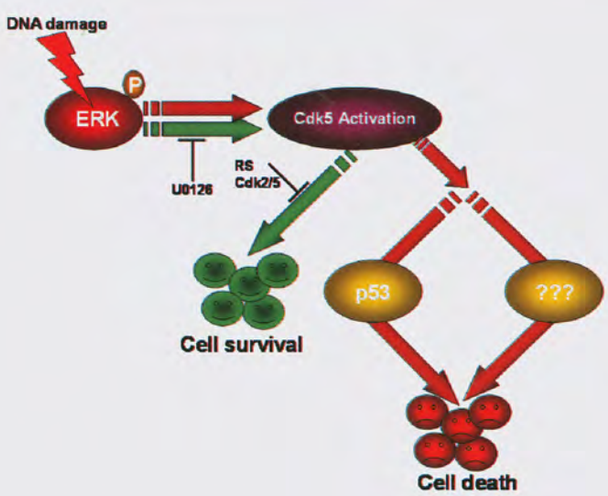
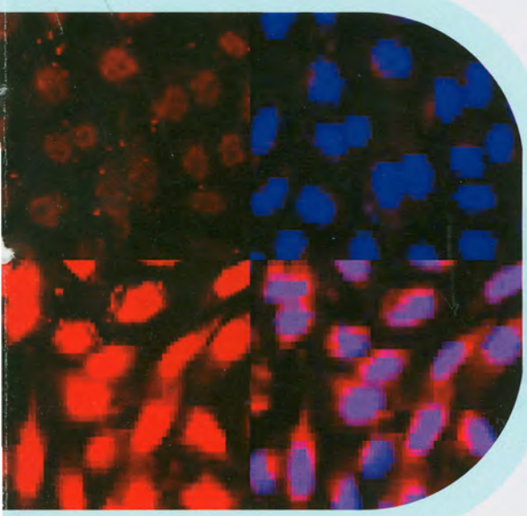


Fig. 3. Proposed model for regulation by Cdk5

Our data provide a strong basis to state that MCF-7 and MDA MB-231 cells express functional Cdk5 protein. The basal activity of Cdk5 is essential for proliferation of these cells. We propose a novel mechanism of ERK induced cell death involving Cdk5 as a molecular target of ERK in carboplatin treated MCF-7 and MDA MB-231 cells. Moreover, ERK activation is associated with cell death in both the cell lines via Cdk5 activation, independent of p53 status. The Cdk5 mediated downstream cell death pathway may be p53 dependent, as well as p53 independent (Fig. 3). Overall, this work assigns a key role to Cdk5 as one of the regulators in carboplatin induced cell death and suggests that Cdk5 can be potentially used as a novel target of chemotherapeutic drugs for better treatment of breast cancer.

Future work

Effects of chemotherapeutic drugs on cell lines derived from various human solid tumors will continued to be investigated.



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

Padma Shastry
padma@nccs.res.in

Participants
Sri Gowry Das G, *SRF*
Radha Pujari, *SRF*
Mruthyunjaya. S, *SRF*
Dr. Sonia Kale, *PDF*
Jayashree C. Jagtap, *Technical Officer A*

Collaborators
Dr. Anjali Shiras
Dr. Avinash Pradhan, KEM Hospital, Pune
Dr. Rumma Manchanda, KEM Hospital, Pune.

Background

For many decades, the mean survival of patients with Glioblastoma multiforme (GBM) - the most malignant form of brain tumors- has been less than a year despite advancements in conventional treatment regimes. This grim scenario has led the clinical researchers to focus on understanding the glioma biology and devise improved strategies to combat this aggressive tumor. Macrophages comprise an important component of the immune system against tumors. Macrophages are recruited and remain at the site of tumor (referred to as Tumor Associated Macrophages -TAMs) constituting a major proportion of the tumor mass. TAMs exert influence on tumors by secretion of a variety of molecules such as chemokines, growth factors, Matrix Metallo-proteinases (MMP) and cytokines like Tumor Necrosis Factor (TNF-α), IL-1β and IL-6. Studying the microenvironment of the tumor is therefore important in understanding gliomagenesis. TNF-α is an activator of the major survival pathways NF-κB and PI3K/Akt. Malignant gliomas constitutively express high levels of activated Akt and NF-κB which correlate with the aggressive nature and resistance of these tumors.

Aims and Objectives

1. Identification of the downstream targets of Akt and NF-κB pathways in TNF-α-mediated responses in gliomas.
2. To understand the mechanism(s) that contributes to resistance in these tumors with focus on activation and consequence of Akt/mTOR in gliomas.

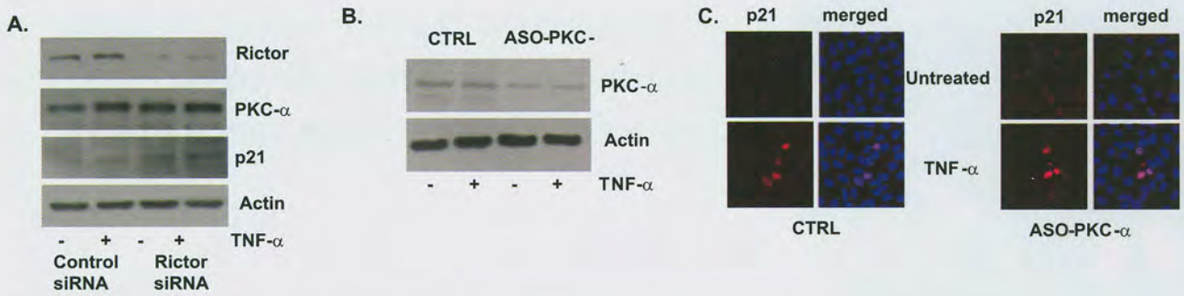


Fig. 1. Rictor PKC-α and p21. **(A)** Untransfected and Rictor siRNA transfected LN18 cells were treated with TNF-α (10ng/ml) for 12hr. Whole cell lysates were subjected to immunoblot assay for the expression of rictor, PKC-α and p21. **(B)** Untransfected and PKC-α ASO transfected LN18 cells were treated with TNF-α and assessed for the expression of PKC-α **(C)** Expression and localization of p21 in untransfected and PKC-α ASO transfected LN18 cells treated with TNF-α p21 was probed using Cy-3 labeled secondary antibody. The nucleus was stained with DAPI (blue). Magnification 63X.

Work achieved

The PI3K/Akt pathway is an important survival pathway that has been reported to be constitutively active in gliomas. For complete activation of the pathway to occur, Akt requires to be phosphorylated at two major sites- T308 and S473. Of the pathways that are activated by Akt, the mTOR pathway is emerging to be the focus for understanding glioma biology. mTOR directly or indirectly affects many important functions such as translation, actin remodeling, survival and growth by forming complexes with raptor (mTOR Complex 1) or rictor (mTOR Complex 2). While the mTOR/raptor interaction and its downstream signaling is reported, little is known about mTORC2 and rictor during TNF-α-mediated response. As rictor is over expressed in gliomas and increases mTORC2 assembly, the aim of the present study was to investigate the role of rictor in TNF-α-mediated functions such as proliferation and invasion in gliomas,

In many cell systems, rictor-mTOR complex is shown to modulate the phosphorylation of PKC-α at S657. As TNF-α enhanced the activity of rictor, we examined the effect of its activation on PKC-α in human glioma cell lines. LN-18 cells treated with TNF-α demonstrated increased expression of PKC-α.

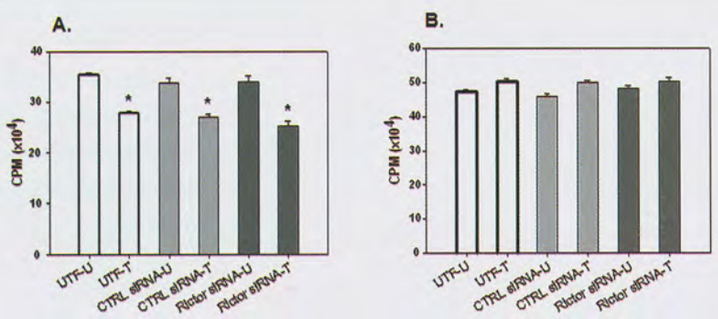


Fig. 2. Rictor-upregulated p21 did not affect TNF-α-induced inhibition of proliferation. Untransfected (UTF), control siRNA (CTRL) and Rictor siRNA transfected LN18 **(A)** and LN229 **(B)** cells were treated with TNF-α (10ng/ml) for 24hr. Proliferation of the cells was determined by tritiated thymidine incorporation assay. U-untreated, T-TNF-α treated. *p<0.05 compared to untransfected, untreated cells.

Interestingly, knock down of rictor enhanced the level of PKC-α significantly and was further increased upon treatment with TNF-α. In many tumors including gliomas, PKC-α plays important regulatory role in cell cycle progression by affecting the CDKi-$p21^{waf1/cip1}$. Rictor-silenced cells showed significant increase in the expression of $p21^{waf1/cip1}$ as compared to control transfected cells and the level was further augmented with TNF-α treatment (Fig. 1A). However, down regulation of PKC-α by antisense oligonucleotide (ASO) showed no effect on p21waf1/cip1 indicating that rictor-regulated $p21^{waf1/cip1}$ was independent of PKC-α (Fig. 1B, C). We earlier reported that TNF-α-induced inhibition of proliferation in LN-18 cells was $p21^{waf1/cip1}$-dependent. It was therefore expected that the increased expression of p21waf1/cip1 on silencing rictor would result in a corresponding inhibition of proliferation. Our data with control transfected and rictor-silenced cells of both LN18 and LN229 as assessed by tritiated-thymidine incorporation assay suggested that an increase in PKC-α and $p21^{waf1/cip1}$ levels on silencing rictor, was not efficient in controlling proliferation (Fig. 2). More importantly, ablation of rictor increased MMP-9 secretion and activity (Fig. 3A). Knockdown of rictor had no effect on TNF-α-induced MMP-9 activity. These functions were independent of PKC-α as cells transfected with antisense oligonucleotides directed to PKC-α-did not mimic rictor-silenced cells (Fig. 3A, C). Further studies are aimed at understanding the TNF-α-induced pathway and the role of rictor/mTORC2 in secretion and activity of MMP-2 and MMP-9.

Future work

To study the involvement of mTOR components in biological activity of TNF-α- and IL-1β in context with tumor progression, aggressiveness and invasive potential of gliomas.

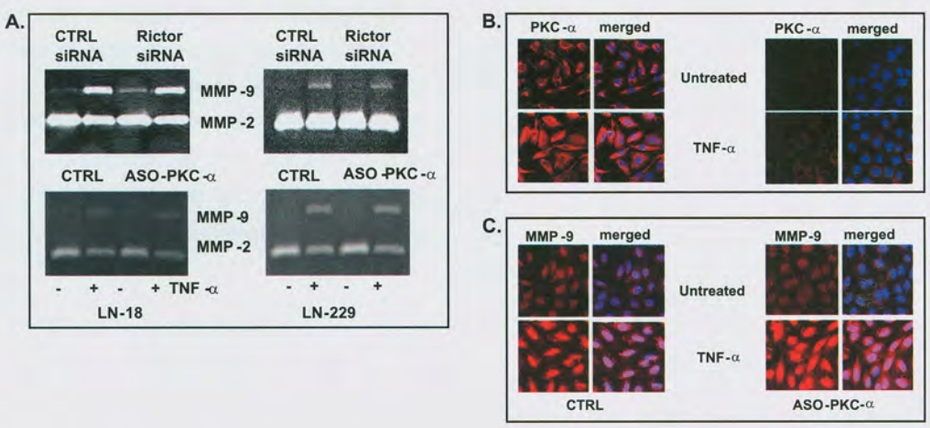


Fig. 3. TNF-α induced MMP-9 is independent of PKC-α. **(A)** Untransfected and Rictor siRNA or PKC-α ASO-transfected LN18 and LN229 cells were treated with TNF-α (10ng/ml) for 12hr. Conditioned medium was subjected to zymography analysis for MMP-9 and -2 activity. Expression of **(B)** PKC-α, **(C)** MMP-9 in untransfected and PKC-α-ASO transfected LN18 cells treated with TNF-α (10ng/ml) for 12hr. MMP-9 was detected by staining with Cy-3 labeled secondary antibody. The nucleus was stained with DAPI (blue). Magnification 63X.

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

Anjali Shiras
anjalishiras@nccs.res.in

Participants
Ms. Navjot Kaur, *SRF*
Mr. Sivaranjan Chettiar, *SRF*
Mr. Ganeshkumar Rajendran, *SRF*
Ms. Sandhya B., *SRF*
Dr. Varsha Shepal, *Technical Officer A*

Collaborator
Dr. L.C. Padhy, TIFR, Mumbai

Background

Recent studies have shown that ncRNAs make up a large proportion of mammalian transcripts, and that the ratio of ncRNA to protein coding RNA increases with the complexity of the organism, suggesting important functional roles for ncRNAs. Because ncRNAs are typically expressed at lower levels than protein coding mRNAs, they are known to fulfill a regulatory role with diverse cellular functions that include transcriptional activation, gene silencing, imprinting, dosage compensation, translation, silencing, modulation of protein function and acting as riboswitches. Some ncRNAs have been shown to be developmentally regulated, while expression of other ncRNAs is correlated with various disease states. With an aim to study novel genes in melanoma genesis, we generated an expression cDNA library from mouse melanoma cells - Clone M3. From this Clone M3 cDNA, a new kind of molecule M3TR was identified that was analyzed and found to be a non-coding RNA (ncRNA). This non-coding RNA obtained through experimental cDNA library screening and bio-computational methods, displays transforming potential on ectopic expression and is expressed as a functional anti-sense transcript complementary to repeats throughout the genome. Interestingly, it shows preponderant expression in the embryos and brain suggesting that its expression is biologically relevant with important roles during development and neurogenesis. Our work focuses on using different approaches to look into the role of this RNA during normal homeostasis, development and tumorigenesis.

Aims and Objectives

1. To characterize the non-coding RNA M3TR, with respect to its role in cell proliferation
2. To study the biological function of M3TR by ectopic expression studies in human and mouse cells
3. To understand the molecular mechanisms and signaling pathways elicited by M3TR that lead to cell transformation.

Work Achieved

Previous work from our lab identified a 612 base-pair (bp) cDNA, termed M3TR, from Clone M3-melanoma cells. The M3TR transcript was apparently derived from the transcription of a genomic segment close to the band XA6 in mouse X-chromosome (Fig 1) and functioned as an enhancer of cell growth. Expression profiling using reverse transcription polymerase chain reactions (RT-PCR) demonstrated that Ginir ncRNA was a poly-adenylated nuclear RNA. More significantly, its expression was not confined to the parental M3 melanoma cells but extended to other cells such as NIH3T3 cells and to the cells of the developing mouse embryonic tissues. One of the aspects of our study was to determine if M3TR is part of a larger transcript, to clone that transcript and to examine its ability to induce cell proliferation. Northern blot analyses in conjunction with strand-specific reverse transcription and qPCR revealed two novel transcripts, one antisense referred to as M3TR-as and one sense transcript, M3TR (Fig 2). In independent experiments set up to evaluate the role of M3TR and M3TR-as transcripts in vivo, we found that when the same numbers of NIH-M3TR and NIH-M3TR+M3TR-as cells were inoculated into nude mice, they grew into different sized tumors over a fixed duration of three weeks (Fig 3). Collectively, these data demonstrate that while M3TR is a positive modulator of cell cycle progression, the anti-sense transcript M3TRas retards tumor growth by interfering with sense transcript function of promoting cell cycle progression. In our study, we demonstrate that M3TR is a member of a pair of sense and anti-sense transcripts (SAST) playing opposing roles in the regulation of cell cycle, tumor suppression and oncogenesis. An ectopic and sustained expression of the M3TR-sense RNA (M3TR) induces premature cell cycle progression, replicative stress, genomic instability and oncogenic transformation by targeting the functions of the tumour

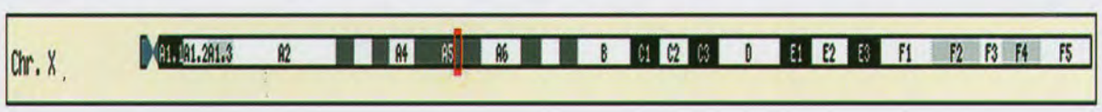


Fig. 1. Localization of M3TR (GenBank accession no. EF649772) to Chromosome X near band XA6 in mouse X-chromosome.

suppressor protein fragile histidine triad (fhit). By contrast, the antisense transcript (M3TR-as) modulates M3TR functions as demonstrated by its ability to retard the growth of tumors induced by M3TR. Collectively, these data identify a novel mechanism to regulate cell cycle progression and suggest that some of the cancers might originate from deregulated expression of a pair of SAST. Our results provide support to the hypothesis that mechanisms like SAST may be effective in orchestrating gene regulation, chromatin remodeling, animal development and stem cell differentiation.

Future Work

1. Evaluate the role of M3TR in inducing pluripotency and decipher the mechanisms contributing towards establishment and maintenance of stemness
2. Identify the transcriptional targets of M3TR. These targets could be could either be acting singularly or may act through a multitude of target genes possibly belonging to the family of tumor suppressors
3. Study the role of this RNA during normal homeostasis, development and pathogenesis

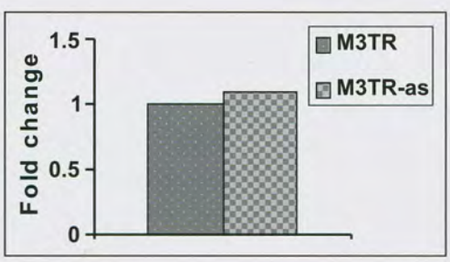
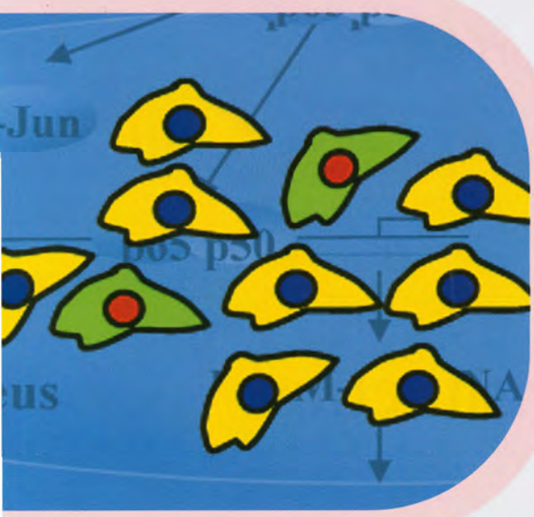


Fig. 2. Expression of sense and antisense transcripts of M3TR using Strand-specific reverse transcription with primers designed for detection of sense and antisense oriented transcripts.

Fig. 3. Attenuation of tumorigenic potential by M3TR-as in Nude mice in comparison to tumors obtained with M3TR-sense cells.)

Research Report

Signal Transduction



Transcriptional regulation of Osteopontin (OPN) and its role in regulation of tumor growth through JAK2/STAT3 and p70S6 kinase signaling pathways: Involvement of stroma-derived OPN in tumor progression

Gopal Kundu
kundu@nccs.res.in

Participants

Ms. Reeti Behera, *SRF*
Mr. Mansoor Ahmed, *SRF*
Ms. Priyanka Sharma, *SRF*
Mr. Vinit Kumar, *SRF*
Ms. Rajinder Kaur, *SRF*
Ms. Remya Raja, *SRF*
Ms. Smita Kale, *SRF*
Mr. Santosh Kumar, *SRF*
Ms. Rosalin Mishra, *JRF*
Ms. Madhvi Mandhania, *JRF*
Ms. Dhanashri Thorat, *JRF*
Mr. Saurabh Pradhan, *Project Student*
Ms. Chaitali Dey, *Project Student*
Ms. Anuradha Bulbule, *Technician-C*

Background

Recent advancement of cancer research focused on the paradigm that tumor progression involves an intricate crosstalk between tumor and the stromal environment. Various groups have demonstrated that stroma-derived factors play a crucial role in fostering the tumor growth. Osteopontin (OPN), a chemokine like ECM associated Small Integrin Binding Ligand N-linked Glycoprotein (SIBLING) family of protein plays an important role in determining the oncogenic potential of various cancers. The role of OPN in various pathophysiological conditions, particularly in cancer, suggested that the variation in post-translational modification such as glycosylation, phosphorylation and sulfation generate the different functional forms that might alter its physiological functions. Recent evidence indicated that OPN regulates tumor growth through induction of COX-2, uPA and VEGF expressions and activation of matrix metalloproteinase (MMP) in various cancer cells. The role of stroma- and tumor-derived OPN in regulation of tumor growth and angiogenesis in various cancers is not well defined. Therefore, it is important to understand the mechanism by which both tumor and stroma-derived OPN control the tumor progression by using various in vitro and in vivo models. Earlier reports have shown that p70S6 kinase and/or JAK/STAT3 are directly involved in regulation of breast tumor growth and angiogenesis. However, the molecular mechanism by which OPN regulates JAK/STAT3 signaling leading to breast tumor growth and cell survival are unknown. Similarly, the roles of p70S6 kinase in regulation of OPN-induced ICAM-1 expression leading to breast tumor growth are not well understood. Moreover, the transcriptional regulation of OPN and the involvement of HDAC in OPN transcription in various human cancers including cervical is not well studied.

Aims and Objectives

1. To investigate whether OPN controls mTOR/p70S6 kinase activation leading to ICAM1 expression and breast tumor progression.
2. To study whether OPN regulates activation of JAK/STAT signaling leading to breast tumor growth and survival.
3. To study the role of stroma-derived OPN in regulation of tumor growth and angiogenesis in melanoma model
4. To examine the role of HDAC in transcriptional regulation of OPN and its role in cervical cancer progression

Work Achieved

In this study, we report that osteopontin induces p70S6 kinase phosphorylation at T421/S424 sites, but does not have any effect on T389 and S371 sites. OPN has no effect on mTOR phosphorylation, however, overexpression of mTOR does not regulate OPN-induced phosphorylation of p70S6 kinase at T421/S424 sites. Moreover, OPN-induced phosphorylation of p70S6 kinase at T421/S424 site is being controlled by MEK/ERK pathway. Overexpression of mTOR/p70S6 kinase suppresses OPN-induced ICAM-1 expression, while treatment with rapamycin augments OPN-induced ICAM-1 expression. These results suggested that OPN induces mTOR/p70S6 kinase regulated ICAM-1 expression which may lead to breast cancer cell motility and tumor growth (Fig. 1).

Deregulation of JAK/STAT3 signaling plays important role in oncogenesis of various cancers. Here, we report that OPN upregulates $\alpha_v\beta_3$ integrin-mediated JAK2 phosphorylation and STAT3 activation in breast cancer cells. Cells

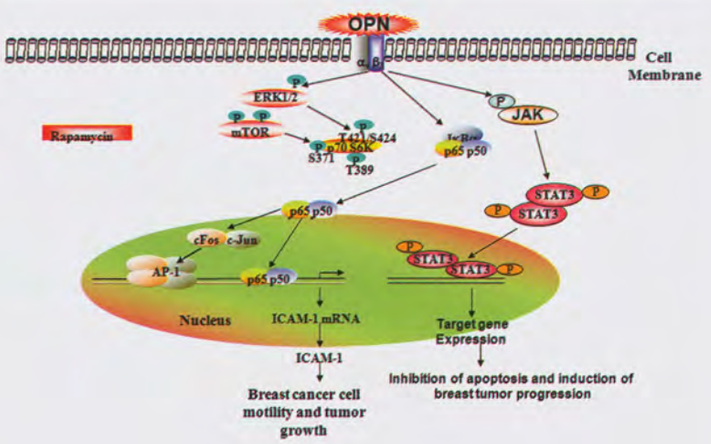


Fig. 1. Schematic representation of Osteopontin regulated p70S6 kinase dependent ICAM-1 expression and JAK/STAT3 activation leading to tumor growth in breast cancer model

transfected with wild type STAT3 enhanced whereas mutant STAT3 suppressed OPN-induced breast tumor cell migration. Treatment of cells with OPN followed by staurosporine revealed that OPN protects the cells from staurosporine-induced apoptosis. Moreover, transfection of cells with wild type (wt) STAT3 upregulates, whereas mutant STAT3 downregulates, Bcl2 and cyclin D1 expressions in response to OPN. Interestingly, STAT3 overexpressing cells when treated with OPN and injected to the NOD/SCID mice, the mice developed enhanced tumor growth as compared to mutant STAT3 injected mice or mice injected with OPN alone. The levels of Bcl2 and cyclin D1 in wt STAT3 injected tumors were significantly higher than controls. Thus, targeting OPN and its regulated STAT3 signaling could be a novel strategy to block tumor growth and may develop an effective therapeutic approach for the treatment of breast cancer (Fig. 1).

In this study, we have demonstrated that deficiency of host OPN effectively curb melanoma metastasis and angiogenesis. Melanoma cells isolated from wild type but not from OPN knockout mice exhibit enhanced growth, motility, enzymatic activity, colonigenecity and invasiveness compared with the parental cell line. The altered behavior correlated with increased melanoma growth, metastases and angiogenesis for wild type but not for parental or knockout isograft derived from melanoma cells upon reintroduction into the mice. Our experimental findings reveal the molecular mechanism underlying the host OPN regulated tumor growth. This study may depict the prognostic significance of host OPN in tumor progression and may help to develop a novel OPN targeted therapy for the management of cancers (Fig. 2).

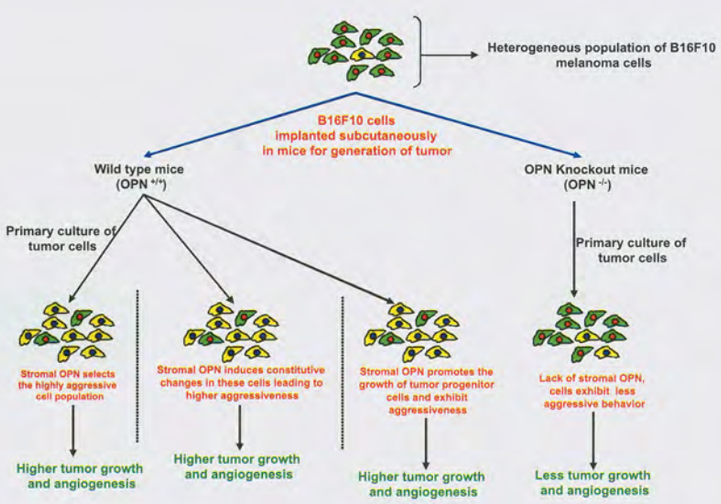


Fig. 2. Stroma-derived Osteopontin plays crucial role in tumor angiogenesis in melanoma model

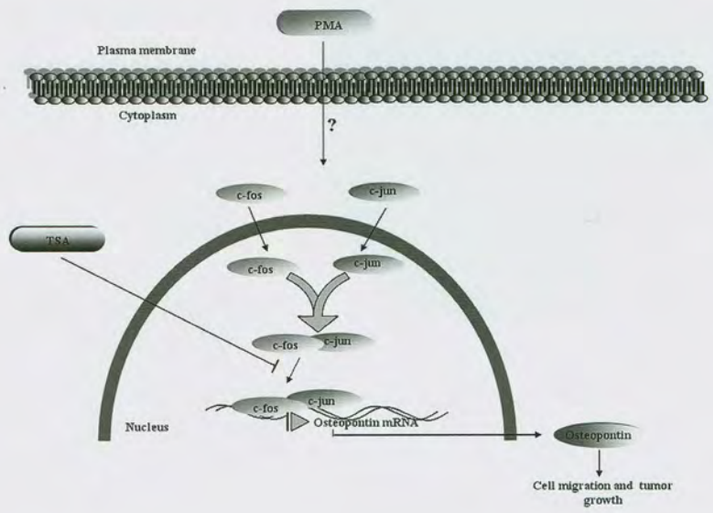
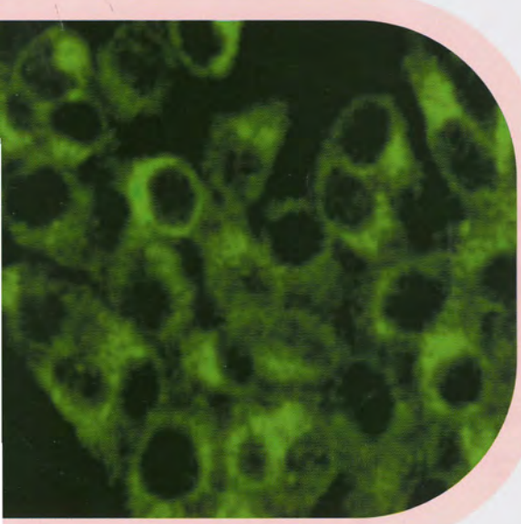


Fig. 3. Transcriptional regulation of osteopontin by HDAC inhibitor in cervical cancer model

In this study, we have examined the effect of HDAC inhibitor (TSA) on OPN transcription using human cervical carcinoma as model. We observed that TSA inhibited PMA-induced c-jun recruitment at the human OPN promoter which was a consequence of suppression of c-Jun expression at the protein level. Our data also identified the previously unrecognized AP-1 binding site in the OPN promoter and is shown to be functionally active. We have also observed that TSA not only suppresses OPN gene expression but also inhibits the expression of other AP-1 regulated genes like cyclin D1 and uPA. Taken together, these results suggest that TSA suppresses c-Jun leading to decreased expression of AP-1 regulated genes like OPN, cyclin D1 and uPA (Fig. 3).

Future Work

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



Membrane Bound Monomer of Staphylococcal α-Hemolysin Induces Caspase Activation and Apoptotic Cell Death Despite Initiation of Membrane Repair Pathway

Musti Krishnasastry
mvks@nccs.res.in

Participants
Saumya Srivastava, *SRF*
Amita Sneh, *SRF*
Neesar Ahmed, *SRF*
Aejazur Rahman, *SRF*
Anil Lotke, *Technician*

Background

Pathogenic microbes actively modify the behaviour of host cells so that they can withstand the rigors of the host environment and establish a suitable niche. Most pathogens act at a distance by producing the virulent factor(s). Pore forming toxins are among the virulent factors that are produced by both gram negative and gram positive bacteria and have been shown to cause significant changes in the cellular signaling of the host cells. Assembly of α-HL of *Staphylococcus aureus*, a potent pore forming toxin, on Jurkat T cells resulted in caspase activation via mitochondrial pathway and oligonucleosomal DNA fragmentation. Furthermore, the caspase activation was not inhibited by zVADfmk or overexpression of Bcl-2 and the process was concluded to be independent of death receptor pathway. However, recent observations were equally surprising that the low doses of α-HL still induced necrotic form of cell death despite caspase activation. Due to these contrasting observations, how α-HL manages to initiate the intrinsic pathway of apoptosis is unclear till date. It is also not clear how the cellular changes are sensed, and the nature of responses evoked by the toxin treated cells. Such knowledge will help us understand how the balance between survival and death pathways is regulated.

In our laboratory, we have clearly shown that the induction of apoptosis by α-HL was due to the presence of the monomeric form of α-HL, or the form that did not undergo conformational changes after binding to the target membranes i.e H35N. Based on the present and published data so far, the reason for caspase activation might be as follows: when α-HL binds to the target cells, only a fraction of the protein converts to lytic pore, while a substantial amount of protein remains as membrane bound monomer and as

non-lytic, pre-pore. In the absence of both the pre-pore and the functional pore, our data clearly highlights the activation of the intrinsic apoptotic pathway. Hence, it is possible that the membrane bound monomer activates the intrinsic apoptotic pathway while a successful pre-pore/functional pore assembly results in classical, necrotic form of death. Interestingly, we have also observed that the binding of H35N also arrests the dynamics of caveolae at cell surface. The observed blockade of caveolae dynamics by α-HL takes place within 2 hr and the process of cell death was initiated in about 8 hr. The question that remained unanswered is what happens in between 2 hr and 8 hr.

Aims and objectives

Delineating the range of cellular changes that takes place immediately after binding of the toxin to the cell surface as monomer and how the cell responds to it.

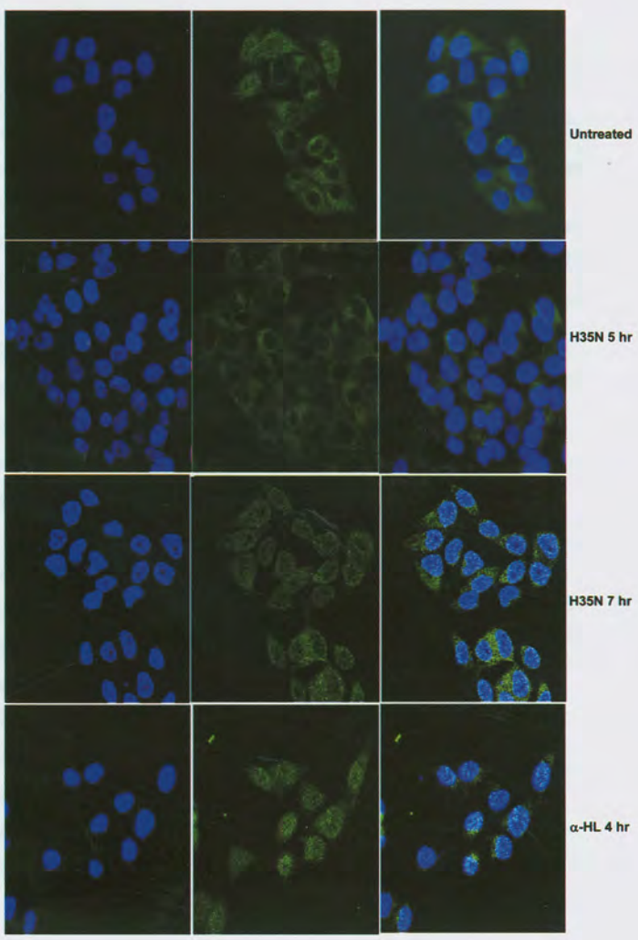


Fig. 1. H35N induced translocation of SREBP-1 to the nucleus. HeLa cells were treated with H35N or α-HL for the following time points and stained with SREBP-1 antibody as mentioned in materials and methods. The extreme left panel shows nuclear staining. The middle panel refers to SREBP-1 staining. The extreme right panel represents the merged image of SREBP-1 with DAPI staining.

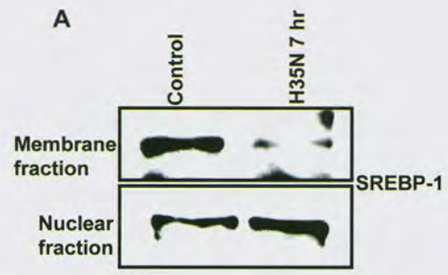


Fig. 2. Immunoblotting analysis of H35N mediated SREBP-1 translocation. HeLa cells were treated with H35N (8 µg/ml) for the following time point. Membrane and nuclear fractions were prepared as mentioned in wang et al. Each fraction (80 µg) were analysed by SDS PAGE followed by Western blotting using SREBP-1 monoclonal antibody.

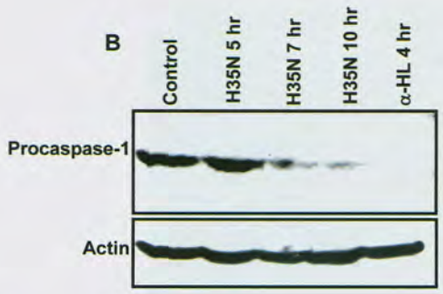


Fig. 3. Immunoblot analysis of H35N induced caspase-1 activation. HeLa cells were treated with H35N (8 µg/ml) or α-HL (800 ng/ml) for the following time points followed by immunoblotting with caspase-1 antibody. The lower panel refers to total actin.

Work Achieved

The blockade of caveolae dynamics by α-HL might be one of the first steps that take place after binding of the toxin to the membrane. At this stage, we anticipated two things to happen: 1) The cell may undergo a repair mechanism to restore the caveolae dynamics, and 2) if the repair mechanism is not adequate to mount an effective survival response, then the cell death process will be initiated. There is evidence in the literature to support this hypothesis. It has been recently shown that binding of α-HL on Hela cells initiates the activation of inflammasome pathway and the translocation of Sterol Regulatory Element Binding Protein (SREBP) to the nucleus which in turn promotes cell survival upon toxin challenge. SREBPs are membrane-bound transcription factors that regulate the expression of genes harboring the Sterol Reponse Element (SRE) in their promoter region and which are involved in cholesterol and fatty acid biosynthesis. SREBPs reside in the ER and are converted into the mature form by S1P and S2P. Mature SREBPs then migrate to the nucleus and induce lipogenic genes which in turn promote cell survival in response to the toxin treatment. We would like to emphasize here that after binding of the toxin to the cell surface, only a fraction of the α-HL that binds as monomer converts itself to the heptameric form. Hence the presence of the membrane bound monomer is inevitable in the above case. To check this hypothesis, we observed the translocation of SREBP-1 protein in Hela cells post H35N treatment. We observed that after 7 hr of H35N treatment, there is translocation of the SREBP-1 protein to the nucleus as compared to control (Fig.1).

The translocation of SREBP-1 from ER to the nucleus is further confirmed by immunoblotting. In Untreated cells, full length SREBP-1 was found in the membrane fraction. The amount of full length SREBP-1 decreased upon H35N treatment from the membrane fraction with the appearance of mature SREBP-1 in the nuclear fraction (Fig.2).

H35N triggers activation of caspase-1

SREBP-1 activation is mediated through caspase-1. Once activated, caspase-1 induces the intermediate targets, which then mediate the processing of SREBPs by S1P and S2P. We therefore analysed the effect of H35N on caspase-1. As shown in Fig. 3, H35N treatment leads to the processing of procaspase-1 with time. Although details of this pathway still remain to be elucidated, our data indicate that the cells, post H35N treatment, initiated the survival pathway to recover from the H35N induced damage. It must be mentioned here that the notable event that occurred after the binding of H35N was loss

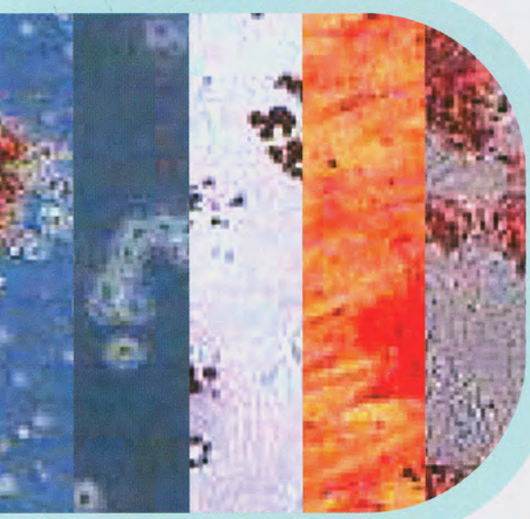
activate the survival pathway in non immune cells. It is assumed that the pathway observed by us is same as that of the one shown by Gurcel and co-workers since vital events observed by all of us are same. It is not clear about the exact role of KIAA0999 and MAP3K2 kinases, which are involved in controlling the dynamics of caveolae at this point of time, including its link to the inflammasome pathway activation. It is becoming clear that caveolae trafficking plays a far greater role in the regulation of cell proliferation and death than is currently known.

Future Work

We will be investigating the kinases involved in regulating the dynamics of caveolae with the help of the external docking protein mutant of α-HL to understand the cell repair mechanisms.

Research Report

Diabetes



Evaluation of islet-neogenic potential of non-pancreatic progenitors for regenerative therapy in diabetes

Ramesh Bhonde
rrbhonde@nccs.res.in

Participants
Sachin.S.Kadam, *SRF (Project)*
Swetha G, *SRF*
Vikash Chandra, *SRF*
Smruti Phadnis, *SRF*
Malati Umrani, *Technician*

Collaborators
Dr. Sanjeev Galande, NCCS, Pune
Dr. Anandwardhan Hardikar, NCCS, Pune
Dr. Surendra Ghaskadbi, ARI, Pune
Dr. Prabha D Nair, SCTIMST, Trivandrum
Dr. B L Prasad,NCL, Pune
Dr. Venkatesan Vijayalaxmi, NIN, Hyderabad
Dr. Shahul A Hameed, Vellore, Tamilnadu

Background

Diabetes is a multi-factorial metabolic disorder caused due to partial/total damage of insulin producing β-cells of the pancreas leading to hyperglycemia. Chronic, uncontrolled, hyperglycemia would also lead to many life-threatening secondary complications such as vasculopathy, nephropathy, retinopathy etc. Current therapies offer better management of diabetes by aiming at tight glycemic control. However none of the existing therapies offer a permanent remedy. Hence there is a need to look for alternative measures. Stem cells display robust proliferation and also the required plasticity to differentiate into various cell lineages. Since adult beta cells have limited potential to replicate, there is a need to replenish the lost beta cell mass by new cells. Adult stem cells offer an attractive source for cell replacement therapy. The present report summarizes the isolation, characterization and evaluation of islet neogenic potential of mesenchymal stem cells (MSCs) derived from non pancreatic tissues of human origin into islet-like aggregates (ILCs) and their role in diabetic reversal. These include MSCs isolated from human adipose tissue, bone marrow, placenta and amnion.

Aims and Objectives

To test the potential of human adult non-pancreatic tissue derived stem cells for differentiation into insulin secreting islet like clusters

Work Achieved

Differentiation of Human Adipose tissue-derived Stem Cells into functional islet-like cell aggregates for cell replacement therapy in diabetes

Insulin dependent diabetes (type-1) is a chronic disease resulting in lifelong dependency on insulin treatments. Pancreatic islet transplantation is a promising possibility for the optimal treatment of type-1 diabetes. However the shortage of cadaveric transplantable islets is a major obstacle to this approach. Mesenchymal stem cells (MSCs) have capacity to differentiate into functional transplantable islets. Adipose tissue (AT) is gaining much attention as the prime source of MSCs for cell therapy by virtue of its ready availability, enormous expandability and ease in isolation with minimum patient discomfort. We have previously shown that murine adipose tissue derived stem cells generate pancreatic hormone expressing islet-like cell aggregates (ICAs). We describe here a homogeneous CD29/CD44/CD90 proliferative population of human adipose tissue-derived stromal cells (h-ASCs) having the ability to differentiate into pancreatic islet-like cell aggregates (ICAs) in addition to their multilineage differentiation potential. Resected fat-tissue

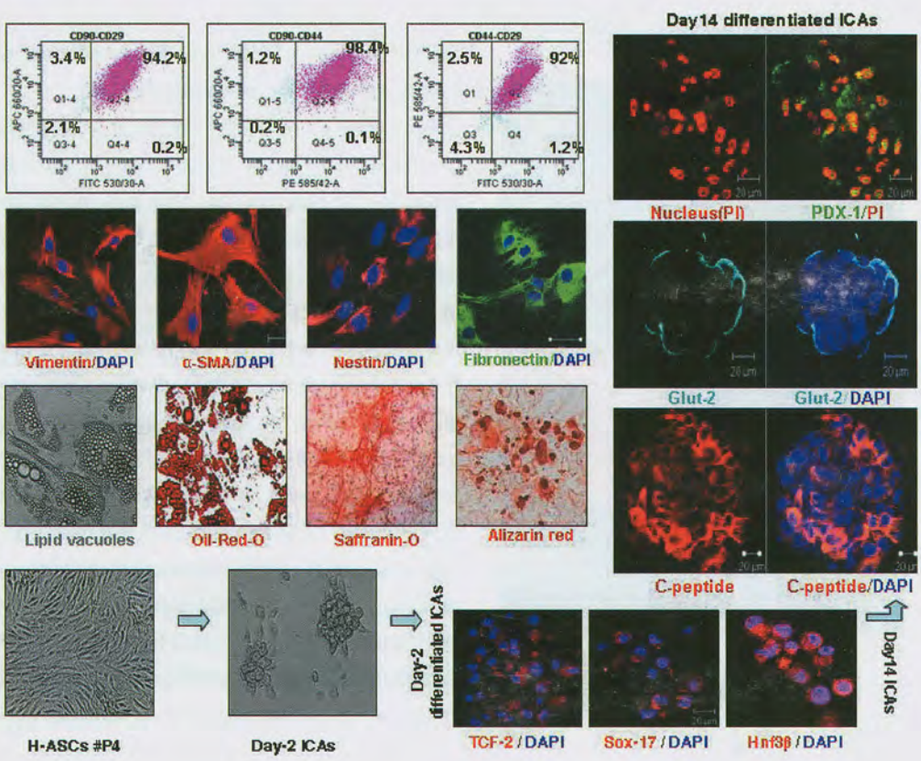


Fig. 1. Characterizations of H-ASCs for mesenchymal markers and CD-markers. In-vitro multilineage differentiation potential of H-ASCs is shown. In addition to mesoderm lineage [Adipogenic (oil red-O), Chondrogenic (saffranin-O), and Osteogenic (alizarin red)] these cells are also shown to undergo differentiation to endoderm pancreatic lineage. Markers for definite endoderm (TCF-2, Sox-17 and Hnf3-β) shown at day-2 as well as markers for Pancreatic endoderm (PDX-1, Glut-2 and c-peptide) are shown at day-14 of differentiation.

samples (n=12, female donor with age group 30-45yr) were enzymatically digested to obtain stromal cell populations. These fibroblast-like cells demonstrate immunopositivity for two mesenchymal proteins (a-smooth muscle actin and vimentin) as well as intermediate filament protein Nestin. We formulated a 14-days stage-specific differentiation protocol to generate c-peptide (14-18%) producing ICAs. In our defined serum free media (SFM) containing ITS in combination with activin A and sodium butyrate induced h-ASCs to differentiate to definitive endoderm (DE). Majority of the differentiated cells (>40%) showed increased expression of primitive endodermal markers like alpha-fetoprotein and nuclear beta-catenin as well as definitive endoderm marker like sox-17, tcf-2 and hnf3-β at day-2 as compared to undifferentiated h-ASCs. Further addition of taurine, GLP-1 and nicotinamide in successive days of SFM cocktail induces pancreatic endoderm (pdx-1, ngn3, neuro-D, pax4, pax-6, isl-1 and glut-2 expressing ICAs). These ICAs demonstrate glucose-stimulated c-peptide release in vitro and can also maintain normal glucose concentrations when calcium-alginate encapsulated day-14 ICAs, transplanted intraperitoneally to streptozotocin (STZ)-induced diabetic immuno-competent mice. Thus h-ASCs can be used as a potential source of autologous stem cells for therapeutic application in treatment of type-1 diabetes.

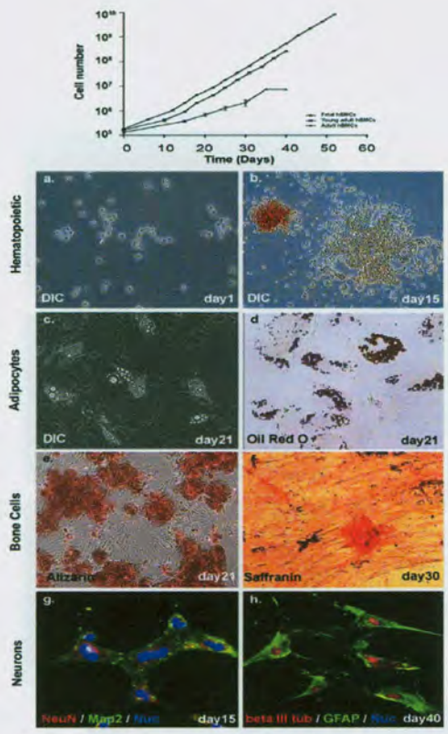


Fig. 2. Growth curve for hBMCs of different age group and multilineage differentiation potential of these hBMCs are shown in figure.

Differentiation of Human Bone Marrow-derived Stem Cells into functional islet-like cell aggregates

Human bone marrow aspirates from fetal, pediatric or adult donors, were processed to generate human bone-marrow-derived mesenchymal cells (hBMCs). We observed that fetal and young donor derived MSCs exhibited higher proliferation rate and long term survival than those derived from the adult donors (Fig. 2). Donor age is thus inversely related to growth potential of hBMCs in vitro. Upon exposure to lineage specific growth factors, hBMCs (irrespective of the donor age) differentiate into multiple lineages, including the endocrine pancreatic lineage without any genetic manipulations. hBMCs migrate to form islet-like cell aggregates (ICAs) that express pancreatic transcription factors (Nkx6.1, Pax6 and Isl1) when exposed to SFM supplemented with growth factors that are involved in pancreatic development. Mesenchymal-Epithelial transition (MET) was evident from the up-regulation of epithelial transcripts (glucagon, pecam1, icam5, etc.) which are expressed in mature pancreatic islets. Although efficient differentiation of hBMCs to endocrine pancreatic lineage was not achieved in vitro, these ICAs mature following transplantation into NOD/SCID mice under the influence of paracrine factors from regenerating pancreas. We found that the transplanted graft was responsive to glucose challenge and produced islet hormones that

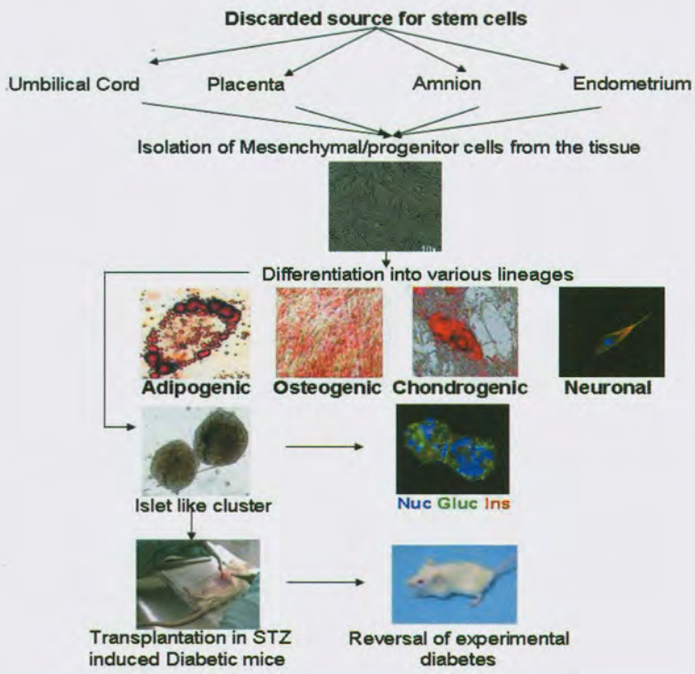


Fig. 3. Flow-chart diagram for derivation of ILCs from different discarded tissue sources.

could maintain normal glucose concentrations in STZ induced diabetic mice for about 3 months.

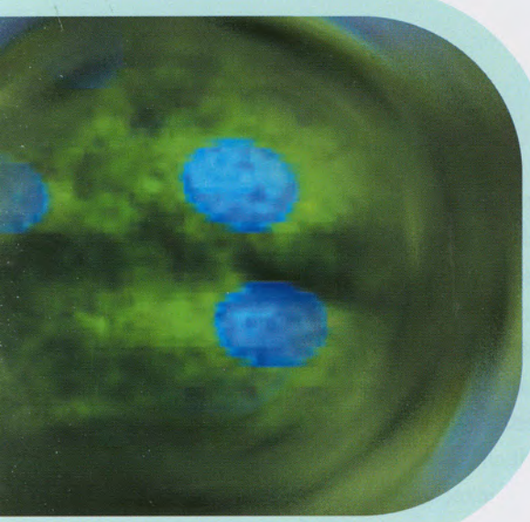
Islet neogenesis from extra embryonic tissue resident mesenchymal stem cells

Mesenchymal stem cells were isolated from human umbilical cord (hUCMSCs), placenta (hPDMSCs), amnion (hAMSCs) and endometrium. They were studied for the expression of mesenchymal markers and found to be positive for markers such as CD44, CD73, CD90 and CD117 and were immunopositve for vimentin, nestin, smooth muscle actin, Stro-1 and desmin (Fig. 3). In addition to mesenchymal stem cell markers, cells from umbilical cord and placenta were also positive for embryonic stem cell markers Oct4 and SSEA-4; indicating a connecting link between embryonic stem cells and adult stem cells making them more amenable for desired lineage specific differentiation. The pancreatic lineage differentiation was tested using SFM containing a cocktail of growth factors and Insulin-Transferrin-Selenium (ITS). Except endometrial MSCs all other tissue derived MSCs aggregated and formed the ILCs in 10 days. These ILCs were found to be positive for diphenylothiocarbazone (DTZ) staining, highly specific for zinc moiety present

in insulin secreting cells, and their immuno-cytochemistry revealed presence of insulin and glucagon. ILCs derived from hUCMSCs, hPDMSCs, hAMSCs were found to be responsive to glucose challenge indicating their ability to synthesize, store and release insulin in response to glucose. Transplantation of newly formed ILCs into STZ-diabetic mice resulted in significant reduction in hyperglycemia and increase in body weight in ILCs derived from all the MSCs except endometrial MSCs indicating their functional status as islet. This normoglycemia condition was maintained for over 3 months follow up before terminating the experiment. In the nutshell, stem cells from fetal membranes represent an exciting field of research of outstanding importance offering novel non-pancreatic, non-invasive and inexhaustible source of stem cells for islet generation. The human islets thus generated from stem cells could form an ideal in vitro model not only for cell replacement therapy in diabetes but also for hypoglycemic screening and toxicological investigations.

Future Work

1. To understand the underlying mechanism of insulin resistance using adipocytes as a model.
2. To compare the adipose tissue stromal cells derived from different sources of human origin such as subcutaneous, retroperitoneal, visceral and mesenteric.



Investigation of mitochondrial dysfunction, oxidative damage and apoptotic cell death stress mechanism in diabetic cardiomyopathy

Sandhya Sitasawad
ssitaswad@nccs.res.in

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

Background

Calcium ions are of tremendous importance for the electrical and mechanical activity of heart and muscles. Calcium antagonists represent an important class of drugs that have a huge potential in cardiovascular medicine. Right from the discovery of the intracellular calcium receptor calmodulin (CaM), its role has been widely implicated in the development of newer pharmacological agents. Calmodulin-controlled intracellular calcium concentration regulates a variety of physiological processes, including control of the cell cycle, apoptosis, gene expression, neurotransmission, memory, synaptic plasticity, and contractile function. Calmidazolium chloride (CMZ), a positively charged, hydrophobic compound and well-established imidazole-based CaM inhibitor, has been tested in the past but discontinued owing to undesired effects. The effects of this drug on diversified cellular events have been investigated in several cell types. CMZ is known to alter calcium signaling, for example, by causing an increase in the intracellular calcium concentration ([Ca]i) in a variety of cells or the release of calcium from mitochondria and the endoplasmic reticulum. Additionally, CMZ was shown to inhibit thapsigargin-induced capacitative calcium entry in thyroid FRTL-5 cells and rat hepatocytes and suppression of angiotensin II or ionomycin-activated capacitative calcium entry without affecting the basal calcium entry in cultured human mesangial cells. CMZ causes cell death in a dose dependent manner in neuronal and hepatic cells. The events that contribute to similar effects of CMZ on the myocardium have not been studied previously. CMZ exerts specific and nonspecific effects on cardiomyocytes. Specific effects of CMZ include blockade of L-type calcium channels, voltage-dependent Na^+ and K^+ channel currents, and Ca^{2+}-release channels in the sarcoplasmic reticulum. Nonspecifically, CMZ causes an increase in membrane

permeability, swelling and uncoupling in mitochondria, and protein degradation, along with structural damage, in the non-recirculating isolated perfused rat heart. Whether these effects of CMZ are exclusively through calmodulin antagonism is not clear and the possibility of calmodulin-independent activity of CMZ could not be ignored. Voltage-operated calcium channels are essential for the cardiac cell contraction and are potential targets for calcium channel blockers. They regulate calcium entry and thereby cell contraction. Calcium accumulation in the cells and mitochondria leads to the opening of high-conductance mitochondrial permeability transition pores resulting in the collapse of the electrochemical potential for H^+, thereby arresting ATP production. This triggers production of reactive oxygen species (ROS) and apoptosis. It is likely that both specific and nonspecific effects of CMZ lead to myocardial cell death. However, the exact mechanism of cardiotoxicity is not clear.

Aims and Objectives

1. To ascertain the role of CMZ in calcium accumulation, generation of ROS and RNS, cytochrome c-mediated poly(ADP-ribose) polymerase (PARP) cleavage, and apoptosis in rat myocardiac H9c2 cells
2. To determine the effect of CMZ treatment on cardiac cell function, contractility indices, and intracellular Ca^{2+} transient properties in isolated ventricular myocytes from adult rats.

Work achieved

During the last year we had observed that nicardipine provides cardioprotection against high glucose-induced (HG)-induced abnormalities in myocyte relaxation, perhaps through their antioxidant effect. Further, we studied the specific effects of CMZ that lead to myocardial cell death. Increasing doses of CMZ resulted in a dose-dependent decrease in cell viability [Fig. 1(I)]. Because the cell viability assay is based on the enzymatic conversion of MTT within mitochondria by succinate dehydrogenase, it indicated that the early signs of CMZ toxicity could be based on an impairment of the mitochondrial respiratory chain. The highly potent calmodulin antagonist CMZ nonspecifically causes calcium aggravation, leading to increased intracellular calcium levels [Fig. 1(II A and B)]. Ca^{2+} plays a vital role in excitation-contraction coupling in the heart; however, a massive accumulation of calcium has been shown to trigger mitochondrial damage, leading to production of ROS and apoptosis. Fluo-3AM fluorescence intensity in CMZ-exposed cardiomyocytes showed that CMZ increases the intracellular calcium accumulation and impedes calcium clearance. This abnormal calcium

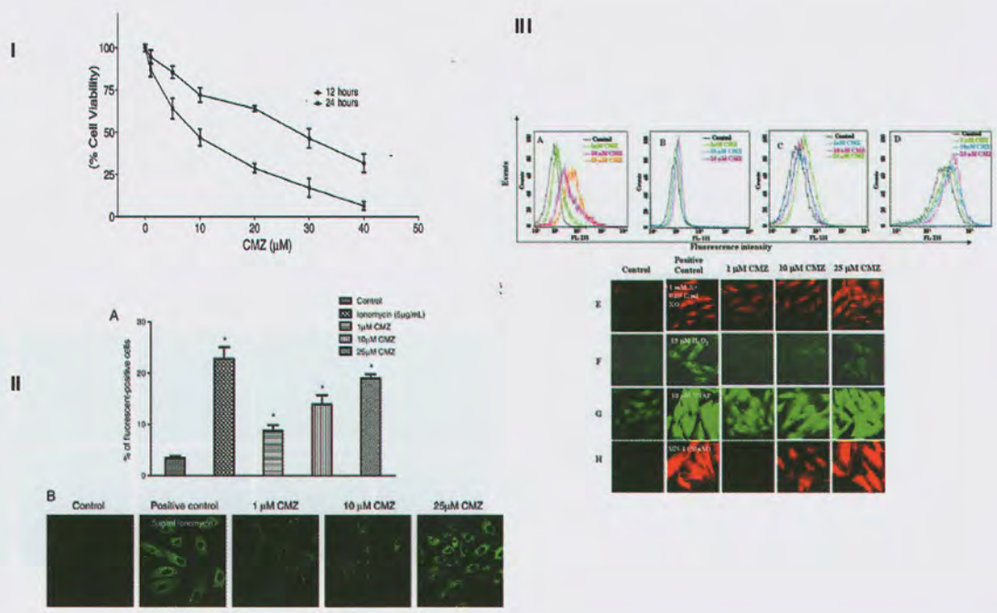


Fig. 1. I. Cell viability of H9c2 cells treated with various concentrations of CMZ for 12 and 24 h. Percentage cell viability was detected using the MTT assay. Data represent means ± SE of three separate experiments. **II.** Effects of increasing concentration of CMZ on intracellular calcium levels on H9c2 cells (30 min). (A) Bar graph represents percentage of fluorescence-positive cells stained with the calcium-sensitive fluorescent probe Fluo-3AM using flow cytometry. Data represent means ± SE of three individual experiments. *significant ($p<0.05$) compared to control. (B) Representative confocal laser scanning microscopy images of cells fluorescently stained with Fluo-3AM. Ionomycin (5 μg/ml) served as positive control for the experiment. **III.** Generation of ROS and RNS in H9c2 cells by treatment with CMZ. (AD) Cells were treated with 1, 10, or 25 μM CMZ for 3 h detected by flow cytometry upon staining with fluorescent dyes: (A) DHE, (B) DCF-DA, (C) DAF-2DA, and (D) DHR-123. (EH) Confocal laser scanning microscopy images of cells fluorescently stained with (E) DHE, (F) DCF-DA, (G) DAF-2DA, and (H) DHR 123. X+XO, H_2O_2, SNAP, and SIN-1 served as positive controls for the experiment.

accumulation leads to the generation of ROS and RNS. Several studies have reported accumulation of ROS in the early stages of apoptosis via depolarization of mitochondrial membrane. Our results indicate an increased intracellular production of ROS and RNS in live H9c2 cells by CMZ in approximately 3 h of treatment [Fig. 1(III A to H)]. During the normal aerobic metabolic process, mitochondria produce highly ROS and efficiently eliminate them by specific enzymatic and non-enzymatic antioxidant systems. Disruption of the mitochondrial respiratory chain could have deleterious consequences for the mitochondrial energy metabolism, which is particularly susceptible to oxidative damage. It is quite likely that accumulated calcium, ROS, and RNS collaboratively cause widespread mitochondrial dysfunction and damage. Additionally, increased levels of peroxynitrite ($ONOO^-$), a destructive nitrogen species, could also account for direct mitochondrial damage. It has also been observed that mitochondrial oxidative insult results from the CMZ-induced collapse of the mitochondrial membrane potential, which was evident after 12 h treatment [Fig. 2(A-D)]. This loss of the mitochondrial membrane potential induces release of cytochrome c into the cytoplasm from damaged mitochondria. Our results show that loss of the mitochondrial membrane potential occurs before cytochrome c release from the damaged mitochondria, implicating the strong role of calcium accumulation and ROS and RNS generation in mitochondrial dysfunction. We observed that CMZ-induced cytolethality is due mainly to apoptosis. Cell cycle

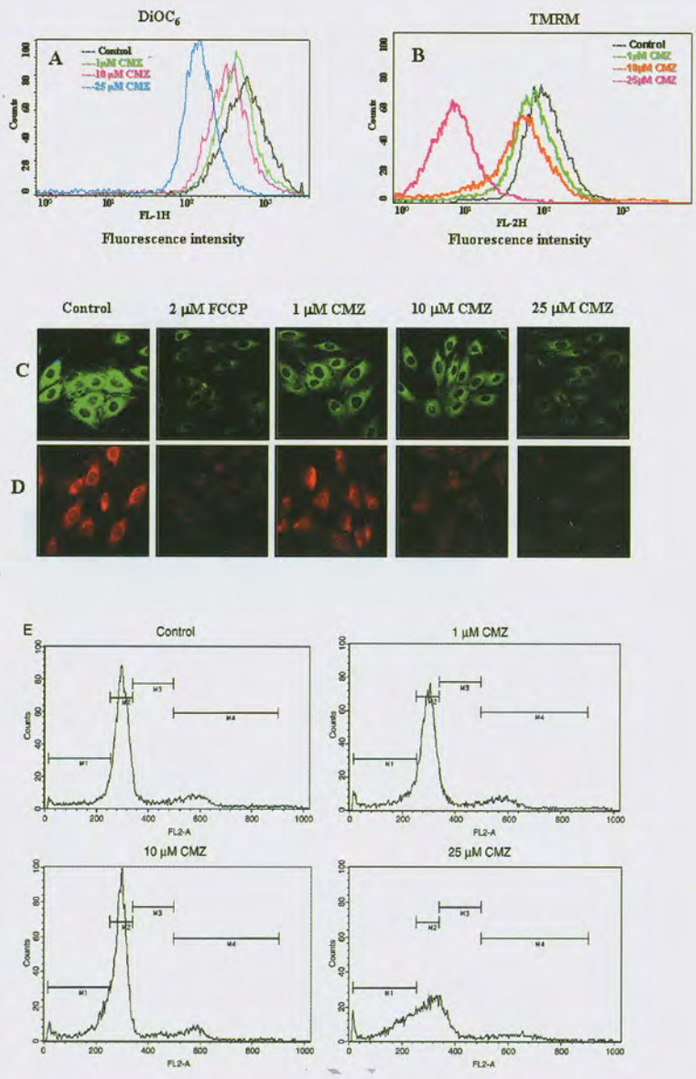


Fig. 2. Detection of loss of mitochondrial membrane potential in H9c2 cells treated with CMZ for 12 h. Bar graphs depict mean fluorescence intensity acquired and detected by flow cytometry upon staining with **(A)** $DiOC_6$ and **(B)** TMRM. Data represent the means ± SE of three individual experiments. *Significant ($p<0.05$) compared to control. **(C** and **D)** Representative confocal laser scanning microscopy images of cells fluorescently stained with (C) DiOC6 and **(D)** TMRM. **(E)** Effects of CMZ on different phases of the cell cycle. Cells were treated with 0, 1, 10, and 25 μM CMZ for 24 h, stained with PI, and assessed on the FL-2A channel of a flow cytometer equipped with a 488-nm laser. The y axis represents the event count and the x axis represents the DNA content of the cells. M1 represents the apoptotic cells in the sub-G0 region, M2 represents the diploid G_0/G_1 population, M3 corresponds to the S phase, and M4 corresponds to the 4n G_2/M population.

analysis with PI staining revealed a dose-dependent increase in the accumulation of cells in sub-G_0 phase (Fig. 2E), and correspondingly, the morphological analysis of nuclei with DAPI staining depicted nuclear and cytoplasmic shrinkage and fragmentation of chromatin indicative of apoptotic cell death (Fig. 5 A-B). Cleaved PARP is another important hallmark of apoptotic death. Native PARP is a 160-kDa nuclear protein that is strongly activated by binding to DNA strand breaks. Increasing evidence indicates that mitochondrial alterations result in the release of cytochrome c into the cytosol, which ultimately leads to PARP cleavage via an intrinsic apoptotic

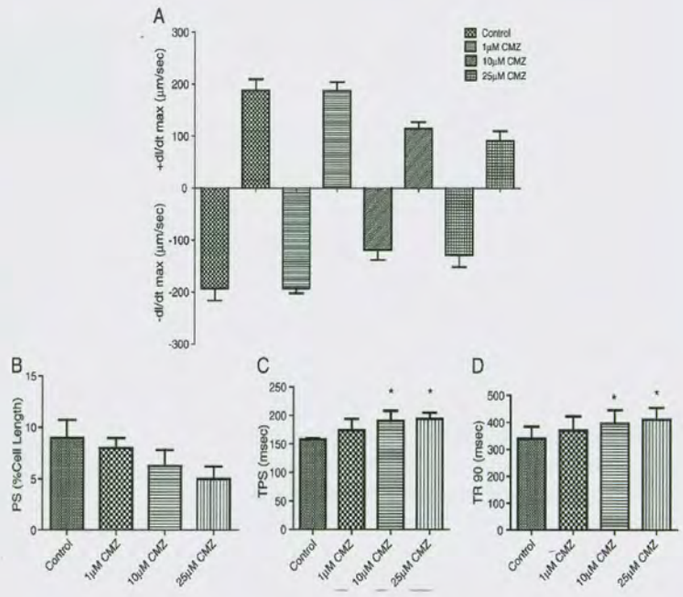


Fig. 3. Effect of CMZ (30 min) on contractile properties of adult rat ventricular cardiomyocytes. **(A)** Mechanical indices are maximal velocities of shortening/relengthening (±dL/dt), **(B)** peak shortening (PS), **(C)** time to peak shortening (TPS), and **(D)** time to 90% relengthening (TR 90). Mean ± SEM, n=3557 cells/group, *p<0.05 vs control.

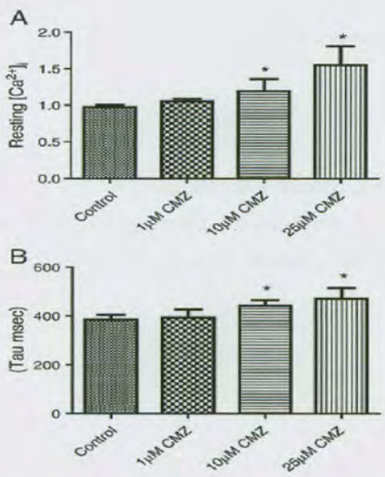


Fig. 4. Intracellular Ca2+ transient properties in Fura-2AM-loaded adult rat ventricular cardiomyocytes cultured for 24 h in control medium and myocytes treated with 1,10, and 25 μM CMZ for 3h. **(A)** Baseline intracellular Ca2+ concentrations. **(B)** Rate of cytosolic free Ca2+ decrease (Tau). Mean ± SEM; n=2638 cells/group. *p<0.05 vs control.

pathway (Fig. 5 C-D). In this study, it has been shown that CMZ increases oxidative activity in the cells by increasing ROS and RNS generation, which leads to a decrease in the mitochondrial membrane potential, causing mitochondrial damage. The damaged mitochondria release cytochrome c that triggers PARP cleavage, subsequently resulting in apoptosis. To our knowledge this is the first report that provides an insight into the subcellular events that are orchestrated in CMZ-induced cardiotoxicity. Drug-induced cardiomyopathy is characterized by systolic and diastolic dysfunction. Impaired diastolic function is the most prominent mechanical abnormality manifested as reduced contractility, prolonged relaxation, and altered function of Ca^{2+}-regulating proteins. This is supported by the reduced peak shortening, ±dL/dt (Fig. 3A-D), and prolonged TR90 in CMZ-treated myocytes observed in our study, which could be underscored by the reduced intracellular Ca^{2+} rise and slowed intracellular Ca^{2+} clearing (Fig. 4 A-B). This result is also in agreement with the previous report by Klockner and Isenberg, demonstrating inhibition of the Ca^{2+} current by CMZ in myocardial cells. These findings indicate that CMZ toxicity contributes to the development of cardiac EC coupling dysfunction at a very early stage.

In conclusion, these results suggest that CMZ induces impaired diastolic function associated with increased intracellular Ca^{2+} rise and prolonged intracellular Ca^{2+} clearing in isolated adult rat cardiomyocytes, resulting in

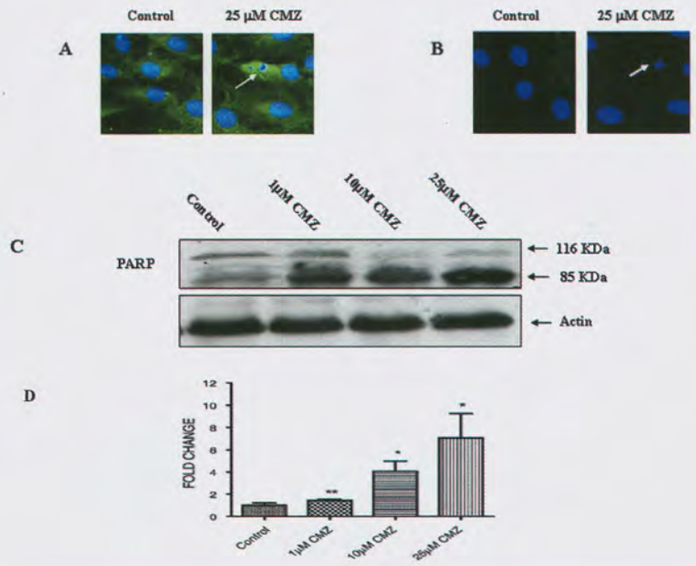
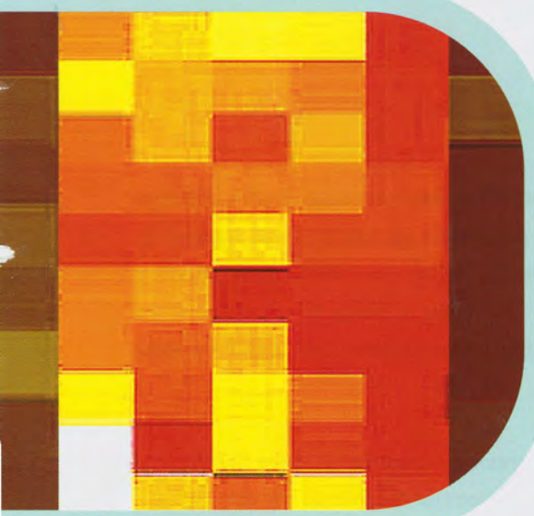


Fig. 5. (A) Cytochrome c immunostaining after treatment of H9c2 cells with 25 μM CMZ for 24 h. The images are representative of 2530 cells each in three separate experiments. **(B)** H9c2 cells stained with 25 μM DAPI. DAPI staining shows nuclear condensation visualized in CMZ-treated cells. Images are representative of 2530 cells each in three separate experiments. **(C)** Western blot showing increase in cleaved PARP expression by CMZ treatment in H9c2 cells using anti-PARP antibody. There was a dose-dependent increase in the cleaved PARP expression after 24 h of treatment. **(D)** Bar graph showing fold increase in the expression of cleaved PARP normalized to β-actin assessed by densitometry. The means ± SE of normalized fold change of cleaved PARP for three independent experiments are shown.

cardiac fatigue. CMZ also causes increased intracellular calcium levels, mitochondrial dysfunction, and oxidative and nitrosative stress in H9c2 cells, producing a dose-dependent growth inhibition. This work provides valuable insight into CMZ- induced cardiotoxicity. It would be an exciting challenge to elucidate the molecular switches that control or block this pathway. This study could form a basis for the development of improved calmodulin antagonists in the future.

Future work

Study the effect of good glycemic control with insulin-mimetic molecules and anti-diabetic pharmaceutical preparations, therapeutic potential of multiple anti-oxidants and calcium channel blockers 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 Hardikar
anand@nccs.res.in

Participants
Amaresh Kumar Ranjan, *SRF*
Mugdha V. Joglekar, *SRF*
Subhshri Sahu, *JRF*
Vishal S. Parekh, *JRF*
Malati R. Umrani, *Technician*

Collaborators
Dr. David Tosh, Bath, UK
Dr. Nageshwar Reddy, AIG, Hyderabad
Dr. Ramesh Dumbare, Pune
Dr. Sasikala M, AIBS, Hyderabad
Dr. Sushil Rane, NIH, USA
Dr. Vinay Joglekar, Shirwal
Dr. Ramesh Bhonde
Dr. Yogesh Shouche
Dr. Sanjeev Galande

Background

Diabetes is caused by the loss of the insulin-producing β-cells in the pancreas. A significant advance in treating diabetes has been the development of a protocol for islet transplantation from Dr James Shapiro and colleagues at the University of Alberta in Edmonton, Canada (the "Edmonton protocol"). However, lack of suitable organ donors for transplantation is still a major issue for the future. A new way to overcome this problem is to try and produce β-cells in the laboratory for transplantation into patients. We have demonstrated earlier that fetal human pancreas-derived cells are better progenitor cells, as these endocrine pancreatic cells can proliferate in vitro and retain an active (H4-Ac, H3-Ac, H3K4-me3,me2) insulin promoter conformation. We also recently demonstrated for the first time that human gall bladder contains a potential source of β-cells that can be used for transplantation into diabetic individuals. The work in our laboratory is focussed on understanding mechanisms that will help us to achieve proliferation of such "committed" cells, which can be used for cell replacement therapy in diabetes.

According to the International Diabetes Federation, there are more than 246 million people worldwide with diabetes (WHO). This figure is projected to rise significantly to 380 million by 2020. India has the largest number of diabetics (over 60 million). Although treating diabetes with insulin injections is reasonably successful, this does not precisely mimic the function of a normal β-cell. Therefore complications such as kidney failure, blindness and heart disease develop. These complications reduce life expectancy, quality of life and more pressure is placed on medical time and healthcare budgets. One way to avoid these complications is through transplantation of pancreatic

islets into diabetics. Islet transplantation procedures have recently improved considerably but one of the main problems is the lack of donor supply of β-cells since demand far outweighs availability. An alternative to donors is making β-cells in the laboratory. Being able to create β-cells is one of the 'holy grails' of diabetes research. It may eventually become possible to make β-cells from different cell sources.

One tissue that has generated enormous interest for us is the human fetal pancreas itself. There are two good reasons for this intense interest. First, the ability to achieve proliferation of endocrine cells by retaining active chromatin conformation makes them as "committed" stem-like/precursor cells. Secondly, these cells are obtained at a time during embryonic development when they are committed to become endocrine pancreas and are also in a phase of active proliferation. In the past year, we have confirmed that these pancreatic islet-derived mesenchymal cells are better progenitor cells that can be induced to differentiate into hormone-producing islet-like cell aggregates (ICAs). Such ICAs can maintain normal glucose concentrations for up to 6 months in diabetic NOD-SCID mice.

Aims and Objectives

1. To understand the maturation of islet progenitor cells following transplantation in mice
2. To assess the potential of FIPC-derived islet-like cell aggregates (ICAs) to maintain normal glucose concentrations in diabetic NOD-SCID mice

Work Achieved

We generated human fetal pancreatic islet-derived mesenchymal cells (FPCs) that are highly proliferative and can be expanded as a homogenous population of mesenchymal cells. These FPCs do not exhibit presence of any islet-specific hormone transcript or protein after million fold expansion in vitro, but yet retain the ability to differentiate back to islet hormone-producing cell aggregates. Initially, we used standard protocol for differentiation that was established in our laboratory during previous studies with slight modifications. FPCs come together/migrate to form cell aggregates that resemble islet like clusters isolated from human fetal pancreas. These events are very rapid and usually occur within 24 hours of exposure to serum free media (SFM). ICAs are observed in culture dishes by day 1, which become compact in next 2-3 days and by day 14, these ICAs are found to contain pancreatic islet hormones: insulin and glucagon. We observe that FPCs from later passages (>passage 15) show limited differentiation potential.

We then looked at the islet hormone transcripts in differentiated ICAs. Pro-insulin, pro-glucagon and somatostatin transcripts were observed at different time points during the period of differentiation (Figure 1 A). Million fold expanded FPCs do not contain any pro-insulin transcript, however, by day 3 of their differentiation pro-insulin transcript starts accumulating and is maintained till day 11 in vitro. On the other hand, FPCs contain modest amount of pro-glucagon and somatostatin transcripts, which exhibit increased abundance by day 11 of differentiation. We compared in vitro differentiation potential of FPCs at different stages of expansion and from different gestational ages for an array of pancreas specific genes using TaqMan-based low density arrays (TLDAs). The profiles were also compared with those in freshly isolated human fetal islets by bi-directional cluster

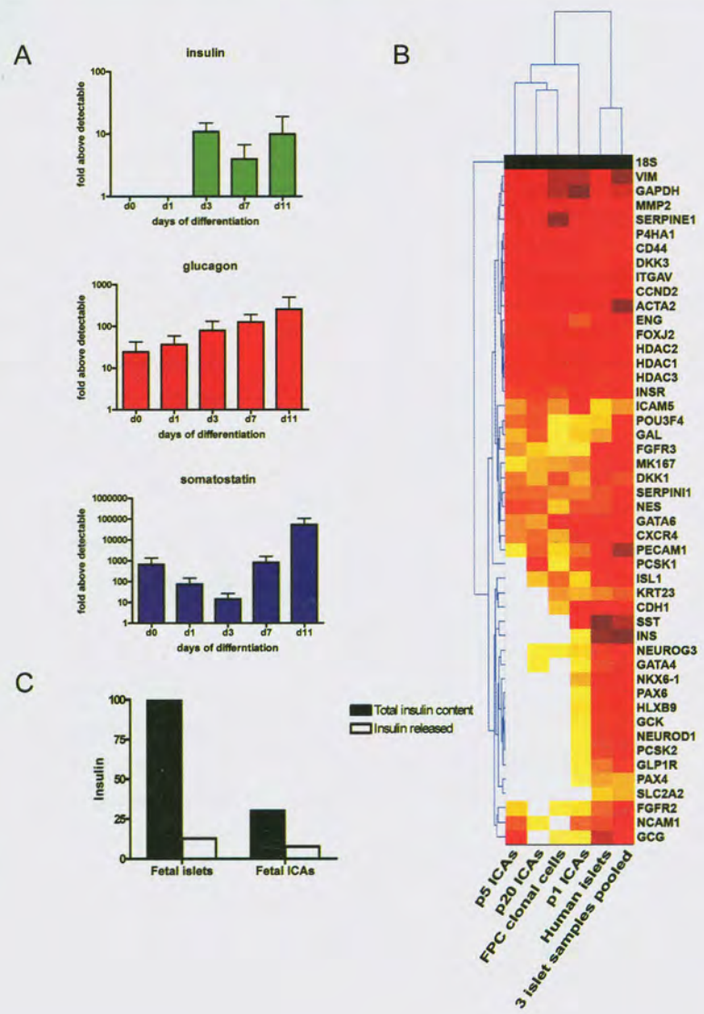


Fig. 1. FPC derived ICAs were aseessed for hormone transcripts at day-0, -1, -3, -7 and -11 during in vitro differentiation **(A)**. Pro-insulin, pro-glucagon as well as somatostatin transcripts show increase in abundance over this period. However this increase is very little as compared to the abundance of these hormone transcripts in fetal islets. Early passage FPC-derived ICAs have pancreatic gene expression profile closely related to that of fetal islets, while late passage ICAs cluster differently and are closer to clonally expanded FPCs **(B)**, suggesting better differentiation potential of early passage FPCs. Total insulin content as well as insulin released in response to glucose is significantly lower in fetal ICAs **(C)**.

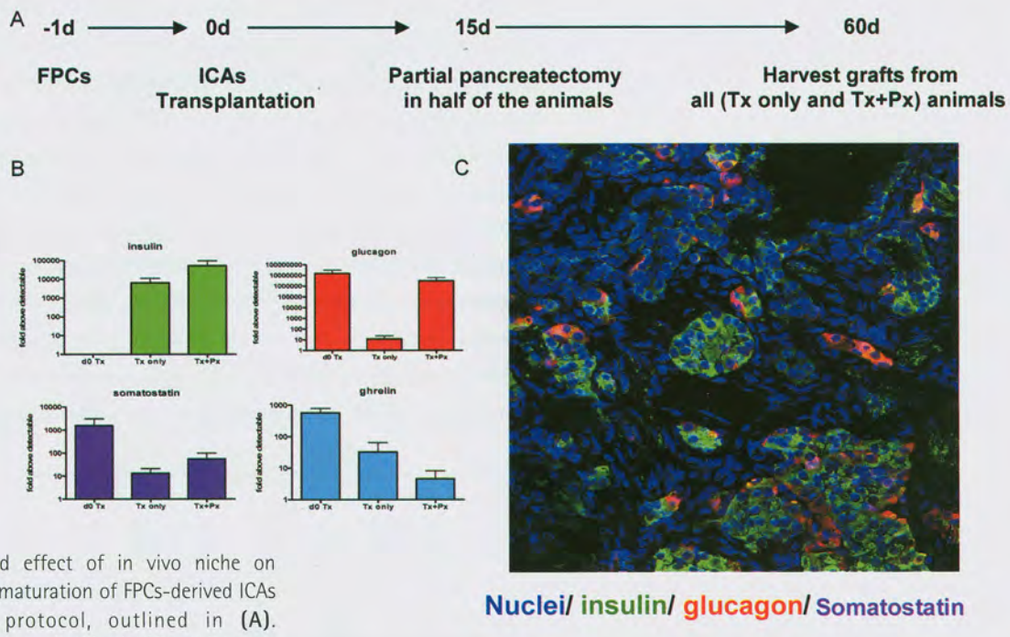


Fig. 2. We studied effect of in vivo niche on differentiation and maturation of FPCs-derived ICAs by following a protocol, outlined in **(A)**. transplantation alone resulted in significant increase in pro-insulin transcript abundance, which was further enhanced in presence of paracrine factors secreted by regenerating pancreas **(B)**. Data is obtained from atleast 8 animals for each of the group (Tx only and Tx+Px). Insulin and glucagon immunopositive cells were observed in the implanted graft in kidney **(C)**.

analysis (Figure 1B). We observe that, the two freshly isolated fetal islet samples cluster together and their expression profile is similar to the ICAs obtained from very early passage FPCs. Late passages FPCs do not differentiate well and their expression matches better with that of clonally expanded FPCs. We then assessed insulin content and insulin release in these ICAs. We observe that total insulin content as well as insulin released as a response to glucose is less as compared to that of freshly isolated human fetal islets (Figure 1C). Overall, we find that FPCs can differentiate into islet hormone-containing ICAs as seen by immunostaining and transcript analysis. However, the amount of insulin produced and secreted is significantly lower as compared to that observed in 3rd trimester fetal human pancreatic islets (Fig.1C).

We then looked if in vivo conditions or paracrine factors secreted within the in vivo niche stimulate differentiation and maturation of FPC-derived ICAs following the experimental design shown in Fig. 2A. We transplanted day 1 ICAs under the kidney capsule of NOD/SCID animals. After 15 days, which we know from our pilot studies is a period required to develop vascularization of transplanted graft, half the number (n=8) of animals were pancreatectomized. At 2 months after transplantations, grafts were assessed for presence of hormone transcripts using real time PCR as well as immunostaining, followed by confocal microscopy. We observe significant increase in the abundance of pro-insulin transcript after transplantation in

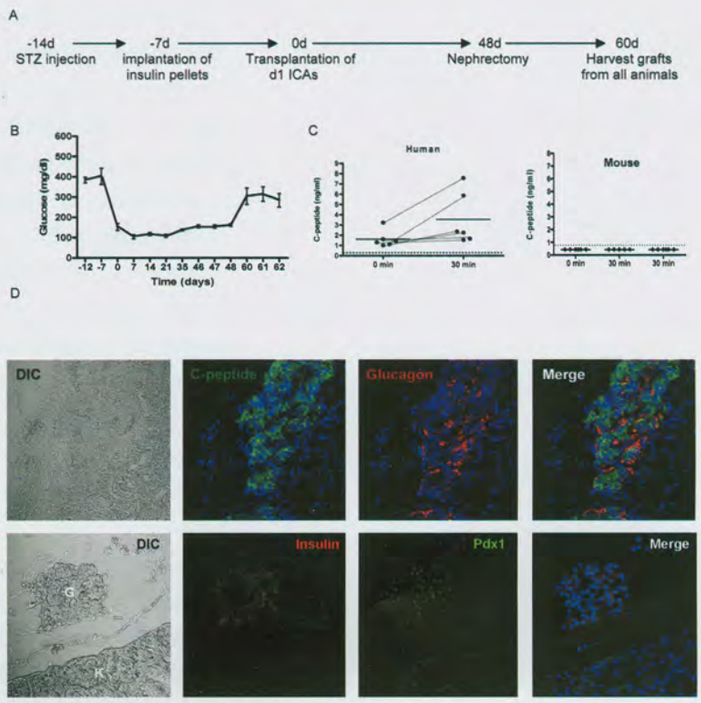


Fig. 3. Functionality of FPC-derived ICAs was assessed by transplanting them in diabetic NOD/SCID animals (n=8) using protocol outlined in **(A)**. blood glucose concentrations were monitored over the period of experiment. Diabetic animals exhibited normoglycemia following transplantation of ICAs, which was reverted back to high glucose concentrations after removal of the kidney bearing ICA graft **(B)**. human c-peptide protein was detectable in the animal sera, while mouse c-peptide was not found **(C)**. ICA grafts also responded to glucose stimulus by secreting more c-peptide 30 min after the injection, albeit at lower level. Grafts were composed of a majority of C-peptide, insulin and pdx Immunopositive cells **(D)**.

vivo, which is further enhanced in animals that underwent pancreatectomy (Tx + Px; Fig. 2B). This indicates that ICAs can differentiate and mature better in vivo and this process is further accentuated in the presence of paracrine factors secreted by the regenerating pancreas. In vitro differentiation yielded maximum of 100 fold increase above detectable for pro-insulin transcript, whereas these in vivo conditions helped in much efficient differentiation (10,000 fold). Surprisingly, same effect was not observed for other hormone transcripts. In fact, the transcript abundance for pro-glucagon, somatostatin and ghrelin was observed at lower levels after transplantation (Fig. 2B). However, pancreatectomized animals showed an increase in pro-glucagon and somatostatin transcripts, suggesting distinct role of regenerating pancreas in differentiation to major islet hormone-producing cells. When we looked at the kidney sections, we observed presence of insulin, glucagon and somatostatin immuno-positive cells in the implanted graft (Fig. 2C). After ensuring the maturation of ICAs in vivo, we next decided to check their functionality by transplanting them in immuno-compromised diabetic NOD/SCID mice. Mice were made diabetic by injecting STZ and then they were maintained normoglycemic by implanting slow release insulin pellets (Linbit, Canada) under the skin (Fig. 3A). This was carried out to achieve

normoglycemia and avoid the risk of glucotoxicity to transplanted grafts in these diabetic animals. Circulating blood glucose concentrations were monitored once a week (Fig. 3B). We found that animals were hyperglycemic after STZ injection (-12 to -7 days). Implantation of insulin pellet resulted in decrease in blood glucose, which was within normal range at the time of transplantation of ICAs (day 0). Effect of these 2 insulin pellets implanted under the skin lasts for a maximum of 14 days from its implantation (day 7), suggesting the normoglycemia achieved in diabetic animals after this period is due to the insulin released from the transplanted ICAs. To confirm this, we carried out unilateral nephrectomy on transplanted mice, wherein the graft bearing kidney was removed after 48 days from transplanatation. We recorded elevated blood glucose concentration in these mice (hyperglycaemic), thus confirming that FPC-derived ICAs were responsible for maintenance of normoglycemia in these diabetic animals. We also analyzed presence of human C-peptide in the sera of these animals and found that there is detectable amount of human C-peptide, which increases after 30 min of glucose injection (Fig. 3C). Though the increase after glucose challenge was modest, this indicates that FPC-derived ICAs can process and secrete insulin. On the other hand, mouse insulin (C-peptide) was not observed in sera of any of the animal, confirming that normoglycemia was achieved by human insulin (C-peptide)-producing cells in the graft. Kidneys bearing ICA grafts were sectioned and immunostained. We observed insulin, C-peptide, glucagon as well as Pdx1 immunopositive cells in the graft (Fig. 3D).

We also observed that FPC-derived ICAs mature and differentiate better in vivo as reported earlier for other cell types. The differentiation was further boosted by at least 10-fold when the pancreatectomy is performed in transplanted animals. This indicates role of paracrine factors secreted from regenerating pancreas that can increase differentiation into β-cell. Our group has earlier demonstrated that the cytosolic extract from regenerating pancreas can control STZ-induced diabetes. Recently, Lee et al 2008, has also shown that adipose tissue-derived stem cells can differentiate into pancreatic lineage using regenerating pancreatic extract. These studies highlight the importance of these paracrine factors and also necessitate their isolation, purification and characterization. Our studies as well as reports from several laboratories demand further research in this area to arrive at a consensus in terms of the best alternative source of cells and the best protocol for directed differentiation with maximum efficiency, to generate a true beta cell type in vitro/in vivo. All these efforts raise a question- 'why is it difficult to generate a true beta or even a beta-like cell in vitro?' We think that a beta cell is a specialized cell that requires association and communication with other islet

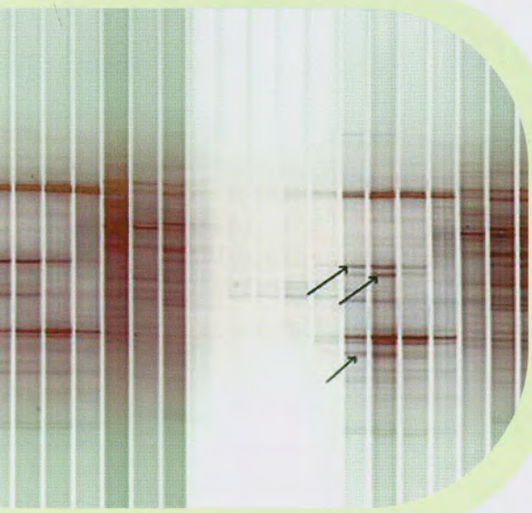
hormone-producing cells, endothelial cells, interactions with matrix proteins and other regulating hormones in order to function properly. It is therefore necessary to study cell-cell, cell-matrix interactions as well as understand β-cell physiology to design new strategies of differentiation.

Studies are already being carried out using clues from pancreas development, using small molecules and also by forced expression of transcription factors. Our ongoing studies using the SOLiD system for assessment of ncRNAs and other small molecules in pancreas development and differentiation may provide insights into in vitro differentiation of these islet-derived progenitor cells. We believe that complete understanding of beta cell biology will enlighten us in designing better strategies for generation of insulin-producing cells in vitro.

Research Report

Biodiversity

Yogesh Shouche 84



Insect and Microbial Genomics

Yogesh Shouche
yogesh@nccs.res.in

Participants

Bipin Chandra Salunke, *RA*
S. Venkata Prasad, *RA*
Kiran Pawar, *RA*
C. P. Antony, *JRF*
Prashant Pandey, *SRF*
Rahul Salunkhe, *JRF*
Nachiket Marathe, *JRF*
Kaushal Sharma, *JRF*
Kaustubh Gokhale, *SRF*
Jay Siddharth, *SRF*
Rasesh Parikh, *SRF*
Pankaj Verma, *SRF*
Ashraf Rangrez, *SRF*
Arvind Gupta, *SRF*
Sajjid Ahengar, *JRF*
Dhiraj Dhotre, *JRF*
Deepak Patil, *SRF*
Shakti Singh, *JRF*
Himanshu Kumar, *SRF*
Arpita Kulkarni Nene, *JRF*
Archana Meshram, *JRF*
Gulab Kirawale, *PA*
Sandeep Valunjkar, *PA*
Girish Kulkarni, *JRF*
Shrikant Pawar, *JRF*
Snehit Mhatre, *JRF*
Avinash Sharma, *JRF*
Gaurav Shimpi, *PA*
Sarang Satoor, *Technician*
Shailesh Sawant, *JRF*
Mandar Rasane, *Technician*

Background

Microbial Genomics

Microorganisms are ubiquitous and form predominant form of life on earth. They not only outdo the eukaryotic cells in number but may also represent about half of the global biomass. Their metabolic, physiologic and genetic diversity is far greater than any other life form. However, very little information is available about their diversity as compared to higher forms due to lack of appropriate methods to study them. In the past few years 16S rRNA gene sequence based methodologies are being used to study microorganisms. These methods allowed us the glimpse of microbial diversity without the need to cultivate them in laboratory. Our laboratory uses these methodologies to understand community structure and function of some unique ecological niches.

Insect Genomics

Malaria is one of the deadliest tropical diseases, affecting 200-300 million people worldwide with a mortality rate of ~1 million people every year. The disease is caused by mosquito mediated infection of protozoan parasites, *Plasmodium*. Controlling the spread of the disease has been impaired due to the evolution of insecticide-resistant mosquitoes and drug-resistant parasites. Availability of genomic sequence information from *Anopheles gambiae*, the African malarial vector, assisted researchers in designing novel anti-malarial strategies along with a better understanding of the interaction with the parasite. *Anopheles stephensi* is a major malarial vector in urban settings in the Indian subcontinent. Despite its importance as malaria vector, very limited genomic sequence information is available in various public databases. Our laboratory has been involved in genomic analysis of this mosquito species.

Collaborators

Dr. H.V. Batra, DRDO
Dr. H.J. Purohit, NEERI
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

Aims and Objectives

Understand the structure-function relationship of microbes in unique ecoystems like hypersaline hyper alkaline lake, insect and human gut.

Work Achieved

Microbial Genomics

Insect Mid-gut

The gut of the insect houses complex and diverse microbiota that are directly or indirectly responsible for health and disease. Dipteran flies are one of the most abundant and important groups of insects, which act as mechanical and/or biological vectors for various microbial agents. Houseflies (*Musca domestica* L.) are cosmopolitan, ubiquitous and synanthropic and live in intimate association with vertebrate pathogens. To explore the bacterial complexity and identity we used both culture dependent and culture independent approaches. Total 136 isolates were partially characterized by the classical microbiological phenotypic, biochemical (BIOLOG and API 20N kits) and molecular methods (16S rRNA gene sequencing). The community structure was explored by 16S rDNA library from the gut of housefly. DOTUR analysis revealed 25 and 56 unique phylotypes or operational taxonomic units (OTUs, at 0.03 evolutionary distance) in cultured isolates and 16S rRNA library, respectively. The OTUs were affiliated with diverse class of bacteria like *Proteobacteria* (α, β, δ and γ), *Firmicutes* and *Bacteroidetes*. Twenty genera were seen in cultured isolates with *Acinetobacter* sp. as a dominant genera followed by *Klebsiella* sp. and *Providencia* sp. Four OTUs from cultured isolates and 21 OTUs from 16S rRNA library showed $\leq$97% similarity, which might represent new genera or species sequences. Clones representing unculturable isolates were composed of 33 genera spanning three phyla with *Aeromonas veronii*, *Bacillus firmus* and *Providencia rustigianii*, as a dominant species. The study of housefly gut microbiota will help to understand the role of gut microbiota in relation to disease spread.

Mosquitoes are medically important arthropod vectors for transmission of numerous human diseases. In particular, dengue has become most important mosquito-borne viral disease in humans in terms of morbidity and mortality. The major reasons for this catastrophic situation are the unavailability of effective vaccines for virus, insecticide resistance in mosquitoes, and the decline in socio-economic conditions in many disease endemic countries. Therefore, we are looking for development of unique control strategies against mosquito-borne diseases like paratransgenesis and development of refractory transgenic mosquitoes. To augment this, we have studied midgut bacteria from different growth stages of mosquito to see how the midgut bacterial community structure changes with the growth of mosquito.

We have constructed 16S rDNA libraries for comparative analysis of midgut bacterial communities from different growth stages of *Aedes aegypti* (from larvae to blood feed adult) and observed the presence of *Serratia*, *Pseudomonas*, *Rahnella*, *Asaia*, *Gluconobacter* in different stages of mosquito life cycle. Real time PCR based quantification of total midgut bacteria, and *Asaia bacteria* was performed and both the approaches revealed a bacterial community shift from *Rahnella* to *Asaia*. Recently it has been reported that *Asaia* always remain associated with *Anopheles gambie* mosquitoes. In our study we found presence of these bacteria only in after blood feed stages and their count increases from 0.15 % (3 day after blood feed) to 60 % (14 day after blood feed). Our study concludes that these bacteria have more potential to adapt to mosquito midgut environment compared to other naturally occurring bacteria, and therefore is a more suitable candidate for future paratransgenesis strategies.

Human Gut

Host and gut microbiota are known to co evolve in human and other organisms. Their interaction is complex and comprehensive understanding of this requires combination of several approaches. Our group has been looking at two major aspects of this interaction.

1. Obesity and gut microbes

Obesity has evolved into a global pandemic syndrome. Apart from genetic reasons, contribution of host gut microbiota in obesity is recently discovered. To accomplish this fact researchers mostly analyzed diet control models of obesity. However, surgically (Sleeve gastrectomy, SG) treated-obesity could prove another interesting weight reduction model. Analysis of gut microbiota from SG individuals could yield microbiota associated with obesity. We have performed such comparative analysis and quantification of dominant gut microbiota in normal (18-24 kg/m^2, *n*=5), obese (25-53 kg/m^2, *n*=5) and the SG treated-obese (*n*=5, 25-36 kg/m^2, Pre-surgery average BMI= 39.8 kg/m^2, Post-surgery average BMI=31.9 kg/m^2, these individuals are undergone sleeve-gastrectomy, which primarily reduces stomach size) individuals. Gut microbial diversity was assessed by constructing pooled 16S rRNA library for each group (n=5) using recently reported universal primers. Bacteria were identified at various taxonomic levels using RDP database. The two major phyla, bacteroidetes and firmicutes were significantly altered in obese and SG treated-obese individuals in comparison with normal. Moreover, bacteria of genus Bacteroides were dominant in obese, but SG treated-individuals showed lower levels similar to normal individuals (Fig. 1). Normal individuals

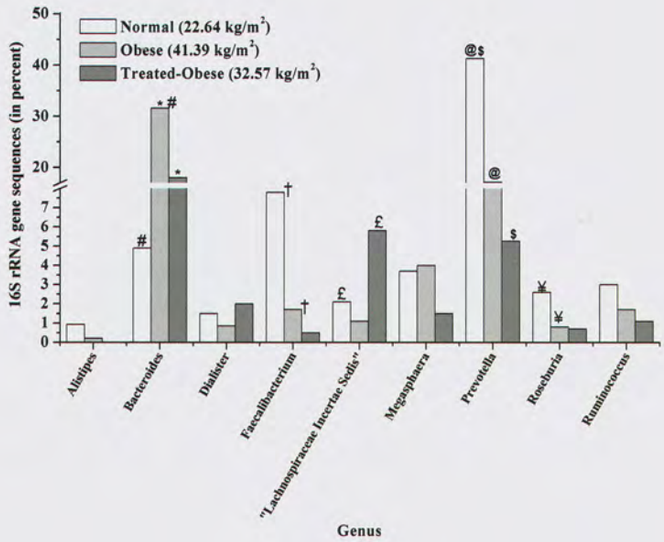


Fig. 1. Genus based bacterial distribution among normal, obese, and SG treated-obese groups based on 16S rRNA gene fragment library approach (Note, only significantly altered microbiota is shown) (*#†£@$¥$P<0.05$).

also showed a higher level of butyrate-producing bacteria (*Roseburia* and *Faecalibacterium*). Prevalence of bacteria of genus Bacteroides in obese and SG individuals is an interesting feature, as their genomes show an enriched set of genes for various glycoside hydrolases, which can help an obese individual to extract more energy from food especially indigestible carbohydrates.

2. Succession of microbial communities after birth

The gastrointestinal tract of infants at the time of birth use to be sterile and during the birth process and soon thereafter, microbes from mother and surrounding environment start colonizing the gastrointestinal tract of the infant until a dense, complex community is established .The type of delivery is presumed to have an effect on the initial acquisition and colonization of infant gut with microbes.

In our study 16S rDNA cloning and sequencing approach was used for comparative enumeration of fecal microbiota of infants born with different method of delivery in District Pune, Maharashtra, India. Twenty four babies stool samples i.e. 12 each for the full term vaginally born breast fed (VB) and full term cesarean section born (CB) infants with initial formula supplementation, at day 7 of their life, were collected. Genomic DNA was extracted and was PCR amplified using 16S rDNA universal primers. Approximately 900 bp fragment of amplified DNA was cloned in pGEM-T easy vector. Subsequently these cloned fragments were sequenced and analysed. Our analysis clearly reveals that fecal microbiota of vaginally born infants had

remarkable dissimilarity with their counterpart CB infants. The most abundant bacterial flora present in the VB infants were *Acinetobacter sp.*, *Bifidobacterium sp.* and *Staphylococcus sp.* While CB infants fecal microbiota was dominated with *Citrobacter sp.*, *Escheria coli* and *Clostridium difficile*. An interesting finding of our present study was recovery of large number of *Acinetobacter sp.*, a noso-comial pathogen, in the feces of the VB infants. However, none of the infants showed any clinical symptoms of the disease. This observation emphasizes the potential risk of Acinetobacter related epidemic outbreak in Indian infants from this region.

Lonar Lake

Lonar Crater Lake is situated in the Buldhana district (Maharashtra, India) in the formerly volcanic, Deccan-Trap geological region. It is a unique aquatic habitat with substantial amount of salts, metals and carbonates. Our endeavour to study the molecular ecology of Lonar Lake started with phylogenetic analysis of the microbial diversity in the lake. Strikingly the results had shown that Lonar Lake entertains huge and uncultured microbial diversity despite of its extreme conditions. Hence, we extended the work to test the genetic and metabolic capacity of this microbial diversity in such alkaline and saline environment using new and advanced molecular ecology techniques such as Stable isotope probing (SIP), SSCP, DGGE, Real time- PCR. We wish to study 1) nitrogen fixation capacity by checking the diversity and abundance (Real time quantification) of *nifH* gene, 2) CO_2 fixation capacity by checking the RuBisCo gene diversity, 3) methane oxidation ability by SIP and DGGE techniques, and 4) Glucose metabolising microbial population by SIP and SSCP.

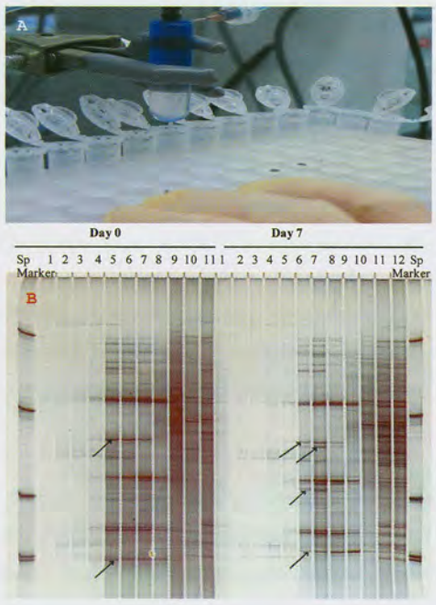


Fig. 2. Stable isotope probe technique A) Collection of fraction of after density gradient centrifugation, SSCP gel image. Numbers 1-11 represent exonuclease treated amplified 16S products from fractions of day 0 and day 7, Sp. Marker is of standard bacterial 16SrDNA. Marked Bands representing the difference in banding pattern of unlabelled.

Microbes from Lonar lake show tremendous and unique diversity of *nifH* genes. A total of 88 *nifH* phylotypes were identified spanning in algal mat, water and sediment. The *nifH* gene library was dominated by δ-proteobacteria followed by *Firmicutes*, γ-*proteobacteria*, *Cyanobacteria* and several unknown bacteria. The *nifH* copies (10^9, 10^8, and 10^{10} in algal mat, water and sediment, respectively) had shown high correlation with 16S rDNA gene copies (10^8, 10^6, and 10^7 in algal mat, water and sediment, respectively) indicating that most of the bacterial population in Lonar Lake have nitrogen fixing capacity. The green like form of RuBisCo gene (commonly found in *Bacteria*) is identified in phytoplankton and sediment but the red like from (found in *Archaea*) is not found and is consistent with our findings that green colouration of water is because of *Arthrosipra species* rather than halophilic *Archaea* unlike in other soda lakes where red colouration is found.

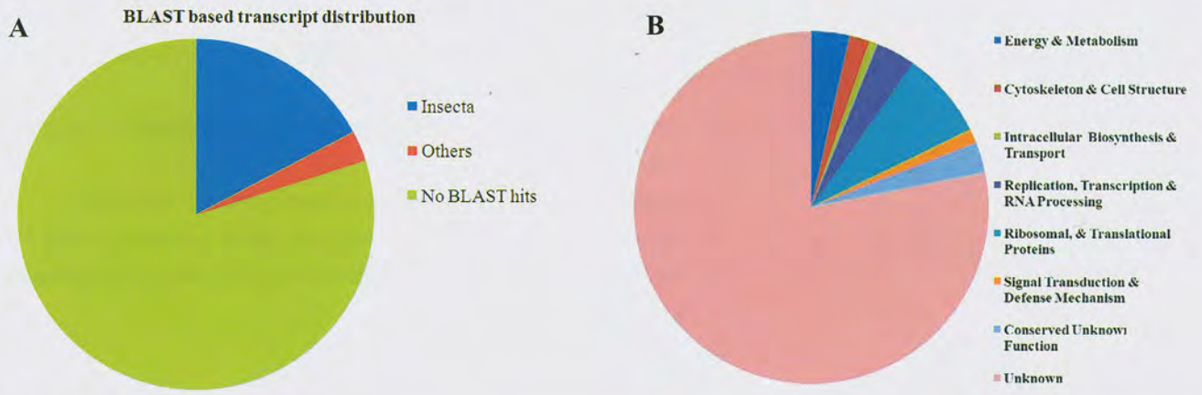


Fig. 3. A. BLASTX based EST distribution among various groups of organisms (e ≥ 10-5). The group "Other" includes organisms such as Bacteria, Human, Plasmodium and Fishes. **B.** Assigned GO (Gene Ontology) Categories to the embryonic ESTs.

The activity of methane oxidation and glucose metabolism was conducted using SIP experiments in collaboration with VTI, Germany and Warwik University, UK. The experiments have shown successful incorporation of ^{13}C in the each experiment with high correlation of ^{13}C-methane and glucose degradation. Further the DNA was extracted from the microcosms and heavy DNA fractions were separated by density gradient centrifugation (Fig. 2). The identification of *Methylomicrobium buryaticum* and *Methylophaga marina* (based on 16S rDNA of heavy fraction DNA) indicates active methane oxidation in Lonar Lake. This is the first report to show methane oxidation at pH >10.0.

Insect Genomics

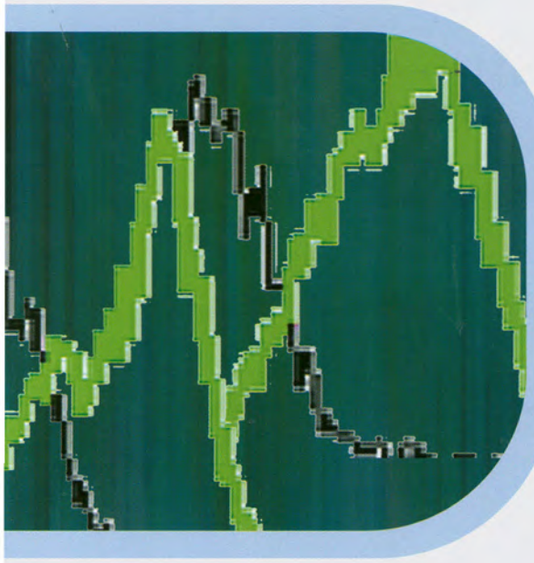
Previously, we generated 15,000 expressed sequence tags (ESTs) from this important insect during two important phases of its life cycle, the sugar-fed stage and *Plasmodium*-infected stage. To characterize and identify more such genes, we further initiated generation, analysis and annotation of ESTs from 16-24 h old embryonic *Anopheles stephensi*. We have sequenced 1140 ESTs from embryonic stage of this insect and identified 1002 unique transcripts or genes. Only a few transcripts (17%) showed homology with organisms from Insecta group and a larger fraction (80%) remained unidentified (See Figure 1). These transcripts also showed a very low homology with our earlier ESTs (27%) from the same insect, indicating their stage specific expression. GO (gene ontology) based classification also identified majority of the transcripts as novel (78%) (Fig. 3a, b). Transcripts encoding ribosomal proteins, translation proteins and energy/metabolic proteins were dominant.

Future Work

1. Exploration of biotechnological potential of microbes present in insect gut using various approaches
2. Understanding the role of mid gut bacterial in the capacity of mosquito to transmit disease
3. Understanding of role of human gut microbes in health and disease
4. Metagenomic analysis of Lonar lake and marine sediment / water for exploration of biotechnological potential in order to dissect the ecological structure

Research Report

Infection & Immunity



Interaction of Monocytes / Macrophages with malarial parasites

D Prakash
dprakash@nccs.res.in

Background

Macrophages are leukocytes, which are present in all tissues of the body. Monocytes are recruited into different tissues where they differentiate into tissue resident macrophages, which perform important functions in tissue homeostasis, the immune and inflammatory responses. Their wide tissue distribution makes these cells well suited to provide an immediate defense against foreign elements prior to leukocyte immigration. Macrophages participate in both specific immunity via antigen presentation and nonspecific immunity against bacterial, viral, fungal, and pathogens.

In the earlier studies, we have shown IL-10 induced immunosuppression by *P.falciparum* hemozoin fed MO/MQ. Other studies have shown alteration in the human / murine MO/MQs functions due to malarial parasites. However, there are no clear reports on the malaria parasite stage specific effect on Co-stimulatory molecules expression, Cytokine profiles, TLRs signaling / MyD88-responses, Dendritic Cell maturation, and Macrophage Migration Inhibitory Factor (MIF). The present study deals with these problems to understand parasite different parasite cycle stages as well as malaria pigment induced manipulations in the *Plasmodium falciparum*-THP-1 (humans) & *P.berghei*-RAW cells (mouse) models.

Aims and Objectives

1. Studies on interaction of *Plasmodium falciparum* / *P.berghei* malaria parasites & its components with MO/MQ & Dendritic cells (THP-1/RAWcells). Expression of MHC molecules, Co-stimulatory molecules etc, DC maturation, Cytokine Profiles etc.
2. Alteration in the intracellular signaling molecules due to phagocytosis of different stages of the parasites i.e ring, trophozoites & schozonts free

hemozoin & synthetic beta-heamozoin molecules. Manipulations of macrophage functions by parasites and its pigment - TLRs signaling / MyD88-responses, Dendritic Cell Maturation, Macrophage Migration Inhibitory Factor (MIF).

Work Achieved

In the present study, we have investigated the Balb/C mice derived macrophage cells line- RAW-264.7 interaction with different components murine malaria parasite Plasmodium berghei in context of RAW cell proliferation, cell viability, cytokine profiles and expression of co-stimulatory molecules.

The adherent RAW macrophage cells proliferation (3H-Thymidine incorporation) is inhibited when the cells phagocytosed with P.berghei infected cells (P.RBC), N-RBC, Pb-Hemozoin & Beta-Hematin. The cells also show less viability by MTT assay. However, the treated and controls cells shows more than 95% viability by Trypan Blue dye exclusion assay. The less viability (MTT) in treated cells is due to different number of proliferating cells.These results indicate that the RAW cells fed with these components are not toxic to the cells but inhibits the cell proliferation at different levels.

Normal RAW cells when fed / treated with different malaria components does not induce secretion of IL-6, IL-10 & IL-12 or TNF alpha expect P-RBC & PB-HZ induces 2-fold increase. Normal cells stimulated with LPS produces very high

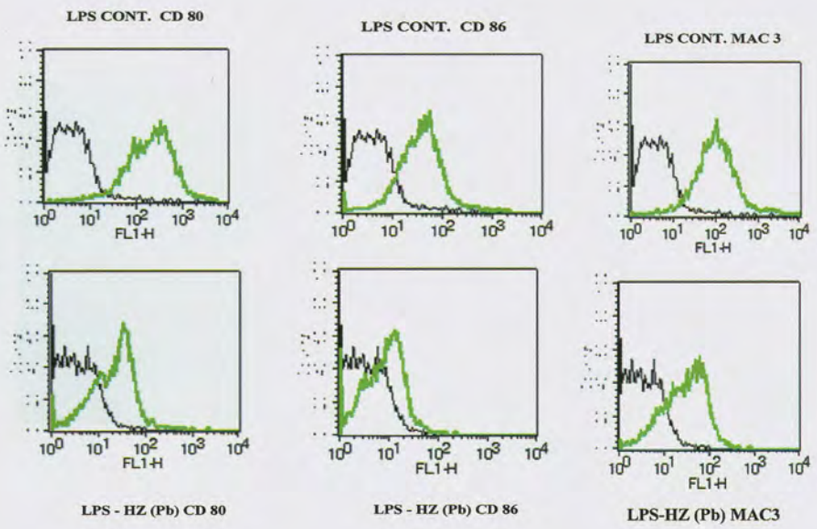


Fig. 1. RAW 264.7 cells were first treated with LPS and cultured for 48h. The cells were stained with FITC-labeled anti-mouse CD 80, CD 86 and MAC 3 for FACS analysis. The macrophage cells show increased expression of these molecules (top row). The expression of these molecules reduced when fed with Plasmodium berhei HZ (bottom row)

amount of IL-6, IL-10 & TNF but not IL-12. LPS stimulation after RAW cells treated /fed with Pb-HZ & B-Hematin induces production of IL-6 & IL12 but inhibits the production of IL-10 P.*berghei*-HZ decreases TNF secretion but B-Hematin increases the cytokine production, P.berghei fed - LPS treated cells decreases IL-10 but increases IL-12 production

The LPS treated cells shows increased expression of all CD 80, CD 86, and MAC 3. The up regulation of the molecules in LPS-treated cells were >3 fold (CD80), >2 fold of (CD 86) and (MAC-3). RAW cells treated with LPS, when fed with *P.berhgei*- HZ down regulate the expression of CD 80 by 5.6 fold, 2 folds of CD 86 and > 2 folds of MAC 3 molecules as compared to LPS treated control cells. The studies will be extended to alteration in the intercellular signaling molecules due to malaria parasites and its components.

Future Work

1. Interaction malaria components with MO/MQ & Dendritic cells and its effect on the Co-stimulatory, MHC molecules, DC-maturation & Cytokine Profiles etc.
2. Alteration in the intracellular signaling molecules due to different stages of i.e ring, trophozoites & schozonts, free parasite hemozoin & synthetic beta-heantin molecules on TLRs signaling / My D88-responses, Dendritic Cell Maturation, NF-Kb, MAPK, CIITA/Macrophage Migration Inhibitory factor(MIF) etc.

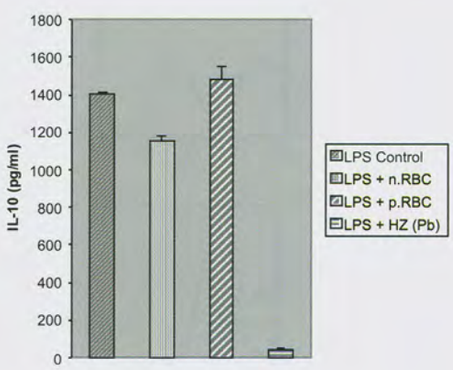
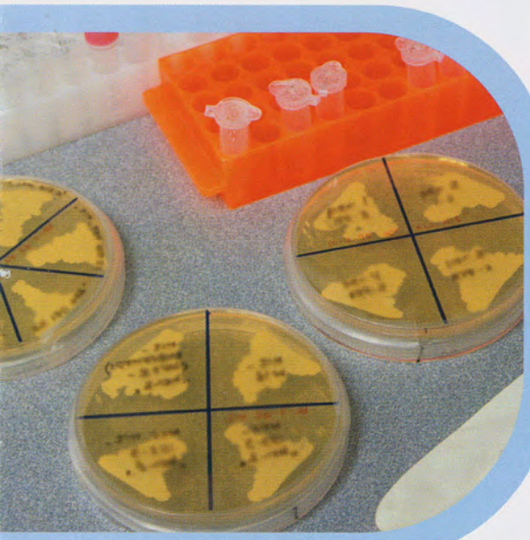


Fig. 2. RAW 264.7 cells were first treated with LPS and cultured for 48h. Then the cells were fed with n-RBC, p-RBC or P.berghei-HZ and cultured. The cell supernatant (48h) shows increased levels of IL-10 cytokine in control as well as in n-RBC & p-RBC fed cells supernatant. But the P.berghei HZ inhibits the IL-10 secretion



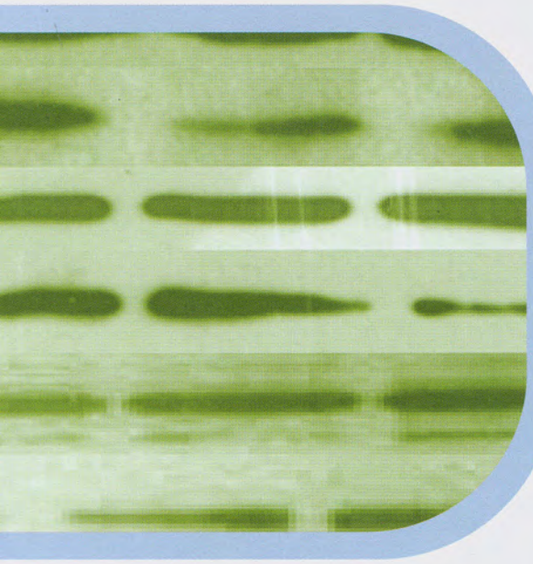
Signaling requirements for differentiation of CD8$^+$ T-cells and memory generation

G C Mishra
gcmishra@nccs.res.in

Participants
Debargh Dutta, *SRF*
Rupesh Srivastava, *SRF*

Collaborators
Dr. Cecilia D. NIV, Pune.
Dr. Mohan R. Wani

Dengue virus (DV) infection causes dengue fever, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The hyper-activation of dendritic cells (DC), monocytes, macrophages and T lymphocytes is thought to play an important role in the immunopathogenesis of DV infection. We sought to determine whether DV infection of monocyte-derived DC (DC1) and plasmacytoid-derived DC (DC2) and their associated T cell responses could play a role in immunopathology. Our results demonstrated that the expressions of costimulatory and regulatory molecules were higher on DV-DC1 than on DV-DC2. Priming of mice with DV-DC2 showed generation of regulatory T cells (T reg cells), whereas priming with DV-DC1 induced a strong proliferative response with high levels of Th1 like cytokines ex vivo. CD4$^+$ T cells from DV infected mice upon secondary challenge with DV-DC1 showed high TNF-α level with marked decrease in their proliferation, suggesting activation-induced cell death (AICD). No difference in T cell proliferation was noticed when challenged with DV-DC2. Furthermore, in vitro studies confirmed that DV-DC1 enhanced proliferation of anti-CD3ε stimulated CD4$^+$ T cells and IFN-γ secretion, while DV-DC2 suppressed CD4$^+$ T cell proliferation. In addition, infected DCs activated with TLR9 and CD40 agonists enhanced CD4$^+$ T cell activation and granzyme B expression on CTL. Our observations indicate for the first time that DV infects both DC1 and DC2 but affects their immunomodulatory function differently, and upon secondary DV exposure of DC1 high TNF-α secretion and concomitant T cell suppression contribute to the immunopathology observed in DHF and DSS.



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

Debashis Mitra
dmitra@nccs.res.in

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

Collaborators
Dr. Samir Brahmachari, IGIB, New Delhi
Dr. Inderpal Singh, NIPER, Mohali
Dr. K. K. Bhutani, NIPER, Mohali
Dr. Sekhar Chakrabarti, NICED, Kolkata
Dr. Syed N. Kabir, IICB, Kolkata
Dr. Bhaskar Saha
Dr. G.C. Mishra

Background

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS), which is defined by a reduction in the number of CD4+ T cells (less than 200 cells/μl) and the onset of opportunistic infections. The incidence of HIV infection has reached alarmingly high levels worldwide including India. The therapeutic strategies being used at present can reduce the viral load remarkably but are not the ultimate answer to AIDS patients. Our group has been working on different aspects of HIV, related to viral pathogenesis, immune response and drug discovery. The primary objective is to gain more understanding of the virus and its interaction with the host cell, which may lead us to new antiviral strategies.

Aims and Objectives

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

Work Achieved

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

We have shown previously that Tat can also transactivate the LTR promoter by directly binding to NFκB enhancer sequences present in the LTR. Tat binding to the NFκB enhancer sequence could be the basis not only for TAR-independent transactivation of viral LTR but also for modulation of cellular gene promoters. Tat has been actually shown to modulate expression of a

number of host cell proteins. In order to look for Tat induced modulation of cellular expression, we have used a real time PCR based array of pathway specific genes, using Tat transfected cells. We have found that several genes of different pathways are modulated by Tat. Furthermore, in order to analyze the recruitment of Tat on the cellular gene promoters in infected cells, we have performed ChIP on chip analysis using HIV-1 infected cells. Tat seems to occupy a plethora of cellular promoters. Studies are underway to look for the plausible role of Tat-NF□B interaction in the regulation of these genes.

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. This interaction seems to be necessary for Nef mediated upregulation of viral gene expression. As Hsp40 is normally associated with Hsp70, we have also studied the role of Hsp70 in HIV pathogenesis. Our results indicate that Hsp70 is also present in the Nef-Hsp40 complex. Furthermore, Hsp70 over expression inhibits viral gene expression whereas its down regulation leads to increase in viral gene expression and replication. Thus it seems that these two proteins reciprocally regulate viral replication where Hsp70 acts as an antiviral factor whereas Hsp40 works as a pro-viral factor. In addition, we have now shown that Heat shock factor-1 (HSF-1), a regulator of HSP gene expression also plays an important role in viral gene expression.

Accumulating evidence indicates that miRNAs of both viral and host origin may influence host-virus interaction in a variety of ways: as direct modulators of viral replication, as factors affecting viral susceptibility and as indirect modulators of cellular genes that influence viral propagation. Prof. Brahmachari's group at IGIB have earlier predicted sites in the HIV-1 genome that can potentially be targeted by human encoded miRNAs using consensus target prediction, and proposed the possibility that the cellular levels of these miRNAs might determine disease progression following HIV-1 infection. These included two miRNAs of highly related sequence hsa-miR-29a and hsa-miR-29b that could potentially bind to the region coding for the accessory protein, Nef of HIV-1. The predicted target site of hsa-miR-29a and 29b located 407 bases into the nef transcript is highly conserved in sequence in all clades of HIV-1 (A, B, C, D, F and H) barring the outlier group clade O. The

importance of the nef gene in establishing a persistent HIV-1 infection prompted us to experimentally test anti-HIV-1 potential of hsa-miR-29a and 29b. We have carried out over-expression and down regulation of hsa-miR-29a and hsa-miR-29b to test the effect of these miRNAs on virus replication. Over-expression of hsa-miR-29a significantly inhibited both Nef expression and virus production whereas modest inhibition was observed with hsa-miR-29b (Fig 1). This data clearly shows that hsa-miR-29a inhibits Nef expression and viral replication in HEK293T cells. As T cells are primary target of HIV-1, we then used human CD4+ T cell line, Jurkat, for analyzing the role of hsa-miR-29a and 29b in virus replication. Jurkat cells were nucleofected with the miRNA clones along with pNL4.3 viral clone and analyzed for Nef and p24 expression. In Jurkat T cells, both hsa-miR-29a and 29b clones significantly inhibited Nef expression and virus production (Fig 1). These results, taken together, showed that these human miRNAs not only inhibit Nef expression but also virus replication.

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

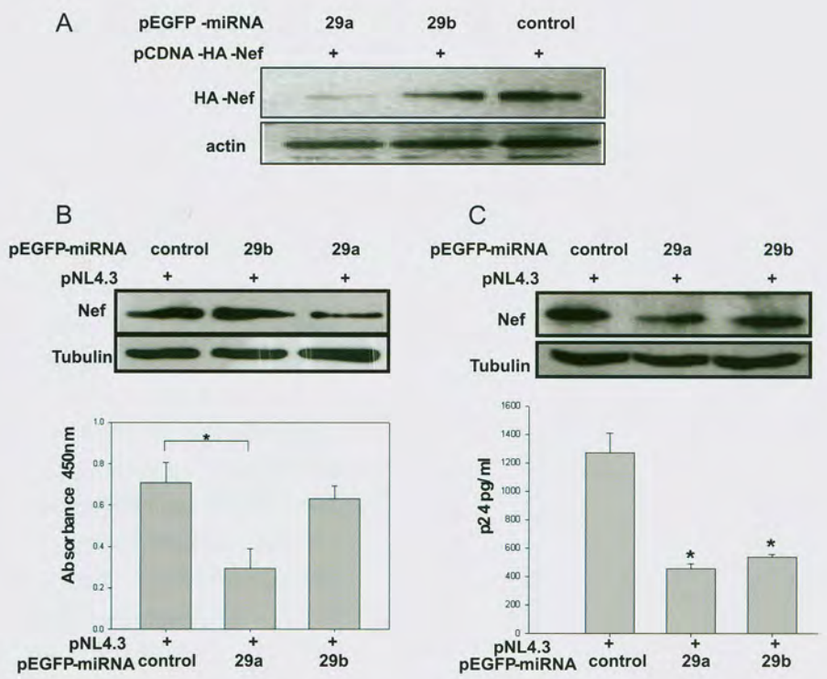


Fig.1. hsa-mir-29a and b inhibits Nef expression and HIV-1 replication. **(A)** Nef expression was inhibited by hsa-miR-29a and 29b. HEK293T cells were co-transfected with miRNA clones or control vector along with pCDNA-HA-Nef using calcium phosphate precipitation. After 36 hours of transfection, cells were lysed and expression of Nef was analyzed by immunoblotting using HA antibody. Immunoreactive actin bands were used as loading control. (B and C) hsa-miR-29a and hsa-miR-29b miRNA clones inhibited virus production in HEK293T (B) and Jurkat cells (C). Cells were co-transfected with miRNA clones or control vector along with HIV-1 molecular clone pNL4.3. Cells were lysed post-transfection and expression of Nef was analyzed by immunoblotting using Nef antibody (upper panels); culture supernatant was used for p24 antigen ELISA (lower panels). Asterisks in **(B)** represent significant p-value of 0.016 for inhibition mediated by 29a compared to control. The difference observed with 29b is not significant. Asterisks in **(C)** represent significant p-value of 0.014 and 0.016 for inhibition by 29a and 29b respectively, as compared to control vector.

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 indicate modulation of complex IV subunits in HIV infected apoptotic cells. We have been looking in to the role of different complexes of the oxidative phosphorylation system in HIV induced T cell apoptosis and our recent results also show modulation of activities of these complexes during HIV induced T cell apoptosis.

CD40-CD40L signaling in HIV infection

Impaired antigen presenting (APC) function is thought to be a critical component of HIV associated immunodeficiency. However, the mechanisms underlying these defects have not been clearly understood. In particular, it is not well established whether these defects are directly due to infection of APC by HIV, or exposure of APC to HIV gp120 or are consequence of dysregulation of $CD4^+$ T cells, as interaction between activated $CD4^+$ T cells and APC is crucial for optimal activation. Among the various ligand receptor pairs important for $CD4^+$ T cell APC communication, CD40-CD154 interaction is very important. CD40 is a member of the tumor necrosis factor (TNF)-receptor super family, which is constitutively expressed on the surface of APC, including B cells, monocytes/macrophages, and dendritic cells (DC). CD40 ligand or CD154, a member of the TNF superfamily, undergoes tightly regulated inducible expression on the surface of $CD4^+$ T cell as a result of signaling via T-cell receptor. CD40-CD154 interactions are critical for the induction and regulation of cell-mediated immunity. Binding of CD40L to CD40 expressing antigen presenting cells (APCs) promotes interleukin-12 (IL-12) and interferon ($IFN\gamma$) secretion that controls T cell-mediated activation of APCs, and regulates differentiation of $CD8^+$ T cells into effector cells. The activity of IL-12 is strongly dependent on APC interaction with activated $CD4^+$ T cells, which again is a potent inducer of $IFN\gamma$ production. Defective activation of APC by T cells that do not express CD40L could thus represent a primary event in the establishment of immunosuppression. The cytoplasmic region of CD40 bears two major signaling domains. Accumulating evidence suggests that CD40 signaling requires the association of either or both domains with binding proteins termed TNFR-associated factors (TRAF). CD40 signaling attributes the initiation of the cascade to its trimerization. It is believed that only trimerized CD40 is able to bind CD40L, which is also in a trimeric form. Since the cytoplasmic C-terminus of the CD40 molecule lack intrinsic enzymatic activity, the signaling via CD40 is mediated through interaction

with TRAFs, which again act as adaptor proteins promoting the recruitment of signaling molecules into a complex. These adaptors link CD40 to multiple downstream pathways that include phosphoinositide3-kinase (PI3K), phospholipase Cγ (PLCγ), mitogen-activated protein kinase (MAPKs), etc.

We have used a human monocytic cell line THP-1 for analyzing cell signaling during HIV infection and the role of CD40-CD40L interaction. Monocytes express CD40, a costimulatory molecule that is known to be involved in modulation of IL-12 and IL-10. We have observed modulation of ERK1/2 phosphorylation in HIV-1 NL4.3 infected THP-1 cells based on the virus concentration used for infection. Our results with CD40 ligation also show modulation in ERK1/2 phosphorylation. We have also observed gradual decrease in CD40 expression in THP1 cells with increase in NL4.3 virus concentration. Thus, our results till date indicate a possible role for ERK1/2 pathway in CD40 expression and signaling during HIV infection of monocytes.

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

The current therapeutic strategy involving the use of reverse transcriptase and protease inhibitors in combination (HAART) has proven to be useful in controlling the virus but is not sufficient to eradicate the virus from the patients. Extensive work is being done throughout the globe to identify new ant-HIV therapeutic strategies. One of the strategies has been to identify anti-HIV compounds in natural resources. We are working along with NIPER for screening of compounds isolated from medicinal plant extracts and new synthetic compounds for identification of anti-HIV molecules along with their potential to be used as a microbicide. More than three hundred extracts, fractions, isolated and synthetic compounds have been screened till date. A number of novel derivatives of quinoline 2, 4-diol and dimeric phloroglucinol molecule have shown potent anti-HIV activity. Further characterization of the activity is in progress.

Future Work

Our results till date indicate that heat shock proteins, specifically HSP40 and HSP70 play an important role during HIV-1 infection. We are now trying to elucidate the role of all the other heat shock proteins in HIV pathogenesis, which will provide us a comprehensive knowledge about the role of HSPs in HIV infection and pathogenesis. In addition, we are trying to elucidate the role of HSF-1 in virus replication and pathogenesis. We are also continuing characterization of some of the other Nef interacting clones identified by

yeast two hybrid system and identifying their functional relevance in HIV lifecycle. Furthermore, we are studying the recruitment of Tat protein on the chromatin during HIV infection that might lead to the elucidation of the mechanism of Tat mediated regulation of both vital and cellular gene expression. Identification of differentially expressed genes and their relevance to HIV induced cell death is being continued, with a focus on mitochondrial oxidative phosphorylation system in infected cells. We have also initiated studies on the role of CD40 and CD40L in HIV infection with emphasis on the study of CD40 mediated signaling during HIV infection. Finally, studies are in progress to identify novel anti-HIV molecules, both from plant sources and synthetic chemistry, with the objective to identify novel lead molecules with potential for use as anti-HIV microbicides.

Host-pathogen interactions

Milind Patole
patole@nccs.res.in

Participant
Vishal Kalel, *SRF*

Background

Search of different PTS2 sequences is an ongoing process and with bioinformatics tool new topogenic signals have been discovered from genome sequences of plants, yeast and human. Although kinetoplastids are important human parasites, the information related to PTS2 sequences is inadequate. Therefore in this study, attempt has been to verify different PTS2 sequences from known glycosomal enzymes and their ability to target reporter proteins to glycosomes and mammalian peroxisomes.

Aims and Objectives

To elucidate functionality of the kinetoplastid peroxisomal targeting signal-2 in mammalian cells.

Work Achieved

Our studies conclusively establish that PTS2 motif of Leishmania hexokinase (LmHKPTS2) is necessary and sufficient for its transport to glycosomes. Surprisingly, LmHKPTS2 as well as other Leishmania glycosomal proteins failed to import EGFP to mammalian peroxisomes when tested in vitro using EGFP fusion constructs. Mutational analysis suggested that PTS2 is not a mere sequence-based motif but also a structure-based motif, where all amino acid residues play important role in receptor binding. These results suggest that Leishmania and human PTS2 motifs and possibly their receptors may not be identical. The studies in this line will help to strengthen the notion of developing drug discovery programme for glycosome biogenesis. Expression of about 100 glycosomal genes was found to be very similar in two different morphological forms of Leishmania. NMR studies indicate that PTS2 sequence from human thiolase enzyme is a linear structure. Its comparison is being made with Leishmanial PTS2 sequence.

Future Work

1. To study interaction of PTS2 sequences from Leishmania with its receptors from human and Leishmanial cells
2. To study different peroxins from Leishmania in glycosome biogenesis.

CD40 signaling in the regulation of immune response

Bhaskar Saha
sahab@nccs.res.in

Participants

Sunil Martin, *SRF*
Raghu Kumar Penke, *SRF*
Neetu Srivastav, *SRF*
Raki Sudan, *SRF*
Sushmita Pahari, *SRF*
Srijit Khan, *SRF*
Mohammed Mumtaz Nayyer, *SRF*
Uddipan Sarma, *Project Assistant*

Background

Immune responses to auto-antigens, allo-antigens, pathogens and tumor antigens are regulated by activation of T-cells. It has been shown that the interaction between T-cell-expressed CD40-ligand (CD40-L) and antigen presenting cell-expressed CD40 plays a crucial role in T-cell activation. However, it was shown previously that $CD4^+$ T cells express CD40 but the functional significance remained unknown. On the other hand, the role of $CD8^+$ T cells in Leishmania donovani infection remains debatable and CD40-deficiency makes the mice susceptible to this parasitic infection. Therefore, we tested whether $CD8^+$ T cells express CD40 and the role played by CD40 in these cells.

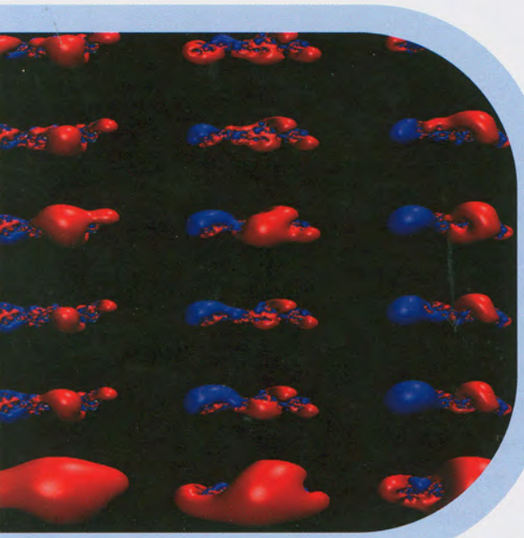
Work Achieved

The concept of suppressor T cells [Ts] and contra-suppressor T cells [Tcs] was introduced almost thirty years ago to explain the observed immuno-regulatory phenomena in various models of transplantation, autoimmune diseases, infectious diseases, hypersensitivity reactions and cancer. However, as the identity of these cells and their molecular mechanism of action were not defined, the concepts of Ts and Tcs gradually faded away. The role of the professional suppressor was revived when Sakaguchi *et al* observed an unexpected autoimmune disease due to the loss of T cells with suppressor functions in neonatally thymectomized mice. Later studies identified $CD4^+CD25^+$ cells as T-regs, which may also express GITR, Foxp3 and $CD127^{dim}$ as additional possible markers, mediating immuno-suppression via IL-10. In contrast, despite high IL-10 production in *Leishmania donovani* infection that causes the disease visceral leishmaniasis, the involvement of T-regs in the infection remains controversial. Paradoxically, $CD4^+$ T-regs require CD40-

CD40-ligand interaction for its generation but the deficiency in either of the molecules renders mice susceptible to *Leishmania* infection. We showed previously that $CD4^+$ T-regs were kept in check till ten days after *L. donovani* infection (D10) but executed their suppressive functions 21 days after the infection (D21) resulting in accelerated parasite growth; elimination of these cells reduced the infection. On the other hand, although $CD8^+$ T cells are implied in host-protection in visceral leishmaniasis, whether these cells keep T-regs in check during the first ten days after infection but lose control at a later phase of the infection remains unknown. Therefore, in this study, we addressed the role of these cells in the regulation of T-regs during the phase-specific anti-leishmanial immune response as a function of CD40 expression.

We have observed that $CD8^+CD40^+$ T cells executed CD40-dependent cytotoxicity against $CD4^+$ T-reg cells. CD40 signaled through Ras, Phosphatidyl-inositol-3 kinase (PI-3K) and protein kinase C (PKC) resulting in NF-κB-dependent induction of granzyme B and perforin, the mediators of the cytotoxic function of $CD8^+$ T cells. Adoptive transfer of these cells reduced the *Leishmania donovani* infection in susceptible BALB/c mice. These $CD8^+$ contra-T-reg cells were apoptosed by IL-10. Thus, $CD8^+CD40^+$ T cells function as contra-T-reg cells modulating the course of an infection.

As elimination of suppressive T cells is shown to result in tumor regression and reduction in pathogen burden in the host, we demonstrate the functional existence of a novel counter-suppressive T cells, termed contra-T-reg cells that by eliminating the T-regs, relieve the suppressive effects of these cells on the host-protective immune response and substantially control the parasite burden in a CD40-dependent manner. It is possible that these are the $CD8^+$ T cells that help maintain the vaccine-induced anti-leishmanial protective T cell memory. The results demonstrate a peripheral regulation of contra-T-reg cells but do not exclude the possibility of fixing the lineage during thymic selection. Since hyper-activation of the contra-T-reg cells may suppress the T-regs preventing graft acceptance and invoking self-reactivity, targeted elimination of contra-T-reg cells can be a novel therapeutic principle.



Role of viral complement control proteins in immune evasion

Arvind Sahu
arvindsahu@nccs.res.in

Participants

Malik Reza, *SRF*
Vivekanand Yadav, *SRF*
Kalyani Pyaram, *SRF*
Muzammil Ahmad, *SRF*
Archana Kadam, *Project Assistant*
Snehal Chavan, *Project Assistant*
Richa Shukla, *Project Assistant*
Sunil Raut, *Project Assistant*
Ashish Kamble, *Project Assistant*
Yogesh Panse, *Technician*

Collaborators

Jayati Mullick, NIV, Pune
Debashis Mitra, NCCS, Pune
Dimitrios Morikis, UC Riverside, USA

Background

The complement system is an ancient yet highly effective immune surveillance system against a wide array of pathogens including viruses. It is known to directly assail viruses by means of opsonization leading to aggregation, phagocytosis and lysis. In addition, it also promotes virus specific B and T cell responses. Owing to the intense threat that the complement system poses, many viruses have learned to escape from the complement surveillance to become successful human pathogens. The formidable complement evasion mechanisms developed by viruses include encoding of structural and/or functional homologs of host complement regulators, capturing of host complement regulatory proteins and usage of host complement receptors for cellular entry. Our laboratory has significantly contributed to the identification and functional characterization of structural homologs of host complement regulators encoded by pox and herpesviruses. Our current emphasis is on understanding the molecular basis of complement inhibition by complement regulators encoded by variola virus (SPICE), vaccinia virus (VCP), *Herpesvirus saimiri* (sCCPH) and Kaposi's sarcoma-associated herpesvirus (Kaposica). In addition, we are also looking into the role of these proteins in viral pathogenesis.

Aims and Objectives

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

Work Achieved

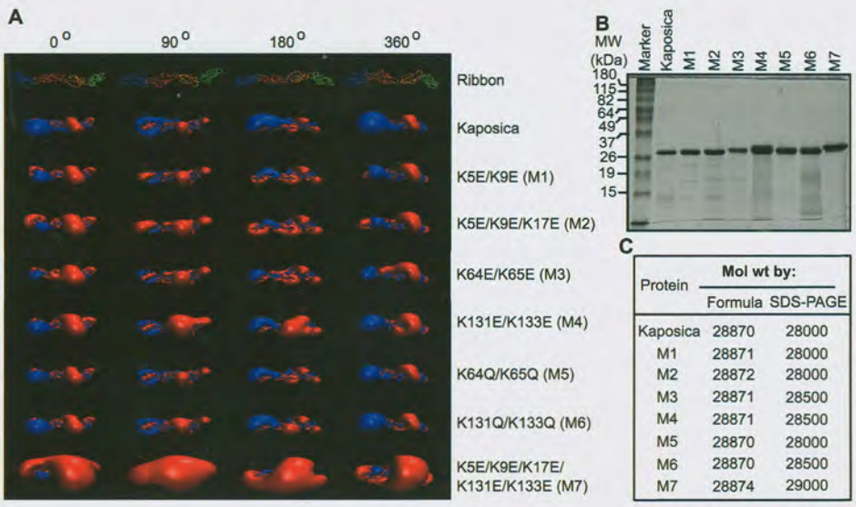
Physicochemical analysis of viral complement regulators revealed that spatial distribution of electrostatic potential is conserved in both herpes as well as poxviral complement regulators. We therefore utilized Kaposica as a model protein to understand how charge distribution on the CCP domains influences the functioning of viral regulators. Structurally, Kaposica is made up of 4 tandem repeats of complement control protein (CCP) domains connected by 4-residue linkers. The CCPs 1 and 4 carry positive charge around them, while CCP 2 is neutral and CCP 3 is negative. In addition, positive potential is found in the linker regions between CCPs 1-2 and 2-3. We thus conducted a systematic mutagenesis study to decipher whether overall positive electrostatic potential of the molecule or of its individual domains influences its various complement regulatory activities and whether it has differential effect on various functional activities of Kaposica.

To design mutants for this study we first generated a homology structure of Kaposica utilizing crystal structure of VCP as a template. The various theoretical mutants were then designed and electrostatic potential calculations were made by Poisson-Boltzmann equation to study the effect of these mutations on spatial distribution of electrostatic potential. The mutants with the least number of mutations and largest effect on electrostatic potential were selected and expressed for experimental analysis (Figs. 1 & 2).

Does overall positive electrostatic potential guide the cofactor activities of Kaposica?

Kaposica supports inactivation of C3b and C4b (the subunits of C3 convertases) by the serine protease factor I by acting as a cofactor. To elucidate the importance of overall positive electrostatic potential of Kaposica in performing the function of a cofactor, we expressed a series of seven mutants with decreased overall positive electrostatic potential and analyzed their C3b and C4b cofactor activities by using a fluid phase assay. In this assay, human complement protein C3b or C4b was incubated with equal concentrations of Kaposica or each of its mutants in the presence or absence of factor I and inactivation of C3b or C4b was assessed by quantitating the cleavage of C3b/C4b.

If the overall positive potential guides cofactor activities then negation of the overall positive potential should result in loss of cofactor activity. Consistent with this premise, the mutant possessing the most overall negative electrostatic potential (M7; Fig. 1) was completely inactive for C3b and C4b cofactor activities. However, the other mutants did not follow this trend.



Protein	Mol wt by:	
	Formula	SDS-PAGE
Kaposica	28870	28000
M1	28871	28000
M2	28872	28000
M3	28871	28500
M4	28871	28500
M5	28870	28000
M6	28870	28500
M7	28874	29000

Fig. 1. Isopotential contours of Kaposica and its mutants illustrating the spatial distribution of electrostatic potential, and SDS-PAGE and mass analysis of purified Kaposica and its mutants.

Mutants M1 and M2, wherein the positive potential on the CCP1 was reduced, showed no difference in C3b and C4b cofactor activities in comparison to the wild type protein. Further, replacement of positive residues with negative or neutral residues at the linkers between CCPs 1-2 (M3 and M5) and CCPs 2-3 (M4 and M6), which resulted in similar or lesser overall decrease in positive potential compared to mutants M1 and M2, led to about 3- to 6-fold loss in C3b cofactor activity and up to 2.5-fold loss in C4b cofactor activity. Our data therefore suggest that the decrease in activity observed by imparting negative potential in the molecule depended more on the site of negation rather than the negation of overall positive potential in the molecule.

Does overall positive electrostatic potential direct the decay-accelerating activities?

In addition to supporting the protease-mediated inactivation of subunits of C3 convertases (C3 cleaving enzyme), Kaposica also regulates classical and alternative pathway C3-convertases by accelerating their decay. We, therefore, next compared the decay-accelerating activities of Kaposica and the various mutants to evaluate whether the overall positive potential influences these activities. To measure the decay activities, the classical (C4b,2a) and alternative pathway (C3b,Bb) C3-convertases were formed on sheep and rabbit erythrocytes, respectively, using purified complement proteins. These enzymes were then allowed to decay in the presence of increasing concentrations of Kaposica or each of the mutants and the remaining C3-convertase activity was estimated by measuring the hemolysis following addition of EDTA-sera (a source of C3-C9).

The data showed that like cofactor activities, the M7 mutant that possesses maximum negative potential, displayed only residual classical pathway decay-accelerating activity (CP DAA). Among other mutants, N-terminal positive potential negation mutants (M1 and M2) did not show any effect on the CP DAA, but linker negation mutants (M3, M4, M5 and M6) showed considerable reduction in CP DAA. However, as in case of the cofactor activities, there was no correlation between CP DAA and overall positive potential, suggesting that positive potential at the linkers and not the overall positive potential is important for driving CP DAA. In case of alternative pathway C3-convertase decay-accelerating activity (AP DAA), unlike the CP DAA and cofactor activities, all the mutants except M1 showed considerable reduction in AP DAA, suggesting that AP DAA is more sensitive to negation of positive electrostatic potential.

Influence of electrostatic potential of individual domains of Kaposica on its cofactor and decay activities

The data obtained from the first set of mutants indicated that decrease in functional activities of Kaposica depended more on the site of negation of positive potential (e.g., linkers) rather than negation of overall positive potential in the molecule. Hence, we next sought to determine the influence of alteration of electrostatic potential on each of the CCP domains on the

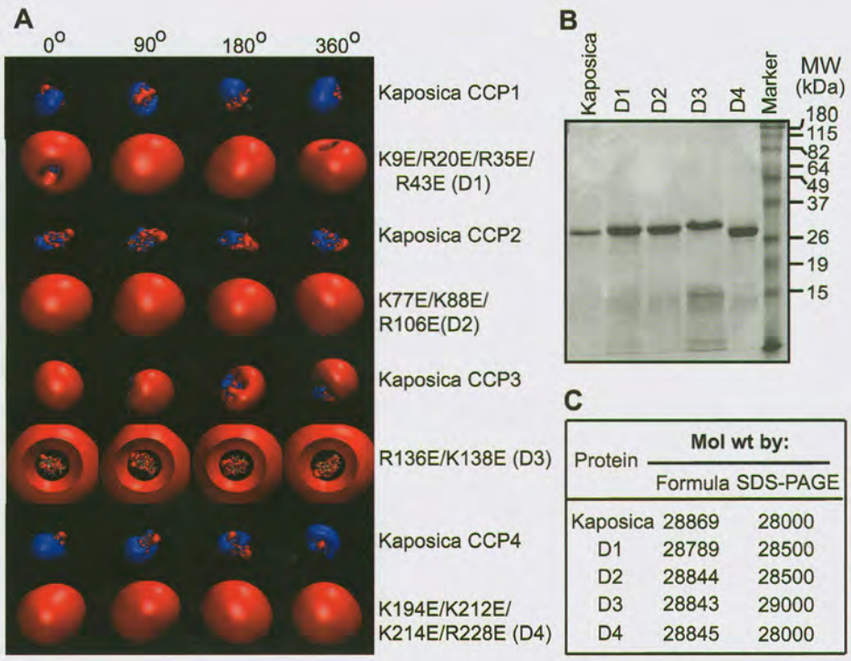


Fig. 2. Isopotential contours illustrating the spatial distributions of electrostatic potential on different CCPs of Kaposica and its mutants in isolation.

cofactor and decay activities. For this purpose, a set of four mutants were designed and expressed wherein electrostatic potential of the individual domains with positive (CCP1 and CCP4) or neutral (CCP2) potential was changed to negative, and the domain with negative potential (CCP3) was mutated to increase its negativity (Fig. 2).

The data showed that the effect of reversal of positive potential in various domains of Kaposica was greater on C3b cofactor activity than on C4b cofactor activity. All the mutants (D1-D4) displayed considerably decreased C3b CFA compared to Kaposica. In contrast, except D1, none of the mutants displayed >2-fold decrease in C4b CFA. These data therefore suggested that maintenance of intrinsic potential on all the domains is critical for C3b CFA as increase in negative character of any of the domains is not tolerated for C3b CFA. Such, however, is not the case for C4b CFA as except CCP1, negative potential was well tolerated on domains 2-4.

Examination of the domain potential reversal mutants on the CP C3-convertases showed that changing positive potential of CCP1 to negative completely eliminated its CP DAA indicating that positive potential at CCP1 of Kaposica is indispensable for this activity. Imparting negative electrostatic potential at CCP2 (D2) and CCP3 (D3) did not lead to any significant change in CP DAA. An unexpected finding was that the D4 mutant that carried negative potential instead of positive potential at CCP4 of Kaposica displayed a remarkable 4.5-fold increase in CP DAA.

The assessment of AP DAA of the CCP domain mutants revealed that imparting negative electrostatic potential on any of the four CCP domains led to either very residual or considerable decrease in activity. Thus like C3b CFA, maintenance of intrinsic potential on all the four CCP domains of Kaposica is also critical for AP DAA.

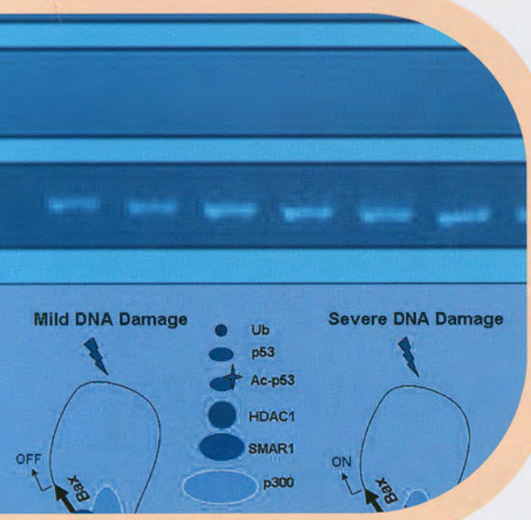
Together these data provides functional relevance to conservation of positive electrostatic potential in CCP 1 and 4 as well as in the linker regions of various viral complement regulators.

Future Work

1. Structural basis for species specificity in poxviral complement regulators.
2. Fine mapping of functional sites in VCP, Kaposica and sCCPH.
3. Role of VCP in viral pathogenesis.

Research Report

Chromatin Architecture & Gene Regulation



Gene regulation by tumor suppressor MAR binding protein SMAR1

Samit Chattopadhyay
samit@ncccs.res.in

Participants

Surajit Sinha, *SRF*
Sreenath K, *SRF*
Sunil K Malonia, *SRF*
Sandeep Singh, *SRF*
Sulabh Kharbanda, *SRF*
Kiran K Nakka, *SRF*
Rakesh Sharma, *Project student*
Sandeep Bhat, *Project student*
Nidhi Chaudhary, *JRF*
Sijo Varghese, *JRF*
Bhawna Yadav, *JRF (LTMT)*
Riya Uthup, *SRF*
Bhalchandra P Mirlekar, *Project SRF*
Mukil M, *Project Assistant*
Devraj Mogare, *Technician C*
Chhavi Jain, *Project student*

Collaborators

Siddhartha Roy, IICB, Kolkata
H.K Prasad, AIIMS, New Delhi
U.D Gupta, NJIL & OMD, Agra
Subeer Majumdar, NII, New Delhi
Pawan Sharma, ICGEB, New Delhi
Uday K. Ranga, JNCASR, Bangalore
Tanya Das, Bose Institute, Kolkata
Gaurishankar Sa, Bose Institute, Kolkata
Debashis Mitra, NCCS, Pune

Background

The eukaryotic interphase chromatin is a highly organized structure. Specific scaffolding proteins form complexes with DNA and play pivotal role in DNA packaging. An important feature of DNA packaging involves folding of the chromatin into loop domains, which are periodically attached to the nuclear matrix through binding to specialized DNA sequences called Matrix Attachment Region or MARs. We study how proteins that specifically bind to MARs regulate genomic DNA organization and nuclear biochemical functions such as transcription, recombination, splicing, repair etc. Past several years our lab has been engaged in understanding the role of nuclear matrix and associated proteins in pathophysiological processes. We have focused on one such novel matrix associated protein SMAR1 that is down-regulated in human breast cancer. It acts as a global repressor for many genes including Cyclin D1, IκBα and CK8 by directly recruiting HDAC1-mSin3a dependent repressor complex. Our findings reveal that SMAR1 functions in two different ways to regulate global gene expression. First, it acts as a transcriptional repressor and second, it modulates the transactivation potential of transcriptional co-activators NF-κB and p53. Additionally, NF-κB and p53 regulates various transcription factors involved in oncogenic transformation. These cofactors globally affect various signaling pathways leading to activation of genes that onset the process of tumorigenesis. Thus, a change in the level of SMAR1 as is seen during cancer progression is directly correlated with dysregulation in signaling pathways associated with aberrant expression of oncogenes. We are therefore focusing our research work in understanding global gene regulation by SMAR1.

Aims and Objectives

1. Identification of factors regulating SMAR1 mRNA and protein levels.
2. Regulation of p53 transcriptional activity by SMAR1 that act as switch in cell cycle arrest and apoptosis
3. SMAR1 mediated repression of HIV-1 LTR
4. Regulation of T_H1-T_H2 lineage commitment

Work Achieved

Identification of factors regulating SMAR1 mRNA and protein levels

The regulation of mRNA stability has emerged as a critical control step in determining the cellular mRNA level, with individual mRNAs displaying a wide range of stability that has been linked to discrete sequence elements and specific RNA-protein interactions. We have earlier reported that SMAR1 expression is downregulated in higher grades of breast cancer and cancer derived cell lines like MCF-7, HBL-100, ZR 75.3 and ZR 75.1. In our recent studies, we have identified the presence of a variant 5' UTR of SMAR1 in MCF-7 and ZR 75.1 cell lines. This form of SMAR1 (ϕ17) is different from its wild type counterpart (ϕ1) in that the ϕ17 SMAR1 lacks an 18-mer stem-loop structure in the 5' UTR at the junction of the first and the second exon of the gene. We have also identified that this stem-loop structure is critical for formation of nucleoprotein complexes on the 5'UTR, rendering SMAR1 mRNA stable upon Prostaglandin A2 treatment. Now, we have identified heat shock protein HSP70 as one of the factors that bind to SMAR1 5'UTR upon PGA2 treatment. HSP70 binds to a novel site on the stem of SL1 structure. We demonstrate that HSP70 is essential for stabilization of ϕ1 SMAR1 transcript, the depletion of which results in decreased mRNA stability. We propose a mechanism wherein the PGA2 induced HSP70 binds to the SL1 of SMAR1 UTR, stabilizes the transcript and results in increased SMAR1 protein synthesis (Fig. 1). Thus, PGA2 mediated induction of SMAR1 can be therapeutically targeted in cancer cells.

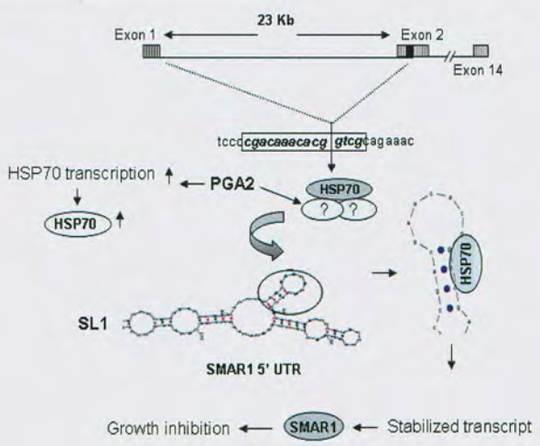


Fig. 1. Schematic representation of events leading to SMAR1 stabilization post PGA2 treatment

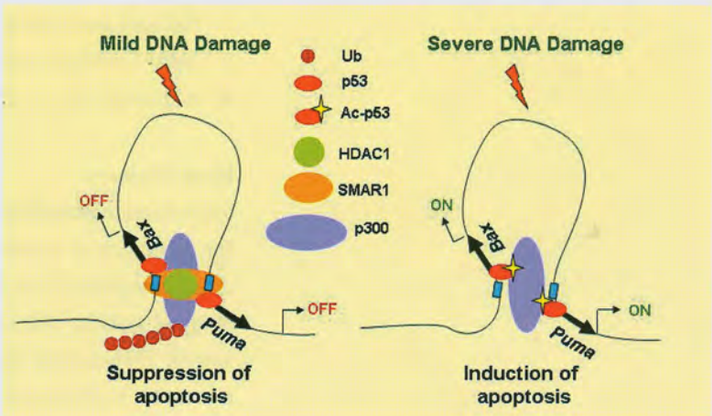


Fig. 2. Model showing the MAR element (blue box) of BAX and PUMA promoter juxtaposed to each other causing the looping out of the intervening sequence. SMAR1 (orange) binds to this identical MAR element along with HDAC1 (green) to p300 (purple) and p53 (red) forming a repressor complex switching off transcription after mild DNA damage (suppression of apoptosis). After severe apoptotic DNA damage, SMAR1 is no longer bound to the MAR element facilitating p53 acetylation (p53-Ac) by p300 and transcription of BAX and PUMA (induction of apoptosis).

Regulation of p53 transcriptional activity by SMAR1 that acts as switch in cell cycle arrest and apoptosis

Earlier, we have shown that SMAR1 is a p53 target gene and also regulates p53 stability in the nucleus by phosphorylation. In our recent studies, we show that SMAR1 modulates p53 dependent cell cycle arrest and apoptosis by regulating the expression of two key apoptotic molecules Bax and Puma in response to UV DNA damage. Under conditions of low dose UV DNA damage, SMAR1 inhibits the expression of Bax and Puma by binding to an identical 25 bp MAR element in their promoters, thereby inhibiting apoptosis and favoring cell cycle arrest. Both BAX and PUMA are located at the same chromosome locus 19q13.3 and harbor identical MAR element suggesting that SMAR1 regulates Bax and Puma through intrachromosomal interactions (Fig. 2). Additionally, we also show that SMAR1 inhibits p53 acetylation by promoting p300 ubiquitination and therefore further impedes acetylation dependent transactivation of BAX and PUMA. At higher doses of UV irradiation, SMAR1 is sequestered by PML into PML nuclear bodies (NBs) and therefore cannot suppress Bax and Puma expression leading to apoptosis. Thus, translocation of SMAR1 into PML-NBs acts as a switch in determining cell cycle arrest and apoptosis. Together, our data suggest that SMAR1 acts as an apoptotic check point regulator and identifies 25 bp MAR as a novel DNA damage responsive cis element.

SMAR1 Represses HIV-1 LTR mediated transcription through chromatin remodeling

Nuclear Matrix and Matrix Attachment Regions (MARs) have been implicated in the transcriptional regulation of host as well as viral genes, but their precise role in HIV-1 transcription remains unclear. Here, we show that >98% of HIV

sequences in GenBank contain a consensus MAR element in their 5' LTRs. We further define the role of this MAR in determining the state of viral transcription and show by MAR-binding assays that the transcriptionally silent HIV LTR has a strong propensity to bind to nuclear matrix. The MAR-binding protein SMAR1 aids in tethering LTR-MAR to nuclear matrix thereby enforcing transcriptional silencing. We have also characterized the minimal DNA sequence in the LTR-MAR to which SMAR1 binds and recruits the HDAC1/Sin3A corepressor complex thereby repressing LTR-mediated transcription. By chromatin immunoprecipitation we show the kinetics of HIV-1 transcription wherein upon activation by PMA/TNF-α, SMAR1 along with HDAC1/Sin3A is dislodged from the LTR in a time-dependent manner with concomitant increase in acetylation of histones at H3K9 and H4K16 and decrease in tri-methylation at H4K20 and enhanced recruitment of hyperphosphorylated RNAP II (Fig. 3). Interestingly, overexpression of SMAR1 reduces virion production, while its knock down induces basal HIV gene expression. Cyclopentanone prostaglandins were earlier shown to inhibit HIV-1 transcription through yet unknown mechanism. We demonstrate the anti-HIV activity of PGA2 is subdued upon knockdown of SMAR1. Therefore, PGA2

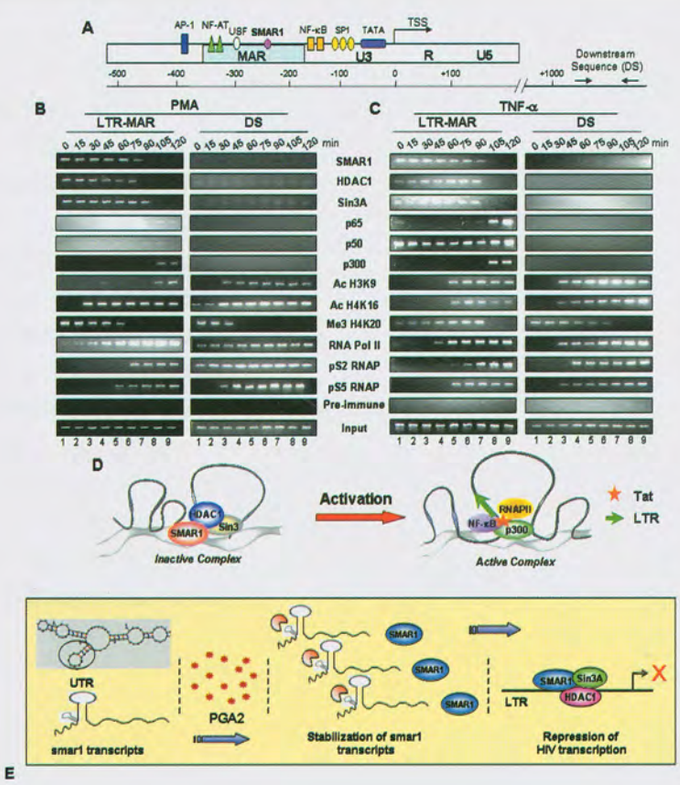
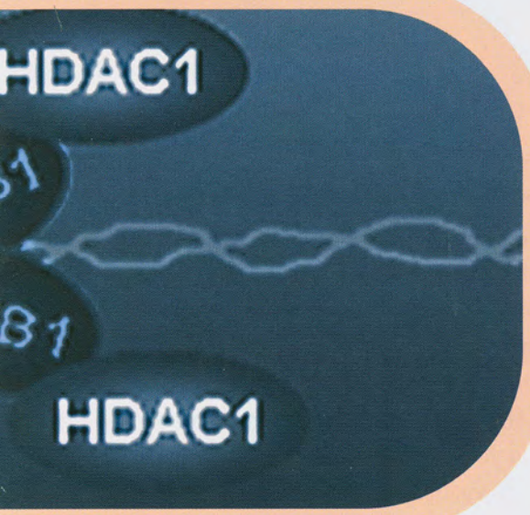


Fig. 3. Models showing mechanism of repression of HIV-1 LTR mediated transcription by SMAR1. **(A)** Schematic representation of HIV-1 LTR with relative binding positions of various transcription factors. **(B** and **C)** Chromatin Immunoprecipitation experiments showing binding of various transcription factors and associated Histone modifications at HIV-1 LTR. ACH-2 cells having a latent HIV genome were activated with PMA/TNF-α and factor binding kinetics analyzed at indicated time points. **(D)** Model depicting occupancy of LTR promoter by SMAR1-HDAC1-Sin3A complex leading to transcriptional latency. Upon activation by PMA/TNF-α, this complex is replaced by NF-κB which leads to activation of transcription. **(E)** Schematic representation of Prostaglandin A2 (PGA2) mediated inhibition of HIV-1 transcription through stabilization of smar1 transcript.

which is known to stabilize SMAR1 mRNA, inhibits HIV-1 transcription by enhancing SMAR1 protein levels. We further show that PGA2 when treated with suboptimal dose of AZT enhances the anti-HIV activity of AZT. Thus, nuclear matrix along with SMAR1 and its associated corepressor complex play determining role in regulating HIV-1 transcription by alternative compartmentalization of the LTR.

Role of SMAR1 in T helper (T_H) cell differentiation

The development and function of the T lymphocyte lineage are regulated tightly by signaling pathways that involve lineage-restricted cell surface receptors, intracellular signaling molecules and nuclear transcription factors. Naïve T helper cells differentiate into two subsets, T_H1 and T_H2, each with distinct functions and their respective cytokine profiles. We demonstrate that SMAR1 facilitates T_H differentiation towards T_H2 lineage commitment by inhibiting T_H1-specific transcription factor T-bet, encoded by *Tbx21*. SMAR1 forms a corepressor complex along with HDAC1/SMRT/RBP-Jκ and occupies CSL binding consensus site on *Tbx21* promoter in turn preventing Notch1 (NICD) recruitment and enhanced T-bet transcription. This mechanism is further triggered through recruitment of GATA-3 and positive regulator CBP/p300 onto *SMAR1* promoter. Additionally, SMAR1 transgenic mice exhibited a defective T_H1 induction as evidenced by decreased IFN-γ, IgG2a levels and T-bet expression. Thus, we suggest SMAR1 as a key player in driving the fate of naïve $CD4^+$T cells ($CD62L^{hi}$, $CD44^{low}$). During polarization of naïve $CD4^+$T cell to T_H2 lineage, GATA3 the master regulator of T_H2 type response, is induced which in turn binds to SMAR1 promoter thereby transcriptionally inducing SMAR1. SMAR1 is then recruited to T bet promoter along with a corepressor complex. Thus we propose that SMAR1 acts as a switch in regulating these kinds of responses. Further, we are now trying to address the implication of this in disease models. *M. tuberculosis* infection manifests strong T_H1 type immune response. Taking *M. tuberculosis* as a model we find that SMAR1 transgenic mice exhibits susceptibility to *M. tuberculosis* infection *in vivo*. Additionally, SMAR1 transcript and protein level goes down upon infection with H37Rv (*M. tuberculosis* laboratory strain) in human monocytic Thp1 cells. We are now trying to understand the mechanism of SMAR1 downregulation and its consequences in detail.



Mechanisms of global gene regulation by SATB1

Sanjeev Galande
sanjeev@nccs.res.in

Participants

Prabhat Kumar Purbey, *SRF*
Sameet Mehta, *SRF*
Amita Limaye, *SRF*
Dimple Notani, *SRF*
Ranveer Jayani, *SRF*
Praveena R. L., *SRF*
Kamal Gottimukkala, *SRF*
Sunita Singh, *SRF*
Rafeeq Mir, *JRF*
Suman Prasad, *Post Doc*
Shiny Titus, *Post Doc*
Nitin Sonawane, *Technician*
Tanuja Bankar, *Technician*
Sarmistha Kalanke, *Project Assistant*
Shashikant Gawai, *Project Assistant*
Trupti Bhankhede, *Project Assistant*
Amrutrao Deshmukh, *Project Assistant*

Background

Wnt growth factors regulate a variety of developmental processes by altering specific gene expression patterns. Wnt proteins are secreted molecules that coordinate cell-to-cell interactions in many different cell types by binding to a member of the Frizzled (Fz) family of transmembrane receptors. Binding of Wnt to Fz elicits a complex cascade of molecular events culminating in the inhibition of the negative regulatory kinase GSK-3β. Phosphorylation of β-catenin by GSK-3β targets it for degradation via the β-TrCP ubiquitin ligase-proteasome pathway. Dephosphorylated β-catenin accumulates inside the nucleus where it associates with the TCF/LEF transcription factors to induce target gene transcription. In vertebrates β-catenin acts as a transcriptional activator which is required to overcome the transcriptional repression by repressor complexes. The C-terminus of β-catenin is indispensable for the transactivation function, presumably since it harbors binding sites for transcriptional coactivators such as p300/CBP and TBP. Thus, recruitment of chromatin remodeling factors on TCF's genomic targets to modulate the gene transcription appears to be the principal function of stabilized β-catenin.

The T-lineage enriched chromatin organizer special AT-rich sequence binding protein 1 (SATB1) was shown to regulate distant genes by selectively tethering matrix attachment regions (MARs) to the nuclear matrix. Furthermore, SATB1 acts as a 'docking site' for several chromatin modifiers including ACF, ISWI and HDAC1, and these chromatin modifiers were suggested to suppress gene expression through histone deacetylation and nucleosome remodeling at SATB1-bound MARs. SATB1 organizes the T helper 2 (T_H2) cytokine and MHC class-I loci into distinct chromatin loops by tethering MARs to nuclear matrix at fixed distances. The densely looped and transcriptionally active chromatin

structure organized by SATB1 is essential for coordinated expression of the T_H2 cytokine genes. Moreover, SATB1 seems to play a role in dynamic organization of the transcriptionally poised chromatin. SATB1 also regulates gene expression by recruiting various chromatin modifiers to promoters. Interaction between SATB1 and partner proteins is frequently mediated by its N-terminal PDZ domain, which is also important for SATB1 homodimerization. Additionally, SATB1 possesses a MAR-binding domain in its C-terminal half containing a Cut domain (CD) and a homeodomain (HD) that together contribute towards recognition and high affinity binding of MARs. SATB1 regulates a large number of genes involved in T cell proliferation, development, and differentiation. SATB1 itself is differentially expressed in various subsets of T_H cells, however, the role of SATB1 in their differentiation has not been demonstrated. Interestingly, many of SATB1's target genes such as c-Myc and Bcl-2 are also targeted by Wnt/β-catenin, suggesting a functional overlap between Wnt/β-catenin pathway and SATB1.

In this study we set out to unravel the molecular mechanisms contributing towards the functional overlap between SATB1 and β-catenin pathways, especially with respect to their target gene regulation. We show that SATB1 physically interacts with β-catenin and recruits it to its genomic targets. Recruitment of β-catenin alters the transcription of SATB1's target genes in thymocytes. Chromatin immunoprecipitation analysis of SATB1 binding sites (SBSs) in promoters of multiple genes revealed that interaction with β-catenin modulated SATB1 function on its target genes by increasing its occupancy and altering histone H3 lysine 9(H3K9) acetylation. β-catenin-responsive genes are also targeted by SATB1 suggesting that both are functionally linked in the Wnt/β-catenin signaling pathway. Thus, these studies establish SATB1 as a downstream effector in the Wnt/β-catenin signaling pathway.

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 interacts with β-catenin

Gene expression profiles of cells expressing phosphorylation- or acetylation-

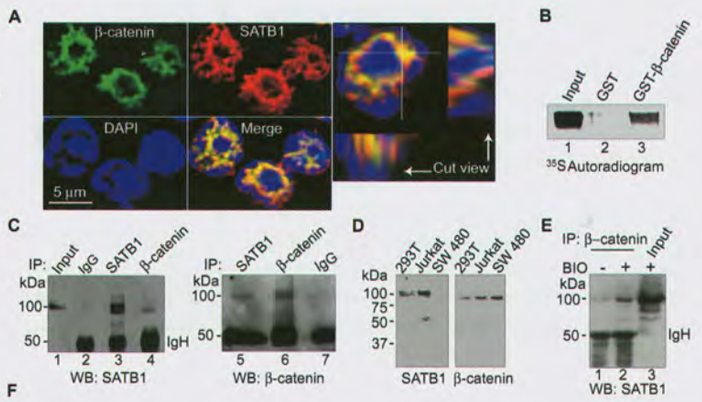


Fig. 1. Delineation of physical interaction between SATB1 and β-catenin. **(A)** Immunofluorescence staining of thymocytes using antibodies to SATB1 (red) and β-catenin (green). DNA counterstaining is performed using DAPI (blue). The cut view panel depicts two perpendicular transverse sections of a triple-stained thymocyte as indicated by white lines, intersecting at the point of the brightest fluorescence signal. **(B)** Direct interaction between SATB1 and β-catenin in vitro. ^{35}S-labeled SATB1 was specifically pulled down after incubation with immobilized GST-β-catenin. **(C)** Coimmunoprecipitation reactions was performed by separately incubating anti-β-catenin and anti-SATB1 antibodies with aliquots of Jurkat nuclear extract followed by western blot (WB) using anti-SATB1 and anti-β-catenin respectively. IgH = immunoglobulin heavy chain. **(D)** Expression levels of SATB1 and β-catenin in HEK 293T, Jurkat and SW480 cells were compared by immunoblot analysis using respective antibodies. The cells were treated with LiCl for 24 h prior to preparation of nuclear extracts. **(E)** Coimmunoprecipitation analysis of nuclear extracts derived from BIO treated (+) and control (-) human thymocytes using anti-β-catenin followed by WB with anti-SATB1.

defective mutants of SATB1 indicated shared target genes with the β-catenin signaling pathway. SATB1 is expressed abundantly in thymocytes and therefore we monitored the subcellular localization of β-catenin and SATB1 in thymocytes upon Wnt induction. Surprisingly, the intra-nuclear immunostaining pattern of β-catenin also resembled the 'cage-like' architecture of SATB1-containing nuclear domains in thymocytes. Optical sectioning revealed that at least part of these signals colocalized across the depth of the nucleus indicating that they occupy similar areas within the thymocyte nucleus (Fig. 1A). To test whether SATB1 and β-catenin actually interact physically or not, we performed in vitro pulldowns using immobilized β-catenin. When SATB1 was passed on GST-β-catenin and GST immobilized on Sepharose beads, SATB1 eluted specifically from the GST-β-catenin affinity matrix suggesting their physical interaction (Fig.1B). Coimmunoprecipitation analysis using nuclear extract from Jurkat T lymphoblastic cells indicated that β-catenin and SATB1 can be immunoprecipitated by antibodies against each other (Fig. 1C). We then tested the effect of Wnt signaling on the physical association of SATB1 and β-catenin. LiCl and 6-bromoindirubin-3'-oxime (BIO) are potent GSK-3β inhibitors that mimic Wnt signaling by stabilizing β-catenin. Upon BIO treatment, increased interaction was observed in human thymocytes (Fig. 1E).

Recruitment of β-catenin upon Wnt signaling alters the transcription of SATB1 and Wnt target genes

To investigate whether SATB1 and β-catenin collaborate functionally we monitored the effect of SATB1:β-catenin interaction on the transcription of multiple genes that are known to be targets of SATB1 as well as Wnt signaling in immature T cells. Wnt signaling was induced in human thymocytes by

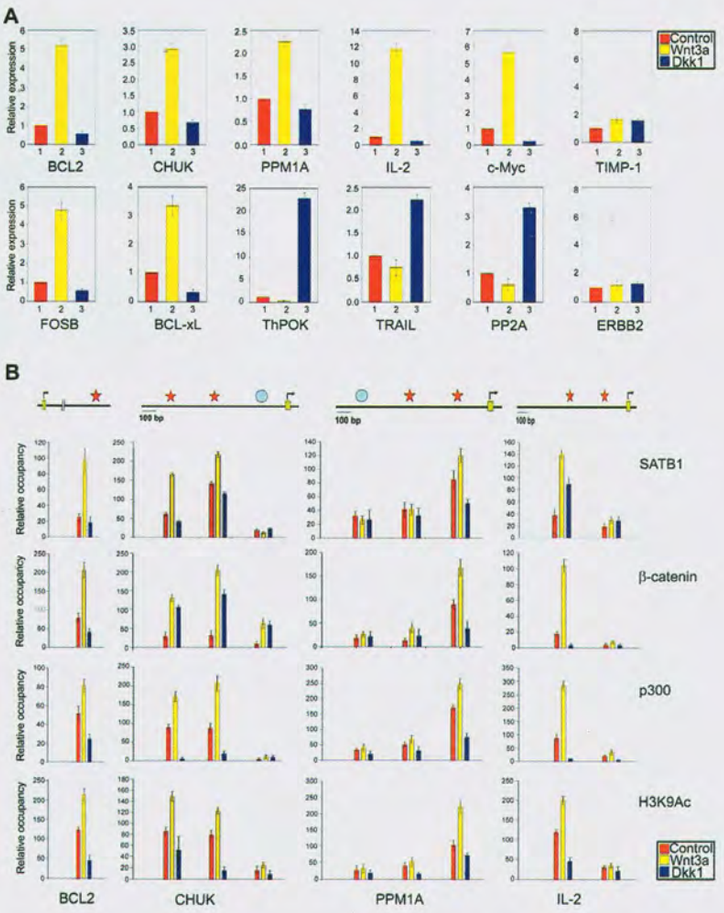


Fig. 2. Wnt signaling results in upregulation of SATB1 targeted genes by recruitment of β-catenin-p300 complex. **(A)** Effect of Wnt signaling on the transcription status of representative SATB1 regulated genes and Wnt regulated genes in thymocytes. Quantitative RT-PCR analysis was performed using RNA extracted from control human thymocytes (bar 1) and thymocytes treated for 48 h with Wnt3a (bar 2), or Dkk1 (bar 3). The values for gene expression in treated cells were normalized with respect to the untreated control which was set to one. Each error bar indicates standard deviation calculated from triplicates. *TIMP-1* and *ERBB2* served as control genes such that TIMP-1 is not regulated by both SATB1 and β-catenin whereas ERBB2 served as SATB1-dependent (49) but β-catenin-independent control gene. **(B)** Occupancy of SATB1, β-catenin, p300 acetyltransferase and H3K9 acetylation across the 1 kb upstream regulatory regions of SATB1 regulated genes *Bcl-2, PPM1A, CHUK* and *IL-2* was monitored by ChIP analysis. Chromatin was isolated from control, Wnt3a or Dkk1 treated human thymocytes and ChIP analysis was performed using indicated antibodies and primers specific for these genes. Relative occupancy was calculated by performing quantitative real-time PCR analysis and normalizing the C_T values with input and IgG controls. Each error bar indicates standard deviation calculated from triplicates. The relative positions of regions analyzed by ChIP are schematically indicated above individual occupancy graphs for respective genes. Stars represent in vitro SATB1 binding sites whereas circles denote non-binding sites. Names of genes are depicted below each column of graphs whereas that of antibodies used for ChIP are depicted on the right side of each row.

treating them with soluble Wnt3a ligand for 48 h and the transcriptional activity of SATB1 targets was monitored by quantitative RT-PCR. The transcription status of multiple Wnt- and SATB1-responsive genes was reversed upon induction of Wnt signaling by addition of Wnt3a in thymocyte culture (Fig. 2A, bar 2). Thus, genes that are downregulated by SATB1 such as *BCL-2, CHUK, PPM1A, c-Myc* and *IL-2* were upregulated upon overexpression of β-catenin or addition of Wnt3a in a manner akin to the known Wnt targets *FOSB* and *BCL-xL* suggesting that these are also β-catenin targets. Furthermore, inhibition of Wnt signaling by Dickkopf (Dkk1) treatment led to transcriptional repression of all SATB1 regulated genes that are also targeted by Wnt signaling (Fig. 2A, bar 3). Among the SATB1 regulated genes, *ThPOK* (ZBTB1-7B) showed reciprocal pattern of regulation. ThPOK was downregulated upon Wnt3a treatment and dramatically upregulated (more

than 20-fold) upon inhibition of Wnt signaling by Dkk1. Among the known Wnt targets in thymocytes, few genes such as *TRAIL* and *PP2A* were downregulated upon Wnt3a treatment and upregulated upon Dkk1 treatment. More importantly, *TIMP1* that is neither targeted by SATB1 nor by β-catenin is not affected by Wnt3a or Dkk1 treatment of thymocytes indicating that this effect is specific to SATB1 and Wnt/β-catenin targets (Fig. 2A). *ERBB2* is a target of SATB1 but not that of β-catenin and therefore is not affected upon Wnt3a treatment further demonstrating that to overcome the SATB1 mediated repression, SATB1:β-catenin functional interaction is essential on SATB1 genomic targets (Fig. 2A). Thus, interaction with β-catenin dramatically alters the transcription status of SATB1 regulated genes, many of which are also known Wnt targets. To study how SATB1 directly regulates expression of these we performed in vitro binding assays to assess the binding status of SATB1 on various regulatory regions from these genes and observed that SATB1 binds to at least one site located within the upstream 1 kb regions of multiple genes. To confirm whether the upregulation of transcription is mediated by SATB1 and factors recruited by SATB1 we performed chromatin immunoprecipitation (ChIP) analysis of promoters of these genes. Chromatin was isolated from control untreated, Wnt3a or Dkk1 treated human thymocytes and subjected to immunoprecipitation using antibodies to SATB1, β-catenin, p300 and histone H3 lysine 9 acetylation (H3K9Ac). The DNA purified from immunoprecipitate was subjected to PCR amplification using oligonucleotide primers flanking various regions within the upstream 1 kb regulatory regions of *CHUK, PPM1A* and *IL-2* promoters and the SBS within the major breakpoint region (MBR) of *BCL2*. ChIP analysis revealed that not all of the in vitro binding sites were occupied by SATB1 in vivo. Strikingly, upon Wnt3a treatment the occupancy of SATB1 was enriched by 1.5- to 4-fold on promoters of genes that are downregulated by SATB1. This change in occupancy is an outcome of Wnt signaling since treatment with Dkk1 resulted in 1.5- to 6-fold decrease in SATB1 occupancy on its in vivo binding sites (Fig. 2B). The changes in occupancy of β-catenin, p300 and H3K9Ac also mirrored that of SATB1 suggesting that SATB1 occupancy is the primary event leading to recruitment of β-catenin and p300 (Fig. 2B). These changes in occupancy were highly specific and restricted to SATB1 binding sites since the regions in proximal *CHUK* and distal *PPM1A* promoters that were not bound by SATB1 in vitro and in vivo did not show any significant change in the occupancy of SATB1, β-catenin and p300 (Fig. 2B). Thus, Wnt signaling results in increased occupancy of SATB1 on its targets which then recruits β-catenin and p300 to upregulate target genes.

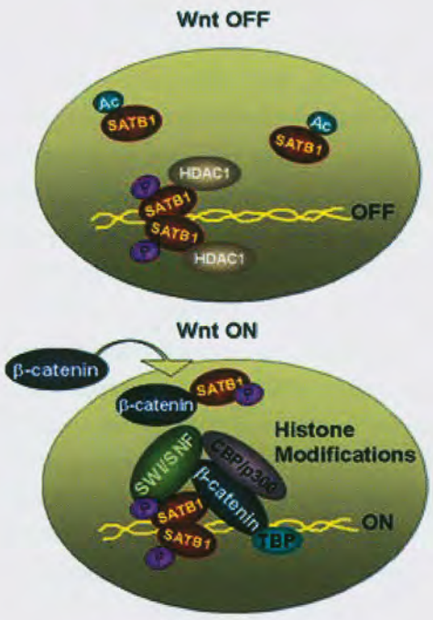


Fig. 3. Model depicting the role of SATB1 in gene regulation upon Wnt/β-catenin signaling. For details see text.

Role of SATB1:β-catenin complex in development and tumorigenesis

Studies of the molecular mechanisms of regulation of gene expression in T cells upon Wnt/β-catenin signaling have been restricted to the TCF/LEF family proteins. Our findings of transcriptional regulation of β-catenin by SATB1 and role of their physical association in regulation of transcription of multiple genes provided an unprecedented clue towards the role of Wnt/β-catenin signaling in T cell development and differentiation. SATB1 is known to orchestrate spatio-temporal expression of multiple genes during T cell development. Upon β-catenin signaling SATB1 is deacetylated thereby it binds to nuclear translocated β-catenin and recruits it to target genes. We found that β-catenin recruits the p300 acetyltransferase to the SBSs of multiple genes, and may further recruit TBP and chromatin remodeling machinery such as SWI/SNF leading to transcriptional upregulation (Fig. 3). Simultaneously, genes which are positively regulated by SATB1 when Wnt is absent are repressed upon Wnt/β-catenin signaling. During tumour progression c-Myc is dysregulated by Wnt and activates the cell proliferation. Since SATB1 no longer represses c-Myc upon Wnt induction, such preferential targeting of SATB1 by β-catenin may constitute an important event facilitating tumour progression. Indeed SATB1 has been shown to reprogram gene expression to promote breast tumour growth and metastasis. The implications of SATB1-mediated orderly deposition of β-catenin and its partners on chromatin towards global gene regulation upon Wnt signaling in various developmental systems and during tumorigenesis await further investigation. Our results provide a molecular mechanism towards understanding how SATB1 governs gene expression at a global level.

Future Work

1. To investigate the role of the SATB1:β-catenin interaction on regulation of transcription of multiple genes by SATB1.
2. To study the role of the SATB1:β-catenin interaction during T helper cell differentiation and also during tumorigenesis.

Support Units

Experimental Animal Facility

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 to provide husbandry, veterinary care and research technical support to facilitate 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 $^{nu/nu}$
NZB
Genetically engineered mutant mice (knock-out, transgenic and mutant mice -**38 lines**)

RATS: WISTAR
LEWIS

RABBITS: NEWZEALAND WHITE

Defined barrier practices are followed scrupulously without any exception or allowance, with access to select personnel, to minimize the risk of microbial infection to the animals housed in the facility.
The breeding program for the propagation of the two different inbred lines viz. BALB/c and C57BL/6 is structured in a three-tier format, i.e. the Foundation colonies (FC), Pedigreed expansion colonies (PEC) and the Production colonies (PC). Other 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 four different mutant mice lines to the existing ones, taking the total now to 38 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 *49* at present. 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 have been extended regularly to Scientists and their group members for the conduct of experiments under IAEC approved projects. The following services have been provided as per user requirements: collection of blood and other samples, immunizations, surgical procedures, injection of tumor cells in nude mice etc., assistance in the writing of Animal Study Protocols, education and assistance regarding interpretations of animal use regulations and the procurement of animals.

The breeding of laboratory animals has been planned to meet the needs of Scientists/Research Scholars for various animal experiments. The details of the laboratory animals supplied for various R & D activities are given below.

01-04-2007 to 31-03-2008

SR.NO.	STRAINS/SPECIES	ANIMALS SUPPLIED
1.	**MICE**	
	BALB/c	6917
	C57BL/6	1399
	SWISS#	594
	DBA/2	03
	DBA/1	395
	Nude (nu/nu)	36
	BALB/c*	34
	FVB/NJ	1182
	NOD-SCID	629
	Mutant Mice	848
2.	**RATS**	
	WISTAR	350
	LEWIS	62
3.	**RABBIT(NZW)**	12

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

Library

The NCCS library has collection in the frontier areas of biotechnology having relevance to NCCS research activities. The library holds approximately eight thousand eight hundred and seventy three bound journals, two thousand two hundred and eighty five books and seventy four NCCS Ph. D. thesis. Currently, it subscribes to seventy five scientific journals and twenty eight other periodicals in print form, and nineteen online scientific journals.

In the development of its collections, the library's priority is to support NCCS research activities. The library collection is expanded in consultation with NCCS scientists. The library's print collections are growing by approximately 900 volumes per year. During the period of 2008-2009, the library has added 127 books and 802 bound volumes of journals to its collection. The library is equipped with Linux based SLIM21 library software for the library house keeping operation, Web-OPAC for online searching of the library documents and has also installed Barcode Technology for circulation (Issue & Return) of the library documents. The library maintains its information (In Hindi & English) on its webpage, which includes NCCS research publication list, library forms, NCCS in news, Ph.D. thesis collection, NCCS alumni and links to free Online Medical database. During the period under review the library has created a Digital Archive of NCCS Ph. D. thesis.

In addition to the above, the library also provides additional documentation facilities such as local area network for library activities, PubMed database access and a number of CD-ROM database including, full text and factual database. The library is listed in the Union Catalogue of Biomedical Serials in India created by National Informatics Centre, New Delhi and continues to be a member of the Medical Library Association of India. It is also a member of DBT online journal consortia "DeLCON" which subscribes to 281 journals from various publishers.

Computer Section

The Computer Centre has currently enhanced its infrastructure as follows -

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

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

- **Procurement of an upgraded Network Antivirus Software Package**
  An upgradation of the current antivirus software is performed thereby providing security to the invaluable data on all the computers of the Institute and for a safe Internet connectivity.

- **Installation of Wi-Fi Networks in all the Labs in Progress**
  The Centre is in the process of Installing Wireless Connections in all the labs. It will soon be functional thereby providing an uninterrupted Wi-Fi connection.

- **Internet Leased Lines Upgradation in Progress**
  NCCS is currently connected to the internet via two leased lines namely one from Reliance (1 Mbps) and the other from VSNL (256 Kbps). The process of upgradation of these leased lines to 2 Mbps is in progress to enhance the internet bandwidth remarkably.

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

a) Providing technical support to more than 120 computers and more than 80 printers. Computer centre provides support such as installations of Operating Systems, Other Softwares and Drivers.
b) Management of the invaluable information in the Institute.
c) Providing Network Support to all the sections and facilities and thereby contributing to the smooth functioning of the routine administrative and research work in the Institute.
d) Providing Support to the students and scientists for their scientific presentations required when attending national and international conferences/seminars. Computer centre helps in the DTP work, CD writing, scanning of images and transperancy printing on color laserjet printer.
e) Regular up-dation of the NCCS website for any changes in the information. Publishing of Tender Notices and advertisements for available posts such as Project Posts, Project Training, Summer Training and other Administrative/Supportive staff. Updating individual scientist webpages.

NCCS Facilities

Central Sterilization Facility

This facility is an infrastructure service department of the institute. It provide services to all the research laboratories, cell repository, media section and other service departments for washing, packing and sterilization of glassware and other research materials. It also supply high grade distilled water to all the sections of the institute. In addition some technical staffs are involved in safe disposal of radioactive and laboratory waste materials.

The team is:

Suresh Basutkar, *Technical Officer C*
Narayan Kadlak, *Technician B*
Pramod Surve, *Technician B*
Dilip Moundekar, *Technician B*
Gayatri Sagare, *Assistant Technician*
Kailash Bhandalkar, *Helper A*

DNA Sequencer

During the year 2008-2009 more than 1,00,000 samples were processed for sequencing on Automated DNA sequencer.

FACS Facility

We have four FACS equipments in the FACS core facility of the Institute under my supervision. These are operated on rotation basis by three dedicated operators.

Team:

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

Cantoll, Calibur and Vantage:

The usage of three analyzer equipments during the period under consideration is summarized in Tabular form:

Equipment	Surface /Intracellular staining	DNA Cell cycle	CBA FLEX	Total Samples Acquired
FACS Canto II	4697	-	140	4837
FACS Calibur	4249	857	-	5106
FACS Vantage	1779	119	-	1898

Samples acquired for outsiders:

We have acquired a total of **470** samples from outside NCCS like for IRSHA, NIV and NCL which included surface staining, DNA cell cycle, intracellular staining and CBA. These were acquired on Calibur and Vantage.

FACS Aria:

FACS Aria is a sorter purchased on DBT funded mega project on "harnessing the potential of stem cells" in the year 2006. From last year it has been used as a dedicated sterile sorter. Various types of samples like surface labeled cells, SP cells, PKH labeled tumor samples, GFP dull, bright, multicolour stained (5 colours) T cells like T regulatory, naïve T cells etc have been sorted successfully. Both two-way and four-way sorting is done on the equipment. Collection in tubes/multiwell plates is well standardized. The usage during the period under consideration is summarized in tabular form below:

EQUIPMENT	SORTING		TOTAL
FACS ARIA	UV laser	BLUE, RED laser	361
	55	306	

FACS Aria II has been purchased in March 2009 and we are in the process of installing it.

Conference /training attended:

Swapnil Walke attended 9th Indo-US Flow cytometry workshop held in NCBS, Banglore from 20-25th July 2008.

Confocal Laser Microscopy

The facility has two microscopes:

1. Zeiss LSM510 system having 4 lasers i.e. UV-Enterprise, 488 Argon, 543 He-Ne, 633-He-Ne. This greatly increases the choice of fluorochromes that can be used. Four colours staining coupled with real time scanning can be done making five parameter studies possible. The number of samples imaged during this year is approximately 1590 in-house and 177 from various other institutes.

2. This year a new advanced Spectral Confocal Microscope, Zeiss LSM510 META, with programmable CO2 incubator and temperature-humidity control was procured. This system comprises of fully motorized and computer controlled Inverted Fluorescence microscope, is being used for regular confocal as well as FRET, FRAP, Live Imaging etc. The Lasers available are Blue Diode laser (405nm), Argon laser (458/477/488/514 nm), 543nm He-Ne and 633nm He-Ne. The spectral detector permits separation of upto eight emission signals, even if the fluorescence spectra are strongly overlapping. The number of samples imaged during this year is approximately 845 in-house and 96 from other institutes. Additionaly, thirteen samples were processed for live-imaging.

Publications & Awards

Publications & Patents

Publications

1. Vaidya AA, Sharma MB and Kale VP (2008). Suppression of p38 stress kinase sensitizes quiescent leukemic cells to anti-mitotic drugs by inducing proliferative responses in them. *Cancer Biol. Ther., 7(8): 1232-40.*

2. Balan S, Kale VP and Limaye LS (2009). A simple two step culture system for the large scale generation of mature and functional dendritic cells from umbilical cord blood $CD34^+$ cells. *Transfusion* (In press)

3. Verma MK and Lenka N (2009). Temporal and contextual orchestration of cardiac fate by WNT-BMP synergy and threshold. *J. Cell. Mol. Med.* (In press).

4. Yogesha SD, Khapli SM, Srivastava RK, Mangashetti LS, Pote ST, Mishra GC and Wani MR (2009). IL-3 inhibits TNF--induced bone resorption, and prevents inflammatory arthritis. *J. Immunol.*, 182: 361-370.

5. Murawala P, Tripathi MM, Vyas P, Salunke A and Joseph J (2009). Nup358 interacts with APC and plays a role in cell polarization. *J. Cell Sci.,* 122(17): 3113-3122

6. Kusumbe AP, Mali AM and Bapat SA (2009). CD133-expressing stem cells associated with ovarian metastases establish an endothelial hierarchy and contribute to tumor vasculature. *Stem Cells*, 27(3): 498-508.

7. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY and Bapat SA (2009). Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells* (In press)

8. Berry NB and Bapat SA (2008). Ovarian Cancer Plasticity and Epigenomics in the acquisition of a stem-like phenotype. *J. Ovarian Res.*, 1:8.

9. Aher NG, Pore VS, Mishra NN, Kumar A, Shukla PK, Sharma A and Bhat MK (2009). Synthesis and antifungal activity of 1,2,3-triazole containing new fluconazole analogues. *Bioorg. Med. Chem. Lett.*, 19: 759-763.

10. Pandey V, Vijayakumar MV, Kaul-Ghanekar R, Mamgain H, Paknikar K and Bhat MK (2009). Atomic Force Microscopy, biochemical analysis of 3T3-L1 cells differentiated in the absence and presence of insulin. *BBA*, 1790(1): 57-64.

11. Upadhyay AK, Ajay AK, Singh S and Bhat MK (2008). Cell Cycle Regulatory Protein 5 (Cdk5) is a Novel Downstream Target of ERK in Carboplatin Induced Death of Breast Cancer Cells. *Current Cancer Drug Targets*, 8(8): 741-52.

12. Dhar S, Reddy EM, Shiras A, Pokharkar V, and Prasad BL (2008). Natural gum reduced /stabilized gold nano-particles for drug delivery formulations. *Chemistry*, 14(33):10244-50.

13. Jain S, Chakraborty G, Raja R, Kale S and Kundu GC (2008). Prostaglandin E2 regulates tumor angiogenesis in prostate cancer. *Cancer Res.*, 68(19): 7750-7759.

14. Ahmed N, Dasari S, Srivastava SS, Sneh A, Ahmad A, Islam Khan M and Krishnasastry MV (2008). Taxol and 10-deacetylbaccatin III induce distinct changes in the dynamics of caveolae. *FEBS Lett.*, 582: 3595-600.

15. Chandra V, G Swetha, Phadnis S, Nair PD and Bhonde RR (2009). Generation of Pancreatic Hormone Expressing Islet like Cell Aggregates from Murine Adipose Tissue-derived Stem Cells. *Stem Cells* (In press)

16. Ishaq Ahmed VP, Chandra V, Sudhakaran R, Rajesh Kumar S, Sarathi M, SarathBabu V, Bhonde RR and Sahul Hameed AS (2009). Development and characterization of cell lines derived from rohu, Labeo rohita (Hamilton), and catla, Catla catla (Hamilton). *J. Fish Dis.*, 32(3): 211-18.

17. Ishaq Ahmed VP, Chandra V, Parameswaran C, Venkatesan R, Shukla R, Bhonde RR and Sahul Hameed AS (2008). A new epithelial-like cell line from eye muscle of Catla catla (Hamilton): development and characterization. *J. Fish Biol.*, 72(8), 2026-2038.

18. Kadam S, Hardikar AA and Bhonde RR (2008). Islet neogenesis from full term placental stem cells - a step towards cell replacement therapy *Placenta*; 29(8): A9.

19. Kadam S, Tiwari S and Bhonde RR (2009). Simultaneous isolation of Vascular endothelial Cells and Mesenchymal Stem Cells from the Human Umbilical Cord. *In Vitro Cellular & Dev. Biol. Animal*, 45(1-2): 23-7.

20. Kadam SS, Pataki SM. and Bhonde RR (2009). Human Fallopian tube harbors multipotent stem cells A possible evolutionary mechanism for assured transport of eggs. *Stem Cells and Regenerative Medicine*, (In press).

21. Murawala P, Phadnis SM, Bhonde RR, Prasad BL. Colloids Surf B (2009). In situ synthesis of water dispersible bovine serum albumin capped gold and silver nanoparticles and their cytocompatibility studies. *Colloids Surf B Biointerfaces* (In press).

22. Datar SP and Bhonde RR (2009). Islet-derived stellate-like cells as a novel source for islet neogenesis in chicks. *Poult Sci.*, 88(3): 654-60.

23. Dorisetty RK, Kiran SG, Umrani MR, Boindala S, Bhonde RR and Venkatesan V (2008). Immuolocalization of nestin in pancreatic tissue of mice at different ages. *World J Gastroenterology*, 14 (46): 7112-6

24. Kumar S and Sitasawad SL (2009). N-acetylcysteine prevents glucose / glucose oxidase-induced oxidative stress, mitochondrial damage and apoptosis in H9c2 cells. *Life Sci.*, 84(11-12):328-36.

25. Lavhale MS, Kumar S, Mishra SH and Sitasawad SL (2009). A novel triterpenoid isolated from the root bark of Ailanthus excelsa Roxb (Tree of Heaven), AECHL-1 as a potential anti-cancer agent. *PLoS One*. 4(4): e5365.

26. Kumar S, Kain V and Sitasawad SL (2009). Cardiotoxicity of calmidazolium chloride is attributed to calcium aggravation, oxidative and nitrosative stress and apoptosis. *Free Radic Biol Med.* (In press)

27. Kode JA, Mukherjee S, Joglekar MV and Hardikar AA (2009). Mesenchymal Stem cells: Immunobiology and role in Immunomodulation and Tissue regeneration. *Cytotherapy*, 11(4): 377-91

28. Sahu S, Tosh D and Hardikar AA (2009). New sources of beta-cells for treating diabetes. *J. Endocrinol.*, 202(1): 13-6.

29. Ranjan AK, Joglekar MV and Hardikar AA (2009). Endothelial cells in pancreatic islet development and function. *Islets*, 1(1): 1-8.

30. Dalvi M, Umrani M, Joglekar MV, and Hardikar AA (2009). Human pancreatic islets demonstrate phenotypic plasticity to generate progenitor / stem cell populations in vitro. *J. Biosci.* (In press)

31. Sahu S, Joglekar M, Dumbre R, Phadnis S, Tosh D and Hardikar AA (2009). Insulin-producing cells in the gall bladder are retained in diabetic humans. *J. Cell and Mol. Med.*, 13(5): 999-1000.

32. Joglekar MV, Joglekar VM, Joglekar SV and Hardikar AA (2009). Human fetal pancreatic insulin-producing cells proliferate in vitro. *J. Endocrinol.*, 201(1): 27-36 (Cover story)

33. Joglekar MV, Joglekar VM and Hardikar AA (2009). Pancreatic islet specific microRNAs are post-transcriptionally regulated during embryonic development. *Gene Exp. Patterns.*, 9(2): 109-13

34. Patil SA, Surakasi VP, Koul S, Ijmulwar S, Vivek A, Shouche YS and Kapadnis BP (2009). Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. *Bioresour. Technol.* (In press).

35. Sobti RC, Kumari M, Sharma VL, Sodhi M, Mukesh M and Shouche YS (2009). Sequence analysis of a few species of termites (Order: Isoptera) on the basis of partial characterization of COII gene. *Mol. Cell. Biochem.* (In press)

36. Kumari M, Sharma VL, Sodhi M, Mukesh M, Shouche YS and Sobti RC. (2009). PCR-SSCP and sequence analysis of three Odontotermes spp. (Order: Isoptera; Family: Termitidae) on the basis of partial 16SrRNA gene. *Mol. Cell. Biochem.*, 100(21): 5132-9.

37. Patel AK, Ahire JJ, Pawar SP, Chaudhari BL, Shouche YS and Chincholkar SB (2009). Evaluation of Probiotic Characteristics of Siderophoregenic Bacillus spp. Isolated from Dairy Waste. *Appl. Biochem. Biotechnol.* (In press)

38. Khandwekar AP, Patil DP, Khandwekar V, Shouche YS, Sawant S and Doble M (2009). TecoflexTM functionalization by curdlan and its effect on protein adsorption and bacterial and tissue cell adhesion. *J. Mater. Sci. Mater. Med.*, 20(5): 1115-29.

39. Sharma A, Pandey A, Shouche YS, Kumar B and Kulkarni G (2009). Characterization and identification of Geobacillus spp. isolated from Soldhar hot spring site of Garhwal Himalaya, India. *J. Basic Microbiol.*, 49(2): 187-94.

40. Desai C, Parikh RY, Vaishnav T, Shouche YS and Madamwar D (2009). Tracking the influence of long-term chromium pollution on soil bacterial community structures by comparative analyses of 16S rRNA gene phylotypes. *Res. Microbiol.*, 160(1): 1-9.

41. Chavan P, Mane S, Kulkarni G, Shaikh SA, Ghormade V, Nerkar D, Shouche YS and MVD (2009) Isolation and characterization of natural yeast flora from different grape varieties used for wine making in India. *Food Microbiol.* (In press).

42. Shivaji S, Chaturvedi P, Begum Z, Pindi PK, Manorama R, Padmanaban DA, Shouche YS, Pawar S, Vaishampayan P, Dutt CB, Datta GN, Manchanda RK, Rao UR, Bhargava PM and Narlikar JV (2009). Janibacter hoylei sp. nov., Bacillus isronensis sp. nov. and Bacillus aryabhattai sp. nov., isolated from cryotubes used for collecting air from upper atmosphere. *J. Systematic & Evolutionary Microbiol.* (In press)

43. Guiyedi V, Prakash D, Fesel C, Jain R, Dzeing-Ella A, Cazenave PA, Kombila M, Mishra GC and Pied S (2009). Correlation of Plasma Soluble Fas Ligand Levels with Severe Anaemia in Gabonese and Indian *Plasmodium falciparum* Malaria Patients. *The Open Tropical Medicine Journal*, 2: 17-23.

44. Ozarkar A, Prakash D, Deobagkar DN and Deobagkar D (2009). Analysis of PfEMP1var Gene Sequences in Different Plasmodium falciparum Malarial Parasites, *Scholarly Research Exchange*, Volume 2009, Article ID 824949

45. Talwar GP, Dar SA, Rai MK, Reddy KV, Mitra D, Kulkarni SV, Doncel GF, Buck CB, Schiller JT, Muralidhar S, Bala M, Agrawal SS, Bansal K and Verma JK. (2008) A novel polyherbal microbicide with inhibitory effect on bacterial, fungal and viral genital pathogens. Int. *J. Antimicrob. Agents*, 32(2): 180-5.

46. Ahluwalia JK, Khan SZ, Soni K, Rawat P, Gupta A, Hariharan M, Scaria V, Lalwani M, Pillai B, Mitra D and Brahmachari SK (2008). Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. *Retrovirology*, 5: 117.

47. Atre AN, Surve SV, Shouche YS, Joseph J, Patole MS and Deopurkar RL (2009). Association of small Rho GTPases and actin ring formation in epithelial cells during the invasion by Candida albicans. *FEMS Immunol. Med. Microbiol.*, 55(1): 74-84.

48. Wadhone P, Maiti M, Agarwal R, Kamat V, Martin S and Saha B (2009). Miltefosine promotes IFN-gamma-dominated anti-leishmanial immune response. *J. Immunol.*, 182: 7146-54.

49. Rub A, Dey R, Jadhav M, Kamat R, Chakkaramakkil S, Majumdar S, Mukhopadhyaya R and Saha B (2009). Cholesterol depletion associated with Leishmania major infection alters macrophage CD40 signalosome composition and effector function. *Nat. Immunol.*, 10: 273-80.

50. Singh K, Sinha S, Malonia SK, Bist P, Tergaonkar V and Chattopadhyay S (2008). Tumor suppressor SMAR1 represses IκBα expression and inhibits p65 transactivation through MARs. *J. Biol. Chem.*, 284(2): 1267-78.

51. Pavithra L, Mukherjee S, Kadreppa S, Kar S, Sakaguchi K, Roy S and Chattopadhyay S (2009). SMAR1 forms ternary complex with p53-MDM2 and negatively regulates p53 mediated transcription. *J. Mol. Biol.*, 388(4): 691-702.

52. Singh VB, Pavithra L, Chattopadhyay S and Pal JK (2009). Stress-induced overexpression of the heme-regulated eIF-2alpha kinase is regulated by Elk-1 activated through ERK pathway. *BBRC*, 379(3): 710-5.

53. Nakka K and Chattopadhyay S (2009). Modulation of chromatin by MARs and MAR binding oncogenic transcription factor SMAR1. *Mol. Cell. Biochem.* (In press).

54. Kaul-Ghanekar R, Singh S, Mamgain H, Jalota-Badhwar A, Paknikar KM and Chattopadhyay S (2009). SMAR1, a phenotypic diagnostic marker for differentiation between cancerous and non-cancerous cells: combined AFM and SEM study. *BMC Cancer* (In press).

55. Purbey PK, Singh S, Notani D, Kumar PP, Limaye AS, and Galande S (2009). Acetylation-dependent interaction of SATB1 and CtBP1 mediates transcriptional repression by SATB1. *Mol. Cell Biol.*, 29: 1321-1337.

56. Notani D, Limaye A, Kumar PP and Galande S. Phosphorylation-dependent regulation of SATB1, the higher-order chromatin organizer and global gene regulator. 2009. In "Transcription Factors: Methods and Protocols". *Methods in Molecular Biology Series.* Humana Press, NY (In press).

57. Singh AK, Yadav VN, Pyaram K, Mullick J and Sahu A (2009). Mapping of functional domains in Herpesvirus saimiri complement control protein homolog: the complement control protein domain 2 is the smallest structural unit displaying cofactor and decay-accelerating activities. *J. Virol.* (In press).

58. Murugaiyan G and Saha B (2009). Protumor vs antitumor functions of IL-17. *J. Immunol.*, 183: 4169 -4175.

59. Tripathy MK and Mitra D (2009). Differential modulation of mitochondrial OXPHOS system during HIV-1 induced T-cell apoptosis: up regulation of Complex-IV subunit COX-II and its possible implications. Apoptosis (In press).

60. Notani D, Gottimukkala KP, Jayani RS, Limaye A, Damle MV, Mehta S, Purbey PK, Joseph J and Galande S. Global regulator SATB1 recruits β-catenin and regulates T helper type 2 differentiation in Wnt/β-catenin-dependent manner. PLoS Biology (In press).

Patents filed / sealed

Manoj Kumar Bhat

Synthesis of new fluconazole analogues containing 1,2,3-triazole moiety and having better antifungal spectrum. Aher, N.G., Pore, V.S., Mishra, N.N., Kumar, A., Shukla, P.K., Sharma, A., and Bhat, M.K. Indian patent application IPO/ 054/NF/2008 Jointly with NCL, Pune, 2008

Samit Chattopadhyay

"Tumor suppressor Activation polypeptide and uses thereof" Indian patent file number: IPR/4.19.20/06083/2006. International patent is filed for USA and Europian countries. Ref: 06083 (PCT).

Memberships/ Awards/ Fellowships

Vaijayanti P. Kale

- Active member of International Society for Stem Cell Research
- Active member of the International Society for Hematology and Stem Cells (ISEH)

Lalita S. Limaye

- Life member
  - Indian Society for Cell Biologists
  - Indian Society for Biotechnologists
  - Indian Women Scientists Association
  - Indian Association of Microbiologists Annual member International Society of Experimental Hematologists
- Fellowships
  - Availed DBT short term overseas fellowship from April 2008-July 2008 by working in Firpo Lab., Stem Cell Institute, Minnesota University, Minneapolis, USA on "cryopreservation of human embryonic stem cells."

Nibedita Lenka

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

Vasudevan Seshadri

- Life member Indian Society of Cell Biology

Mohan Wani

- Chancellor nominee as a Executive Council (Governing Body) member, Maharashtra Animal and Fisheries Sciences University, Nagpur (2009-2012).
- Member of the Executive Committee of Indian Society of Cell Biology (2007-2009).
- Member of CPCSEA for NIV and Raj Biotech, Pune.
- Member of the American Society for Bone and Mineral Research, USA.
- Member of International Chinese Hard Tissue Society.
- Member of Molecular Immunology Forum.
- Life Member of Indian Society of Cell Biology.
- Member of Doctoral Committees of ACTREC, Mumbai and NIV, Pune.

Jomon Joseph

- Member - Indian Society of Cell Biology

Sharmila Bapat

- Awarded National Women Bioscientist Award 2008 by the DBT
- Member - DBT Task Force on Chronic Disease Biology
- Member - American Association of Cancer Research (AACR)
- Member - International Society of Stem Cell Research (ISSCR)
- Member - International Epigenetics Society (earlier DNA Methylation Society)
- Member - Indian Association of Cancer Research (AACR)
- Member - Indian Women Scientists Association

Anjali Shiras

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

Ramesh Bhonde

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

Debashis Mitra

- Executive Member, Microbicide Society of India

Arvind Sahu

- Member of the International Complement Society
- Member of the Molecular Immunology Forum
- Member of the American Society for Microbiology

Samit Chattopadhyay

- Fellow, The National Academy of Science, Allahabad, India, 2006 onwards

Extramural Funding

Vaijayanti P Kale

- Identification of the molecular mechanisms involved in the induction of proliferative responses in primitive hematopoietic cells, DBT (20082011).

Lalita S Limaye

- Harnessing the potential of stem cells: In vitro generation of megakaryocytes and dendritic cells. DBT (2005-2009).
  Co-Investigator: Dr.V.P.Kale

Nibedita Lenka

- In vitro targeting and functional characterization of ES cell derived dopaminergic neurons and exploration of their therapeutic potential. DBT (2004-2008).
- Harnessing the potential of multipotent adult stem cells; Sub project: Exploring the potential of SP cells derived from umbilical cord blood and human bone marrow. DBT (2005-2009).

Mohan Wani

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

Jomon Joseph

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

- Regulation of β-catenin function by the nucleoporin Nup358 in Wnt signaling. DBT (2008-2011).

Anjali Shiras

- Unraveling the role of miRNAs in self-renewal and tumorigenicity of brain tumor stem cells derived from Neuroepithelial tumors of the Central Nervous System (CNS). DBT (2008-2011).
- 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).
- Harvesting the potential of multipotent Stem cells Identification, Development and Characterization of long term Neural Stem cell-lines from adult brain tissue. Program Support Project: DBT (2005-2008).

Gopal Kundu

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

Ramesh Bhonde

- Islet immunoisolation with xenotransplantation and stem cell regeneration to islets as strategies for treatment of diabetes. Nair PD, SCTIMST, Thiruvananthapuram and RR Bhonde, NCCS, Pune. 2005-2009 (DBT).
- Harnessing the potential of adult human stem cells: Differentiation / transdifferentiation of stem cells from pancreatic and non pancreatic sources of human origin. RR Bhonde, NCCS, Pune. 2005-2009 (DBT).

Anandwardhan Hardikar

- Differentiation of human fetal pancreatic progenitor cells for potential use in cell replacement therapy for type 1diabetes. DBT (2007-2010).
- Biliary Duct Stem Cells for Cell Replacement Therapy in Diabetes. Funding agency: UKIERI (United Kingdom and India Educational Research Initiative) jointly funded by the Department of Science and Technology and the British Council, UK. Period of funding: January 2007 to January 2010

Yogesh Shouche

- Development of molecular techniques for identification and typing of indegenous probiotic cultures, DBT (2005-2008).
- Identification, isolation and characterization of Azo dye degrading genes, DBT (2006-2009).
- Cloning, Expression and Production of haeme-proteins by yeast in fermenters for combating nutritional iron deficiency, DBT (2005-2008).
- DNA barcoding of butterflies from Western Ghat. 2007-2010.
- DNA Barcoding of Amphibians from Western Ghat 2007-2010.
- Screening for Bio-molecules from microbial diversity collected from different ecological niches, funded by Department of Biotechnology (2007-2010)
- Characterization of Hox complex and regulatory elements from Anopheles stephensi and Aedes aegypti (2008-2011).
- Establishment of Microbial Culture Collection and Biological Research Centre, funded by Department of Biotechnology (2009-2013)

International

- Methanotrophic communities in a meteor impact crater lake in India, funded by UK India Research and Educational Initiative (UKIERI) (2008-2011)
- Microbial Diversity & Development of Antibiotic resistance associated with industrial waste water treatment, funded by Swedish Research Council (2009-2011)
- Mid gut bacteria in Aedes aegypti and vector competence , Forgarty International Research Collaboration Award

Debashis Mitra

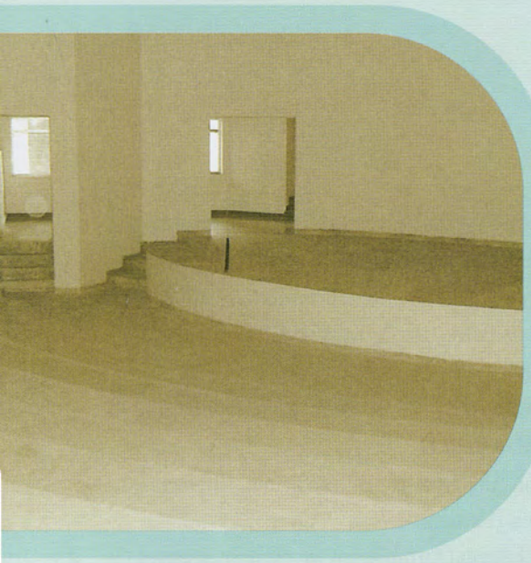
- Identification of anti-viral compounds with potential for development of Microbicides to prevent HIV infection and transmission. DBT (2006 2010). Co-Principal Investigator: Inderpal Singh and KK Bhutani, NIPER, Mohali
- Dissecting the CD40L-CD40 mediated signaling pathway in HIV infection.CMR-DBT (20062009). Co-Investigator: Bhaskar Saha (NCCS) and Sekhar Chakrabarti, IICB, Kolkata
- Characterization of anti-HIV activity of Acaciaside-B and pre-clinical studies towards its development as a potential microbicide-spermicide formulation. DBT (2009-2012). Co-Principal Investigator: Syed N. Kabir, IICB, Kolkata.

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

Samit Chattopadhyay

- Molecular switch in Th1-Th2 response by SMAR1: Its implications in Mycobacterium Tuberculosis infection. DBT project (BT/PR8686/ Med/14/1275/2007). 2007-2010.
- Regulation of HIV-1 LTR mediated transcription by MAR binding protein SMAR1. DBT project (BT/PR8687/Med/ 14/ 1276/ 2007). 2007-2010.



Seminars

Seminars Delivered by Visiting Scientists

1. **Dr. Sudit S. Mukhopadhyay,** Advinus Therapeutics PVT. Ltd., Pune. Mitochondrial Role in Hematopoietic Stem Cell Death and Cancer, August 2008.
2. **Dr. Vivek M. Rangnekar,** Associate Director of Translational Research, Markey Cancer Center, KY, USA. A Paradigm for Cancer-Selective Apoptosis, Professor & Alfred Cohen, M.D., Chair in Oncology Research, September, 2008.
3. **Dr. Arjun Guha,** Department of Biochemisty and Biophysics, University of California, San Francisco, USA. One surprise after another: Studies on the development and function of the respiratory system in Drosophila, September 2008.
4. **Dr. Kailash Chand Pandey,** Dept. of Medicine, SFGH-UCSF, San Francisco, USA. Structure-Function Analysis of Malarial Cysteine Proteases Falcipains, September, 2008
5. **Dr. Nandkumar Khaire,** University of Oxford, UK. Regulation of tumour suppressor activity of Retinoblastoma protein by lysine methylation, October, 2008
6. **Dr. Nagesh Narayan Pandey,** Applications Specialist, Flow-cytometry, Beckman Coulter India Ltd. "MOFLO" The most efficient cell sorter and its applications in scientific research, November 2008.
7. **Dr. Abdur Rahaman,** Research associate, Molecular Genetics and Cell Biology, University of Chicago, USA. Novel role of a dynamin-related protein in the nuclear remodeling of Tetrahymena, December, 2008.
8. **Dr. A.K. Rajasekaran,** Director, Nemours Center for Childhood Cancer Research, Wilmington, DE 19803, USA. Epithelial to Mesenchymal Transition: Role of Na,K-ATPase, January, 2009.

9. **Dr. Anu Puri,** CCR Nanobiology Program, National Cancer Institute at Frederick, NIH, MD 21702, USA. Lipid-Based Nanoparticles for Sustained, Targeted, and Localized Delivery of Cancer Therapeutics, February, 2009.
10. **Prof. David Tosh,** University of Bath, UK. Conversion of liver cells to pancreatic beta cells, March, 2009.
11. **Dr. Samit Adhya,** IICB, Kolkata. Delivery of RNA to mitochondria: implications for gene therapy, March, 2009.
12. **Dr. Yamini Bhusan Tripathi,** Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Scientifically validated new polyherbal formulation for chronic diseases, May, 2009.

Seminars delivered by NCCS Scientists

Lalita S Limaye

- Invited lecture in National Institute for integrated Sciences in Trivendrum in March 09 on " Basics of Flow cytometry"

Nibedita Lenka

- N. Lenka. 2008. Stem cells: The Promises and The Challenges. UGC sponsored State level Symposium on Current Trends in Cellular Communication, Hislop College, Nagpur, India (Invited Speaker, SessionChair).
- N. Lenka. 2008. Stem cells from bench to bedside. BIOZEAL' 08, National Conference on Stem Cell Research, Thadomal Shahani Engineering College, Mumbai, India (Invited Speaker).
- N. Lenka. 2008. To be or not to be - Wnt at the crossroad. 1st International Stem Cell Summit, India 2008, IIT, Madras, India (Invited Speaker, Session Chair).
- N. Lenka. 2009. In vitro generation of dopaminergic neurons from ES cells and exploration of their therapeutic efficacy. 2nd Asian and Oceanian Parkinson's Disease and Movement Disorders Congress, All India Institute of Medical Sciences, New Delhi, India (Invited Speaker).

Mohan Wani

- "Recent advances in stem cell technology and its application in the field of animal health" in Annual Convention of Indian Association of Veterinary Anatomist held at KNP College of Veterinary Science, Shirval, May 8, 2008
- "How close are we to make therapeutic use of stem cells" School of Health Sciences, University of Pune, August 8, 2008.

- "New molecular targets for the treatment of osteoporosis and rheumatoid arthritis", National Seminar on Recent Advances in Preclinical and Clinical Pharmacology, Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, September 19, 2008.
- "Strategies for developing stem cell research", Brain Storming Session on Stem cells, Gujarat State Biotechnology Mission, Gandhinagar, September 25, 2008.
- "IL-3 negatively regulates human osteoclast differentiation and bone resorption" Molecular Immunology Forum held at Alibaug, March 7-9, 2009.

Sharmila Bapat

- Symposium on Cancer Stem Cells on 27-28th July 2008 at Panjab University, Chandigarh.
- "Isolation, Characterization and Application of Cancer Stem Cells" International Meeting on Research in Vision and Ophthalmology, January 15-18, 2009, Hyderabad, India, also moderated the session on New Insights from Cancer Stem Cell Biology
- Molecular and Cellular Medicine on 1st Februray 2009 (Sunday) in Krishna Medical Institute Karad.
- Stem Cells and Cancer Stem Cells Indira Pharmacy College, Pune - SAB 28.2.09
- Ovarian Cancer Stem Cells " Symposium on Ovarian Cancer" on 27th-28th March, 2009 at All India Institute of Medical Sciences (AIIMS).
- Integration of bioinformatic approaches and functional assays in transcription factor target identification - HUGO HGM-2008 to be held during September 27-30 in Hyderabad, India, The theme of the Satellite Symposium "Complex Diseases: Approaches to Gene Identification and Therapeutic Management."
- A Signatorial Mitochondrial Mutation Profile in Ovarian Cancer Stem Cells, Indo-US meeting on Mitochondrial Research and Medicine 12-14 Nov.2008
- Integration of Bioinformatic approaches and functional assays in transcription factor target gene identification, in Emerging Concepts In Cancer Biology: Targeted Therapeutics, Cancer Stem Cells And Nanotechnology. Thiruvananthapuram, December 14 and 15, 2008.
- "International Symposium on Glyco-science, Cell-Engineering & Bioinformatics during the 2nd DBT-AIST, Japan Bilateral Workshop" from 25-26 November, 2008, Hyderabad.

- "Modulation of gene expression in ovarian cancer by active and repressive histone marks" at the AACR Special meeting on "Cancer Epigenetics" at Boston from 27th -30th May, 2008
- "Ovarian cancer stem cells recruit CD133 expressing stem cells towards the establishment of an endothelial hierarchy that mediates long-term tumor angiogenesis", Poster presentation at the International Society for Stem Cell Research, June 10-14, 2008
- "Epigenetic Mechanisms of Gene Regulation in Ovarian Cancer" Invited by BioCity, Turku and Frontiers of Science, Finland, 28th August, 2008

Manoj Kumar Bhat

- Invited talk at Rajiv Gandhi Proudyogiki Viswavidyalaya (University of Technology of Madhya Pradesh) at Bhopal on 8th April 2009, February 2009. Title of the presentation: Cancer therapy: Improvement in chemotherapy and novel drug targets.
- Invited talk at Institute of Life Sciences, University of Hyderabad Campus, Hyderabad on 25th April 2009. Title of the presentation: Enhancement of the therapeutic index of chemotherapeutic drugs for improvement in cancer therapy.

Padma Shastry

- The emerging roles of Akt/PKB and NFkappa B in TNF-□ mediated responses in gliomas. Gowry Das, Sudheerkumar and Padma Shastry. International Symposium on "Novel Strategies for Targeted Prevention and Treatment of Cancer" December 19-20, 2008, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India,
- Trends in Modern Biology- Dept of Zooolgy, Pune University, March 2009. Deregulating the survival pathways: The games cancer cells play-Focus on Neuroblastomas & Glioblastomas- Padma shastry

Anjali Shiras

- Recent advances in Stem Cell Research at Hislop College, Nagpur - 2008.

Gopal Kundu

- Diagnostic and Therapeutic Significance of Osteopontin and other associated genes in Breast and other Cancers. NATIONAL CONFERENCE OF SHANTI SWARUP BHATNAGAR AWARD WINNERS, Indore, 17-19th July, 2009
- Identification of potential novel target(s) and development of target based therapy in various cancers. Indian Institute of Integrative Medicine, Jammu, 11th May, 2009

- Understanding the Molecular Mechanism of Tumor Growth and Angiogenesis in Breast and Prostate Cancers by Chemokine like Protein, Osteopontin. IACS, Kolkata, 23rd April, 2009
- Cancer Awareness in India. 10th April, 09, Bataspur High School Golden Jubillee Celebration, West Bengal, 10th April, 2009
- Therapeutic and Diagnostic significance of Osteopontin and other associated proteins in regulation of vascular angiogenesis, tumor growth and metastasis. CDRI, Lucknow, 30th March, 2009
- Mechanism of Tumor Growth and Angiogenesis in Breast and other cancers: role of environmental factors. Intl. Society of Biotechnology Conference, ISBT-2008, Sikkim Manipal Institute of Technology, Sikkim, 29th December, 2008
- Regulation of tumor growth and angiogenesis by natural product in breast and prostate cancers. Intl. Symp. On Novel Strategies for Targeted Prevention and Treatment of Cancer, JNU, New Delhi, 19-20th December, 2008
- Role of Osteopontin in regulation of tumor progression and angiogenesis in breast and prostate cancers. 77th Annual Meeting of SBC(I)-2008, IIT Madras, Chennai, 18-20th December, 2008
- Mechanism of Tumor Growth and Angiogenesis by Osteopontin in Breast and other Cancers.Vanderbilt University Medical Center, TN, USA, 31st October, 2008
- Regulation of tumor growth and angiogenesis by Osteopontin in breast cancers. Delaware Institute of Biotechnology, DE, USA, 30th October, 2008
- Role of Osteopontin in regulation of tumor growth and angiogenesis in breast and other cancers. VA Medical Center, Kansas City, USA, 29th October, 2008
- Understanding the molecular mechanism of tumor progression and angiogenesis by Osteopontin in breast and other cancers. National Cancer Institute (NCI), NIH, Maryland, USA, 28th October, 2008
- Regulation of Tumor Progression and Angiogenesis by Osteopontin in Breast and Prostate Cancers. Loyola University Medical Center, Chicago, USA, 27th October, 2008
- Understanding the molecular mechanism of Osteopontin-induced angiogenesis. 3rd Mayo Clinic Angiogenesis Symposium, Mayo Clinic, Rochester, USA, 25-26th October, 2008
- Identification of novel targets and development of target based therapeutics in Cancers: Targeted therapy in cancer based on small molecule antagonist. North Bengal University, Siliguri, WB, 17th October, 2008

Anandwardhan Hardikar

- "A unique microRNA seed sequence regulates mesenchymal transition of human pancreatic islet cells", at the Stem Cell workshop organized at National Center for Biological Sciences and JNCSR, Bangalore, October 2008
- "Human Fetal Pancreatic Islets Undergo Epithelial to Mesenchymal Transition to Generate an Islet Progenitor Cell Population" Invited speaker at the Stem Cells and Diabetes Annual meeting organized by University of New South Wales and Stem Cell Network, Australia in Sydney, Australia, November 2008.
- "Pancreatic progenitors for cell replacement therapy in diabetes": Invited speaker at Institute for Life Sciences, Bhubaneshwar, December 2008.
- "The miR-30 family microRNAs confer epithelial phenotype to pancreatic cells": Oral presentation at EMBO meeting in Peebles, Scotland, February 2009
- "Lineage tracing of human pancreatic endocrine cells reveals proliferative potential of islet β-cells" Invited presentation at Center for Regenerative Medicine, University of Bath, UK, March 2009

Yogesh Shouche

- "Microbial Diversity of human gut", at Annual Conference of Association of Microbiologists of India, at Chennai, November 2008

Debashis Mitra

- Heat Shock Proteins reciprocally regulate HIV-1 gene expression and replication. International Conference on Fundamental and Translational Research on HIV/AIDS: Global Perspectives, October 5 - 8, 2008, National Institute for Research in Reproductive Health (ICMR), Mumbai, India.
- Heat shock proteins: Role in HIV-1 gene expression and replication., Conference on Human Viruses and Translational Medicine, November 17-18, 2008, National Institute of Immunology, New Delhi, India.
- Human Immunodeficiency Virus 1 induced T cell apoptosis: Role of Mitochondrial Energy generating system, International Symposium on "Perspective of Cell Signaling and Molecular Medicine" November 27-29, 2008, Bose Institute, Kolkata, India.
- Tat is a pro-HIV modulator that suppresses gp120 specific Immune Response in IL-10 dependent manner. Society of Biological Chemists (India) 77th Annual Meeting, December 18-20, 2008, IIT Madras, Chennai, India

- The fight against HIV: Novel molecules and strategies for inhibiting the virus. IISC Centenary Symposium on Biology and Pathogenesis of Viruses: Molecular Insights, May 4-5, IISc, Bangalore, India.

Arvind Sahu

- Silver Jubilee Year Lecture Series, Central India Institute of Medical Sciences, Nagpur, "Complement: a viral target for immune evasion", December 9, 2008.
- 16th Molecular Immunology Forum, "Influence of electrostatic potential on the complement regulatory functions of Kaposica, the complement inhibitor of Kaposi's sarcoma-associated herpesvirus", March 7, 2009.
- National Seminar on Recent Advances and Future Trends in Immunologicals, Sinhgad College of Engineering, Pune, "Viral complement evasion: the stealth attack strategies developed by viruses". March 14, 2009

Samit Chattopadhyay

- Chattopadhyay S. and Sinha S. "Life and death: Game of a new player SMAR1", International Symposium on "Perspective of Cell Signaling and Molecular Medicine". November 27-29, 2008 at Bose Institute, Kolkata, India.
- Chattopadhyay S., Malonia S. and Sinha S. "To die or not to die: A Decision through chromatin remodeling", 28th annual meeting of the Indian Association for Cancer Research, Indian Institute of Science, Bangalore, 21-24, Feb 2009, Jointly organized by IISc and JNCASR.
- Chattopadhyay S Connecting arrrest and apoptosis: Transcriptional regulation by SMAR1. Seminar presented at Indian Institute of Science on December 26th, 2008.

Conferences / workshops attended / Poster Presentations

Vaijayanti P Kale

1. Communication between Hematopoietic Stem Cells and Mesenchyme Cells is promoted by Gap junctions and Nano-ducts. Vaijayanti P. Kale and L. C. Padhy. Keystone symposium on Stem cell Niche interactions, April 21-26, 2009 Whistler, Canada
2. Identification of domains of human endothelial nitric oxide synthase protein that strongly suppress tumour growth in vivo Vasudha Lakshmanan, Roli Misra, Gunjan Mukherji, Michelle Vaz, L. C. Padhy, Vaijayanti P. Kale 100th annual meeting of American Association of Cancer Research (AACR), April 18-22 2009, Denver, Colorado, USA
   (Ms. Vasudha received a travel grant of USD 2000 on the merit of the abstract)

Vasudevan Seshadri

Presented a poster titled "Characterization of novel insulin splice variant in mice that regulate insulin biosynthesis" at the XXXII Conference of Indian Society of Cell Biology (ISCB), December 4-6, 2008, MACS-Agharkar Research Institute, Pune.

Mohan Wani

1. Navita Gupta: 32nd Conference of Indian Society of Cell Biology, held at MACS Agharkar Research Institute, Pune, December 4-6, 2008.
2. Abstract presented: Gupta N, Pote ST, Tomar G, Srivastava RK, Barhanpurkar AP, and Wani MR (2008) Role of IL-3 in regulation of human osteoclast differentiation and activation (Abstract:P19/81).

Jomon Joseph

XXXII Conference of Indian Society of Cell Biology (ISCB), December 4-6, 2008, MACS-Agharkar Research Institute, Pune.

Sharmila Bapat

1. Anjali Kusumbe, Avinash Mali, S.A.Bapat. CD133+ cells in ovarian cancer in Emerging Concepts In Cancer Biology: Targeted Therapeutics, Cancer Stem Cells And Nanotechnology. Thiruvananthapuram, December 14 and 15, 2008.
2. Rajkumar Singh, Avinash Mali, S.A. Bapat. Development of monoclonal antibodies against ovarian cancer cell membrane allied antigen and their therapeutic implication at the 28 annual convention of Indian Association for Cancer Research and international symposium 21-24, 2009 IISC Bangalore

Padma Shastry

Poster presentation-77th Annual Meeting Society of Biological Chemists, IIT Chennai. Immunomodulatory activity of a novel lectin isolated from a phytopathogenic fungus, Rhizoctoni bataticola. Radha Pujari, Nagaraja N.N.

Anjali Shiras

Participated in June - 2008: Symposium on "Control and Regulation of Stem Cells" at Cold Spring Harbor Symposia, at Cold Spring Harbor Laboratory, New York, USA.

Ramesh Bhonde

1. Poster presented at the 6th annual meeting of International Society for Stem Cell Research (ISSCR), held in Philadelphia, USA from June 11th to 14th 2008 for the abstract titled "Adipose Tissue-derived Stromal Cells for Cell Replacement Therapy in Diabetes "Chandra, Vikash, Bhonde, Ramesh R
2. S. S. Kadam, A. A. Hardikar, R. R. Bhonde. Islet neogenesis from full term placental stem cells - a step towards cell replacement therapy. IFPA meeting 2008 -12th EPG Conference on Placenta, Seggau Castle, Austria. Sept 2008

Anandwardhan Hardikar

1. Stem cells and Diabetes Annual meeting at the University of New South Wales, Sydney, Australia (December 2008)
2. Pancreas Developmental Biology meeting at the Center for Regenerative Medicine, University of Bath, UK on March 2009
3. EMBO workshop in Peebles, UK February 2009

Yogesh Shouche

Annual Meeting of Association of Microbiologists of India, Delhi, 2008

Debashis Mitra

1. International Conference on Fundamental and Translational Research on HIV/AIDS: Global Perspectives, October 5 - 8, 2008, National Institute for Research in Reproductive Health (ICMR), Mumbai, India.
2. Conference on Human Viruses and Translational Medicine, November 17-18, 2008, National Institute of Immunology, New Delhi, India.
3. International Symposium on "Perspective of Cell Signaling and Molecular Medicine" November 27-29, 2008, Bose Institute, Kolkata, India.
4. Society of Biological Chemists (India) 77th Annual Meeting, December 18-20, 2008, IIT Madras, Chennai, India IISc Centenary Symposium on Biology and Pathogenesis of Viruses: Molecular Insights, May 4-5, IISc, Bangalore, India.

Arvind Sahu

1. XXII International Complement Workshop (2008), Basel, Switzerland Sep. 28 Oct. 2, 2008.
2. 16th Molecular Immunology Forum (2009), Alibag.

Samit Chattopadhyay

1. Sandeep Singh received travel grant for poster presentation in 9th International Congress on Cell Biology and the 20th Annual Conference of the Korean Society for Molecular and Cellular Biology, held on 7-10th Oct 2008 at Seoul Korea. Poster title: SMAR1 inhibits the enzyme activity of AKR1a4.
2. Oral presentation by Sandeep Singh in Graduate Student Meet, 2008 titled "A novel cytoplasmic function of tumor suppressor protein SMAR1" held on 27-28th Dec, 2008 at ACTREC, Navi Mumbai.
3. Oral presentation by Sreenath Kadreppa in Graduate Student Meet 2008 titled "Nuclear matrix protein SMAR1 represses HIV-1 LTR mediated transcription held on 27-28th Dec, 2008 at ATREC, Mumbai.
4. Sreenath Kadreppa received full travel grant for poster presentation at 5th International AIDS Society Conference held at Cape Town, South Africa.. Poster titled "Nuclear matrix protein SMAR1 represses HIV-1 transcription through chromatin remodeling".

Conferences / workshops attended by students

1. Ajay A.K., Upadhyay A.K and Bhat M.K.: p53 overexpression in HPV positive cells, role of kinases and phosphatases. Emerging areas in Biosciences and Bioengineering, IIT Mumbai, 26th to 28th February 2009.

2. Ajay A.K., Upadhyay A.K and Bhat M.K.: Human Papillomavirus18E6 prevents stabilization of p53 by inhibition of phosphorylation. 25th International papillomavirus conference & clinical workshop organized by International Papillomavirus Society, Malmo, Sweden, 8th to 14th May 2009.

3. Pandey V., Vijayakumar M.V. and Bhat M.K.: Presence of insulin during differentiation alters basal glucose uptake, lipid profile and membrane topology in 3T3-L1 adipocytes. International Symposium on Emerging Areas in Biosciences and Bioengineering, IIT Mumbai, 26th to 28th February 2009.

4. Ajay A.K., Upadhyay A.K. and Bhat M.K.: p53 Overesxpression in HPV positive HeLa cells. Role of kinases and phosphatases. Indian Association for Cancer Research meeting. IISC Bangalore, 21st to 24th February 2009.

5. Sharma A., Upadhyay A.K. and Bhat M.K.: Chemotherapeutic Drugs Induced Expression of Heat Shock Proteins and Their Role in Survival of Hepatocellular Carcinoma Cells. Indian Association for Cancer Research meeting. IISC Bangalore, 21st to 24th February 2009.

6. Sahu S: "Regeneration and Dedifferentiation" meeting at the Center for Regenerative Medicine, University of Bath, UK on July 2009

7. Joglekar MV: Poster presented at the EMBO workshop in Peebles, UK February 26, 2009

8. Regulation of HIV-1 replication by Heat shock Factor 1, Poster presented by Pratima Rawat, (Best poster award), International Conference on Fundamental and Translational Research on HIV/AIDS: Global Perspectives, October 5 - 8, 2008, National Institute for Research in Reproductive Health (ICMR), Mumbai, India.

9. HIV-1 Tat mediated regulation of cellular gene expression and genome wide recruitment on cellular promoters. Poster presented by Neeru Dhamija (Best poster award), IISc Centenary Symposium on Biology and Pathogenesis of Viruses: Molecular Insights, May 4-5, IISc, Bangalore, India.

10. Kalyani Pyaram attended "4th Winter School in Immunology" at Cochin conducted jointly by Cancer Research Institute, New York and Indo US Science & Technology Forum, New Delhi, July 30 August 5, 2008.

11. Dimple Notani: Delivered oral presentation at the Cold Spring Harbor Meeting on 'Dynamic organization of nuclear function' held during September 17-21, 2008, CSHL, New York.

12. Ranveer S. Jayani: Visiting student at National Institute of Genetics, Mishima, Japan for a collobarative project from March 2009 to July 2009.

13. Sunita Singh: International symposium on DNA-Protein Transactions, IMTECH, Chandigarh; RCAI International Summer Program (RISP) 2009, Yokohama, Japan

14. Kamalvishnu Prasad G.: Participated in Winter School in Immunology held at Cochin, Kerala; Attended ADNAT conference on Chromatin Biology held at CCMB, Hyderabad.

List of students awarded Ph.D.

Mahesh Kumar Verma

Thesis Title: Cardiac differentiation of embryonic stem cells

Guide: Dr. Nibedita Lenka

Ankur Kumar Upadhyay

Thesis Title: Action of chemotherapeutic drugs - Molecular mechanism and role of signaling molecules at cellular level

Guide: Dr. Manoj Kumar Bhat

Rajendra Prasad

Thesis Title: Studies on a novel non-coding RNA M3TR, identified from a human neuro-epithelium cell-line

Guide: Dr. Anjali Shiras

Goutam Chakraborty

Thesis Title: A Study on the role of Osteopontin in regulation of vascular endothelial growth factor (VEGF) expression Angiogenesis and Tumor Growth

Guide: Dr. Gopal Kundu

Shalini Jain

Thesis Title: Studies on the Role of Osteopontin (OPN) in the regulation of cyclooxygenase-2 (COX-2) expression, Tumor growth, and Angiogenesis in prostate cancer

Guide: Dr. Gopal Kundu

Satyabrata pany

Thesis Title: A Comparative Study of alpha-hemolysin and jacalin that influence mammalian cell signaling

Guide: Dr. MV Krishnasastry

Debargh S. Dutta

Thesis Title: Role of dendritic cells in naive T cell activation, proliferation and differentiation and generation of memory

Guide: Dr. G C Mishra

Pavithra Sampath

Thesis Title: Regulation of MAR binding protein SMAR1 under stress: Implications in cell cycle by modulation of ATM-p53-MDM2 pathways

Guide: Dr. Samit Chattopadhyay

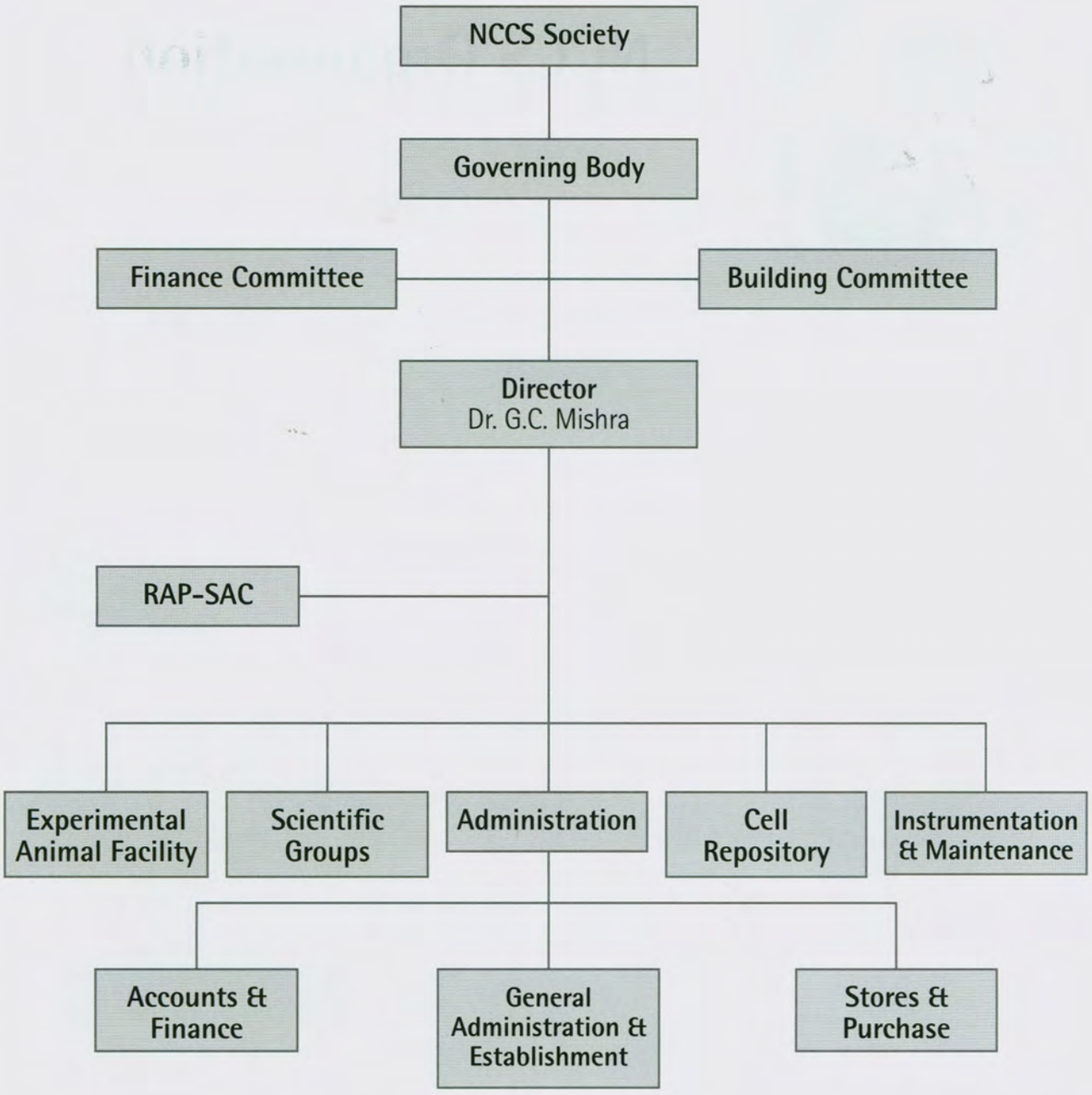
Prabhat Kumar Purbey

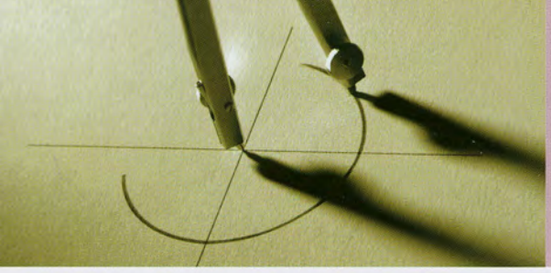
Thesis Title: Structural and Functional characterization of the PDZ-like signaling domain of SATB1

Guide: Dr. Sanjeev Galande

NCCS Organisation

NCCS ORGANIZATION





NCCS Committees

Society

1. **Shri Prithviraj Chavan** — President
   Hon'ble Minister for Science & Technology,
   Minister of Earth Sciences,
   Minister of State PMO,
   Minister of State, Personnel, Public Grievances & Pensions and
   Minister of State Parlimentary Affairs,
   Anusandhan Bhawan,
   2, Rafi Marg, New Delhi 110001

2 **Prof. M. K. Bhan** — Chairman
   Secretary,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

3. **Dr. Vishwa Mohan Katoch** — Member
   Secretary to Govt. of India,
   Department of Health Research
   (Ministry of Health & Family Welfare), and
   Director General, ICMR,
   Ansari Nagar, Post Box 4911,
   New Delhi 110029

4. **Dr. C.M. Gupta,** — Member
   DBT Distinguished Biotechnologist,
   Central Drug Research Institute,
   Chattar Manzil, Post Box No. 173,
   Lucknow 226001

5. **Vice Chancellor** — Member
   University of Pune,
   Ganeshkhind,
   Pune 411007

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 110003

7. **Shri Sukdeb Sinha** — Member
   Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

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 110001

9. **Prof. Saroj Ghaskadbi** — Member
   Head, Department of Zoology
   University of Pune,
   Ganeshkhind, Pune 411007

10. **Prof. Vijay Raghavan** — Member
    Director,
    National Centre for Biological Sciences,
    Tata Inst. of Fundamental Research,
    GKVK, Bellary Road, Bangalore 560065

11. **Dr. Padma Shastry** — Member
    Scientist 'G'
    National Centre for Cell Science,
    Ganeshkhind, Pune 411007

12. **Dr. G.C. Mishra** — Member
    Director,
    National Centre for Cell Science,
    Ganeshkhind, Pune 411007

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 110003

2. **Dr. Vishwa Mohan Katoch** — Member
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   Chattar Manzil, Post Box No. 173,
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4. **Vice Chancellor** — Member
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   Ganeshkhind,
   Pune 411007

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   Joint Secretary & Financial Adviser,
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

6. **Shri. Sukdeb Sinha** — Member
   Adviser, Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

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 110001

8. **Prof. Saroj Ghaskadbi** — Member
   Head, Department of Zoology
   University of Pune,
   Ganeshkhind,
   Pune 411007

9. **Prof. Vijay Raghavan** — Member
   Director,
   National Centre for Biological Sciences,
   Tata Inst. of Fundamental Research,
   GKVK, Bellary Road,
   Bangalore 560065

10. **Dr. Padma Shastry** — Member
    Scientist 'G'
    National Centre for Cell Science,
    Ganeshkhind,
    Pune 411007

11. **Dr. G.C. Mishra** — Member
    Director,
    National Centre for Cell Science,
    Ganeshkhind,
    Pune 411007

Finance Committee

1. **Shri K.P. Pandian** — Chairman
   Joint Secretary & Financial Adviser,
   Department of Science & Technology,
   Technology Bhavan,
   New Mehraulli Road,
   New Delhi 110016

2. **Dr. Srikanth Tripathy** — Member
   Scientist 'F',
   Clinical Sciences Dept.,
   National Aids Research Institute,
   G-Block, P-73, Near Electronic Sadan,
   Bhosari,
   Pune 411026

3. **Dr. Satish Kumar Gupta** — Member
   Staff Scientist VII and Chief,
   Reproductive Cell Biology Laboratory,
   National Institute of Immunology,
   Aruna Asaf Ali Road,
   New Delhi 110067

4. **Prof. Saroj Ghaskadbi** — Member
   Head, Department of Zoology
   University of Pune,
   Ganeshkhind,
   Pune 411007

5. **Shri Sukdeb 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,
   National Centre for Cell Science,
   Ganeshkhind,
   Pune 411007

Building Committee

1. **Dr. S.K. Basu** — Chairman
   Professor of Eminence,
   National Institute of Immunology,
   Aruna Asaf Ali Marg,
   New Delhi 110067

2. **Shri Sukdeb Sinha** — Member
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

3. **Shri B. Bose** — Member
   Management Consultant,
   National Institute of Immunology,
   New Delhi 110067

4. **Dr. A.C. Mishra** — Member
   Director,
   National Institute of Virology,
   Dr. Ambedkar Road,
   Pune 411001

5. **Dr. V.S. Rao** — Member
   Former Director,
   Agharkar Research Institute,
   Pune 411004

6. **Ms. Moushumi Rudra** — Member
   Director (Finance),
   Department of Biotechnology,
   Block No. 2, 7th Floor,
   CGO Complex, Lodi Road,
   New Delhi 110003

7. **Chief Engineer** — Member
   Pune (PW) Region,
   New Central Building,
   Pune 411001

Scientific Advisory Committee

1. **Prof. N. K. Ganguly** — Chairman
   Distinguished Biotechnologist,
   National Institute of Immunology,(THSI),
   Aruna Asaf Ali Marg,
   New Delhi 110 067

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
   CEO
   The Wellcome Trust/DBT India Alliance,
   Banjara Hills,
   Hyderabad 500 034

6. **Dr. C. M. Gupta** — Member
   DBT 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. G.C. Mishra** — Member
   Director,
   National Centre for Cell Science,
   Ganeshkhind,
   Pune 411007

9. **Shri P.Y. Bhusnale** — Convener
   Technical Officer 'C'
   (Engineer-Central Instrumentation)
   National Centre for Cell Science,
   Ganeshkhind,
   Pune 411007

10. **Shri B.G. Acharya** — Invitee
    Officer 'D' (Sr. Officer-Administration)
    National Centre for Cell Science,
    Ganeshkhind,
    Pune 411007

11. **Shri J.P. Singh** — Invitee
    Officer 'D' (Sr. Officer Accounts)
    National Centre for Cell Science,
    Ganeshkhind,
    Pune 411007

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

9. **Shri. S. 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.

11. **Prof. Sandip K. Basu** — Member
Prof 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. **Prof. Vijay Raghavan** — Member
Director
National Centre for Biological Sciences
Tata Institute of Fundamental Research
GKVK, Bellary Road,
Bangalore 560065

14. **Dr. B. Ravindran** — Member
Director,
Institute of Life Sciences,
Nalco Square, Chandrasekharpur
Bhubaneshwar 751 023

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

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

Scientists	: 25
Administrative	: 40
Technical	: 57
Total	: 122

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. Dr. M.S. Patole, and Shri. B.G. Acharya attended a training programme conducted by DoPT regarding the implementation of the reservation policy.

RIGHT TO INFORMATION ACT 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. B.G. Acharya as CPIO for Administrative matters and Dr. D. Mitra as CPIO for Scientific matters. Shri A.D. Patil has been nominated as ACPIO and Dr. G.C. Mishra has been nominated as the Appellate Authority. Shri. B.G. Acharya, A.D. Patil and Dr. D. Mitra attended a training workshop on RTI conducted by the Institute of Public Administration, Bangalore.

IMPLEMENTATION OF OFFICIAL LANGUAGE

NCCS has constituted Official Language Implementation Committee, which meets quarterly and tries to pursue Govt. of India orders in the matter of implementation of Official Language in day to day official work. Maximum staff members have passed Hindi Pragya Exam conducted by Hindi Teaching Scheme Office. Most of the forms have been made bilingual. Noting/Drafting work on many of the files is done in Hindi. On every Monday, all the work in the Library is carried out in Hindi and the same is made mandatory for all library users. Unicode Encoding System has been enabled in most of the computers so that Hindi work can be carried out easily anywhere in the Institute. The

Dr. Onkar Nath Shukla (left), Hindi Officer, IITM, Pune, was the chief guest on the occasion of the Hindi Diwas celebration organized at NCCS on 18th September, 2009. Dr. G.C. Mishra, Director, NCCS (right).

centre also observes Hindi Saptah every year. Essay and Circular writing competitions were held and winners were given cash awards. Guest lecture was also arranged on Hindi Day. Official language activities are strongly supported by the Director.

VIGILANCE MATTERS

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

SECURITY

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

DISCIPLINARY MATTERS

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

COMMITTEES

The centre has formed various committees to assist in its day to day activities. These committees are

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