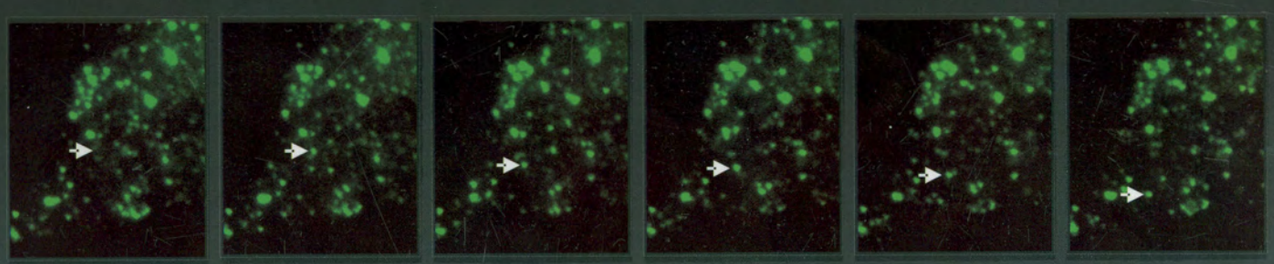
nccs

National Centre for Cell Science



Annual Report 2009-2010

National Centre for Cell Science
Annual Report 2009-2010

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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, other reagents and cell products independently and in collaboration with industry and other organizations.

- Research and development

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

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

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

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

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

From Director's Desk

It gives me immense pleasure in presenting the annual report of National Centre for Cell Science (NCCS) for the year 2009-2010. This report highlights the scientific achievements in the previous year and envisions the future perspectives. As the National Cell Repository, NCCS maintains and distributes cell lines to various universities and research institutions in India, and we have provided over 2000 cell lines in the reporting year. Recent years have witnessed an explosion of information in diverse areas of biology owing to multidisciplinary approaches. This necessitates development of a large number of skilled scientific personnel to make use of the new scientific knowledge accumulated to address the societal needs. Under the human development programme, we have trained over 36 student fellows from all over the country on different aspects of research in modern biology. NCCS has greatly emphasized on Research and Development in the areas of cell biology, signal transduction, cancer biology, diabetes, biodiversity, infection & immunity, chromatin architecture and gene regulation, stem cells, proteomics, bioinformatics and regenerative biology.

As a leading research institution, our prime focus is on addressing the current and emerging public health needs, with special reference to infectious diseases such as Leishmaniasis, AIDS, Tuberculosis and Malaria. In fact, understanding the molecular intricacies of infection and immunity gains greater importance, because of the recent emergence of non-native/new infectious agents. Establishment of any infection depends on how effectively the pathogens can counteract the host innate and adaptive immune responses. Our scientists have identified specific domains in the viral proteins that simulate the complement system and overcome the innate immune response. Furthermore, we have characterized the signaling events resulting from CD40-CD40L interaction upon Leishmania and HIV infections. We have also identified a number of novel anti-HIV compounds that have potential use in microbicide formulations against HIV.

Cell-cell communication is fundamental to the growth and development of multi-cellular organisms. We are studying the regulation of critical players

such as p53, eNOS, osteopontin, mTOR and TNF-a, which are involved in some of the pathways that go awry in cancers. We have identified the key player that mediates communication between the AKT/mTOR and the MEK/ERK pathways. We have also elucidated the signaling events that are involved in the osteopontin-mediated gene expression during tumor progression. Our studies have defined a subset of cancer stem cells that contribute to tumor dormancy and resistance to chemotherapy. We have developed a protein-interaction network model that gives a snapshot of the extensive cross-talk between the diverse signaling pathways in ovarian cancer.

At NCCS, major thrust is also given to 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. Novel methods to isolate, maintain and preserve viable stem cells have been developed by our scientists. We have also developed protocols to successfully differentiate these cells into specific lineages that have potential applications in cell/tissue replacement therapy.

Recent advances suggest a critical role for non-coding RNAs in regulating various cellular processes. Interestingly, we characterized a non-coding RNA that is involved in tumorigenesis, and understanding the molecular mechanism of its action is underway.

SATB1 and SMAR1 are two nuclear-matrix-associated proteins that modulate gene expression by regulating chromatin remodeling and transcription. Identification and characterization of SMAR1 interactome have shed light into the understanding of its mechanism of action. Furthermore, a novel isoform of SATB1 that could be responsible for inducing dynamic chromatin remodeling during T cell activation has been identified.

NCCS has established a Microbial Culture Collection centre for the conservation and exploitation of the biodiversity in India, with a special mandate from DBT. The microbial distribution in various habitats has been studied using the meta-genomic approach. The variations in the gut microflora of mosquito, housefly and humans, and their effect on the physiology of the host have been studied.

Our scientific productivity is reflected in the number of publications in several prestigious international journals, and in the current year we have over 90 scientific papers to our credit. The research activities in the institute are augmented by an excellent experimental animal facility that procures and maintains animals, and provide technical support to the scientists. NCCS has recently recruited scientific personnel in the areas of proteomics and bioinformatics to complement the current research activities and to explore new horizons. I am very pleased to state that the construction of buildings for cell repository and the student hostel is in the final stages. In addition to the institutional research funds, NCCS scientists have been

successful in obtaining peer-reviewed competitive grants from various national and international agencies. NCCS has also established Indo-French, Indo-German, Indo-Finland, and Indo-US collaborations to enhance our scientific activities.

Our centre considers many different perspectives in establishing research priorities. In the coming years NCCS will continue to focus on important questions relevant to human health, specifically in the area of translational research with a view to benefit the society.

G.C. Mishra
Director

Human Resource Development

During 2009-2010, 38 students joined for pursuing Ph.D under various Scientists. This year 11 student presentations were completed and their admission is confirmed by University. The total number of Ph. D students as on 31.03.2010 was 97.

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

Project Training - 31
Summer Training – 5

During this year 44 research fellows attended seminars/conferences/ symposium conducted by various reputed organizations in India and 8 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 2009-2010, we have supplied 2050 cell lines to 120 research institutions in the country.

Research Reports

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

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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 HSC niche has not been defined in molecular terms owing to its heterogeneous cellular composition comprising of cells like adipocytes, osteoblasts, reticular cells, endothelial cells etc.

Adipocytes and osteoblasts form important components of the HSC niche and these two cell types are known to affect the HSC growth in an opposite manner: the adipocytes are considered as negative regulators of hematopoiesis while the osteoblasts are known to exert positive effects. It is therefore conceivable that there could be a constant flux operative in the niche leading to a dynamic modulation of the cellular composition of the microenvironment, thereby allowing both quiescence and proliferation of the HSC taking place in a regulated manner. However, the precise nature of the signaling mechanisms involved in this regulation is not clearly identified. We have taken a two-pronged approach to elucidate the specific contribution of these individual cells types on the regulation of hematopoietic stem cells. In the first approach we propose to use adipocytes or osteoblasts prepared from the naïve MSCs and examine their effect on the HSC regulation, either singly or in varying combinations. The second one involves the use of MSCs having an exogenous over expression of PPAR-gamma and Runx-2 to identify the contribution of the signaling evoked by these master regulators of adipogenesis and osteogenesis respectively.

In addition to these two regulators, we also propose to over express other hematopoietic regulators like BMP4, Wnt5a, Wnt10b, HIF-1α, HIF-2α, eNOS, N-Cadherin, MSX-2, etc. to examine the effect of the signaling emanating from them on the stem cell functions.

These systems, though "reductionist" in nature, may enable us to decipher the contribution of individual cell type in the HSC regulation as well as to get a "signal to effect" correlation of stroma-mediated signals on the HSC fate.

Aims and Objectives

1. To study whether BMP4, a cytokine secreted by the osteoblasts, commits the bone marrow stromal cells to the adipocyte lineage
2. To examine the effect of these differentiated adipocytes on the growth of primitive HSCs when used as feeders in the co-culture system.
3. To examine whether the BMP 4-mediated adipogenesis can be inhibited by pharmacological means.
4. To develop stromal cell lines stably expressing master regulators of hematopoiesis and examine their effect on the stem cell fate.

Work Achieved

BMP 4 induces adipogenic differentiation of marrow stromal cells (MSCs) in a dose-dependent manner:

BMP4 has been reported to be secreted by the osteoblasts present in the niche and is known to commit the naive embryonic stem cells C3H10T1/2 of mouse origin to the adipogenic lineage. We therefore wanted to examine whether BMP4 possesses an ability to commit the naive MSCs to adipocytic lineage.

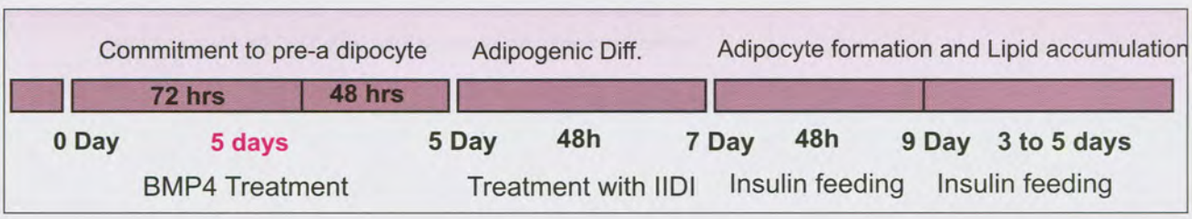
To study the BMP4-mediated commitment of the BM MSCs to adipocytes lineage we have used M210B4, a murine bone marrow-derived stromal cell line, as a model system. The cells were seeded at low cell density and were treated with BMP4 (50ng/ml), or not, till the cells became confluent, and the treatment was further continued for another 48 hours. The cells were then treated with standard adipogenic differentiation medium [IIDI-Insulin (4μg/ml), 3-isobutyl, 1-methyl xanthine (IBMX;500μM), Dexamethasone (0.25μM), and Indomethacin (200μM)] for 48 hours followed by feeding with insulin (2μg/ml) after every 72 hours. After 14 days of culture, the cells were fixed with 10% buffered formaldehyde and were stained with Oil Red-O. The stained adipocytes were counted manually under an inverted microscope (Olympus). It was observed that a significantly higher number of adipocytes formed in the BMP4-treated group as compared to the control (Fig.1A). This result suggested that BMP4 indeed induces the commitment of MSCs to differentiate into pre-adipocytes and leads to the formation of higher number of adipocytes as compared to the control.

We then examined whether the effect of BMP4 on these cells was dose-dependent or not. To study this aspect, we treated the M210B4 cells with an increasing concentration of BMP4 from 1ng/ml to 100ng/ml and scored the adipocytes formed in them. As seen in the Fig. 1B, the number of adipocytes increased with an increase in the BMP4 concentration. This

result confirmed that BMP4 has a dose-dependent adipogenic effect on M210B4 stromal cells.

Treatment of BM MSCs with BMP4 results in an upregulation of adipocyte-specific genes:

To further confirm the BMP4-mediated commitment of BM MSCs to adipocyte lineage at the gene expression level, we carried out PCR



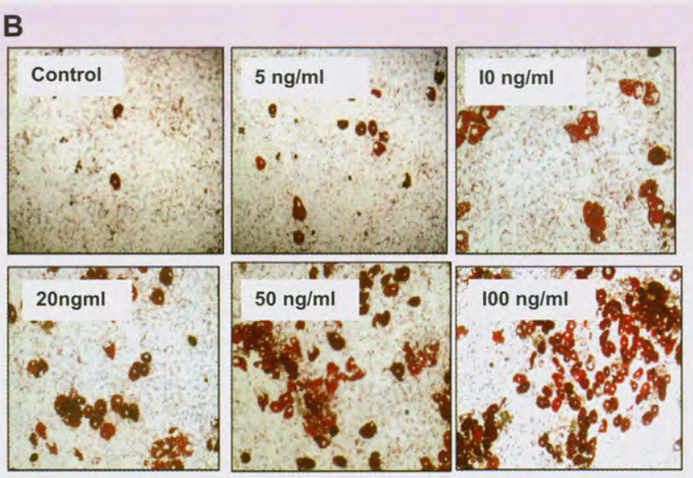
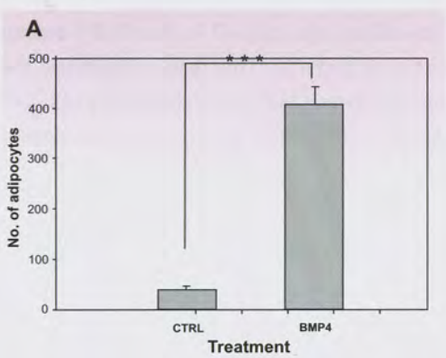
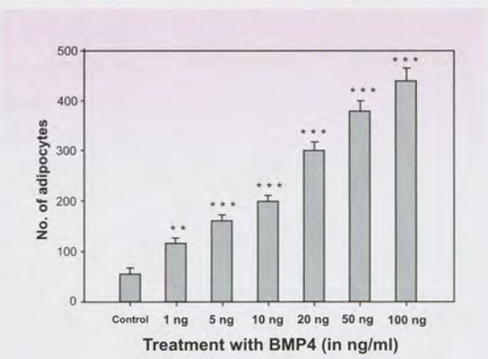


Fig.1. A. M210B4 cells were treated with 50ng/ml BMP4. The cells were fixed and were stained with Oil Red O. Significantly more number of Oil Red O positive cells were observed in BMP4-treated cells as compared to the control cells, clearly indicating that BMP4 commits the bone marrow stromal cells towards the adipocyte lineage.(*** $p < 0.001$). **B.** M2 10B4 cells were treated with increasing concentrations of BMP4, 24 hours after seeding of cells. The cells were fixed and were stained with Oil Red O. Increasing number of Oil Red O positive cells was obtained with an increasing concentration of BMP4; clearly indicating that BMP4 exerts a concentration-dependant adipocyte-commitment effect on the stromal cells. The upper panel shows Oil Red O stained cells and the lower panel depicts the quantitative data (*$p<0.05$,** $p< 0.01$ and *** $p< 0.001$).





experiments to quantitate the mRNA levels of the transcription factors like PPARγ, C/EBPα and aP2 (FABP4) that are known to regulate the process of commitment of MSCs to adipocyte lineage and formation of mature adipocytes with accumulation of lipid droplets. The flow chart illustrated below depicts the process involved in the adipogenic differentiation and the different time points at which the gene expression study was carried out.

We studied the gene expression at 5 day, 7 day and 9 day time point by carrying out real time PCR experiments. We monitored the relative fold change in the gene expression of PPARγ2 and aP2 genes by BMP4 treatment as compared to control using GAPDH as a normalizer. As seen in the Fig. 2 the expression of PPARγ (a) and aP2 (b) was up regulated by BMP4 treatment at all time points examined. This result suggested that the treatment of the BM MSCs with BMP4 results in the activation of transcriptional cascade that regulates the adipogenic commitment of the BM MSCs.

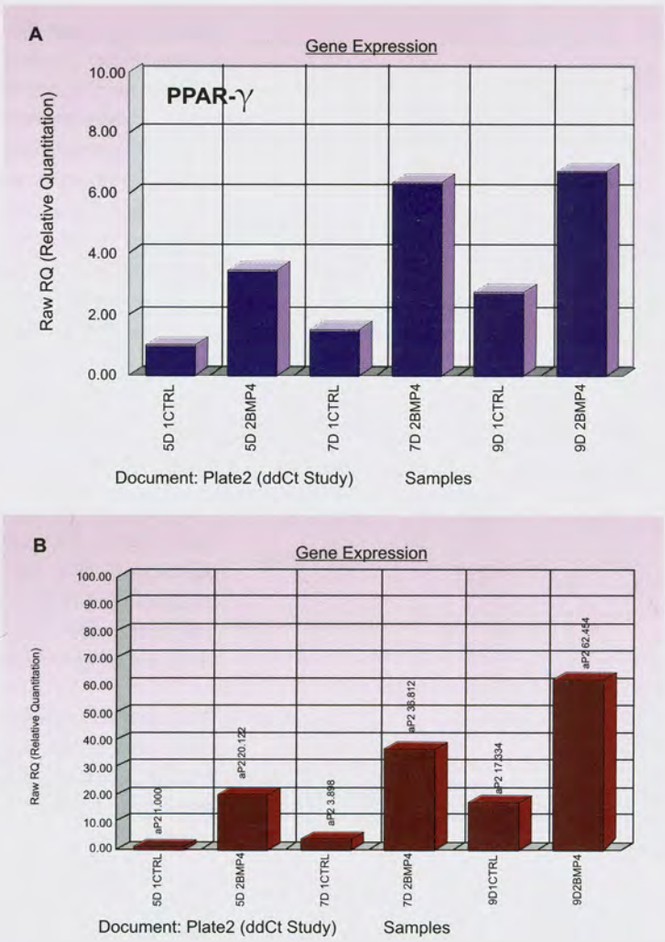
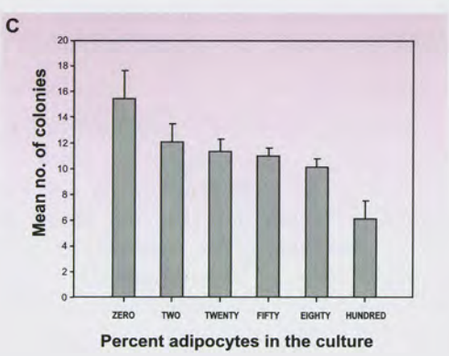


Fig. 2 M210B4 cells were treated with BMP4 or not and the cDNA was prepared on 5th, 7th and 9th day after the treatment. Real time PCR was carried out using PPARγ and aP2 gene-specific primers. A clear up regulation of PPARγ- **(A)** and aP2- **(B)** specific mRNA was seen at all the three time points indicating that the effect of BMP-4 was at transcriptional level. **(C)** An adipocyte gradient was created in the M2 10B4 cells using increasing concentrations of BMP4 and these feeders were irradiated. Mouse MNCs were co-cultured with these feeders. The cultures were maintained by demi-defoliation. The cells were harvested after 2 weeks of co-culture and were subjected to colony formation assay. The number of colonies was found to decrease with an increase in the number of adipocytes in the co-culture suggesting that the adipocytes negatively regulate LTC-type primitive stem/progenitor proliferation in a dose-dependent manner.



Adipocytes generated by the BMP4 treatment negatively regulate hematopoiesis:

Adipocytes have been reported to negatively regulate hematopoiesis, i.e., when the adipocyte number increases, the hematopoiesis decreases and vice versa. On the other hand, they are also reported to maintain quiescence in the hematopoietic stem cells, i.e. they inhibit the HSC differentiation into mature progenitors, thereby maintaining the HSC pool. Cytokines like leptin and adiponectin secreted by the adipocytes are known to have a salutary effect on the HSCs. Thus, the role of adipocytes in the control of hematopoiesis remains equivocal.

We treated M210B4 cells with an increasing concentration of BMP4 and generated a gradient of adipocytes (0, 2, 20, 50, 80, and 100 %). We used this gradient of adipocytes as feeder layers in the co-culture assay to study effect of these adipocytes on the HSC proliferation and hematopoiesis. After irradiation of the feeders, freshly isolated mouse bone marrow MNCs were seeded on them and the cultures were set in the LTC medium. After 10 days of co-culture the cells were harvested and were subjected to the CFU assays. After 14 days, different types of colonies, which represent the different hematopoietic progenitor cells, were scored. As seen in the Fig.2c, we observed a decrease in the CFU colonies formed with an increase in the adipocyte number in the feeder layer. These results showed that the BMP4-induced adipocytes negatively regulated the proliferation of the primitive stem/progenitors. Whether these adipocytes maintain the stem cells in quiescent conditions or not needs to be examined. The data however suggested that a study of pharmacological inhibitors of BMP4-mediated adipogenesis may yield useful information for the reversal of adipogenesis in the in vivo situations and may help in development of therapeutic agents for clinical use.

Study of pharmacological inhibitors for the BMP4- mediated adipogenesis of M210B4 cells:

The process of formation of mature adipocytes from BM MSCs involves two steps; first is the commitment of BM MSCs to adipocyte lineage (pre-adipocytes) and the second is the maturation of adipocytes with the accumulation of lipid droplets. In the present study we have focused our attention on the inhibition of BMP4-mediated "commitment" of BM MSCs to the pre-adipocyte lineage.

We screened an array of pharmacological inhibitors to study whether they can inhibit BMP4-mediated commitment of BM MSCs to the adipocyte lineage. To study this, we treated the cells with BMP4 along with the respective inhibitor only for the initial five days (i.e. the phase of commitment). No inhibitor was added during the treatment with the standard adipogenic differentiation medium and subsequent insulin feedings. Therefore, the observed effect could be attributed to the process of BMP4-mediated commitment of BM MSCs to the adipocyte lineage.

Simvastatin was used in two different concentrations, viz. 0.5μM and 1μM, along with BMP4 during the commitment period and was withdrawn before the addition of the standard differentiation medium. As seen in the Fig.3A, we observed that simvastatin at concentration of 0.5μM and 1μM significantly inhibited the adipocyte commitment process.

Parathyroid Hormone is a well known osteogenic agent and its osteogenic effect is widely studied in vitro as well as in vivo. It is reported that PTH induces the osteogenic differentiation of BM MSCs. BM MSCs differentiate into both

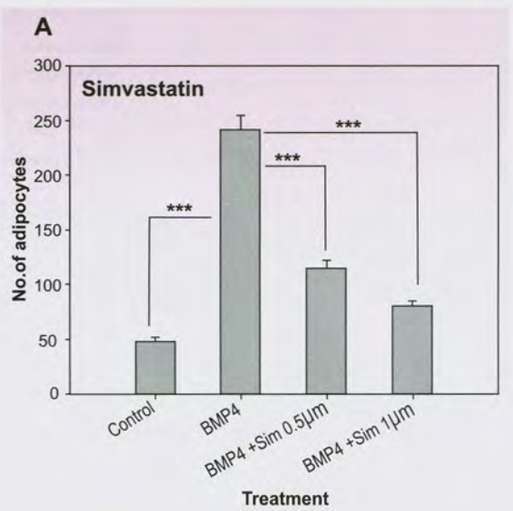
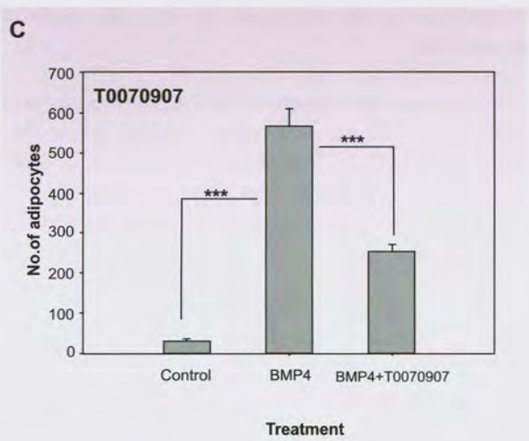
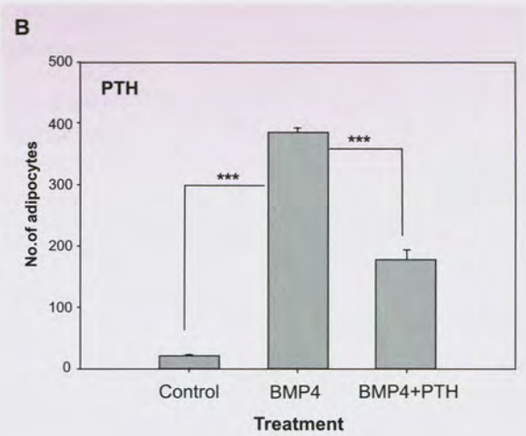


Fig. 3 M210B4 cells were treated with BMP4 in the presence of Simvastatin (0.5μM and 1μM); PTH (0.1μM) or T0070907 (75 μM). The cells were fixed and stained with Oil Red O. A significant reduction in the BMP4-mediated adipogenesis was observed with all these pharmacological agents. (*** p≤0.001)





osteoblasts and adipocytes and these two fates are inversely regulated by expression of Runx2 and PPARγ. Therefore, it is possible that the treatment of BM MSCs with PTH may negatively regulate their BMP4-mediated commitment to the adipocyte lineage so that the osteogenic program can set in. In order to examine this possibility, we treated the cells with PTH (0.1 μM) along with BMP4 during the commitment period and withdrew it before the addition of the standard differentiation medium. As seen in the Fig.3B, we observed that the treatment of cells with BMP4 in the presence of PTH resulted in the inhibition of adipocyte formation as compared to BMP4 alone. This result further suggested that PTH has a dominant negative effect on the BMP4-mediated adipocytic commitment of the BM MSCs.

T0070907 is a PPARγ antagonist. It covalently modifies PPARγ on cysteine 313 in helix 3 of human PPARγ2, inducing conformational changes that block the recruitment of transcriptional cofactors to the PPARγ/RXR heterodimer. Since PPARγ is a master regulator of adipogenic differentiation, we hypothesized that treatment of the cells with T0070907 (0.75 μM) hereafter referred as T00) along with BMP4 may inhibit the BMP4-mediated commitment process. As seen in Fig. 3C, treatment of cells with T0070907 resulted in a significant reduction in the number of adipocytes formed.

These results suggest that pharmacological agents like statins, PTH or T0070907 can be used to inhibit the adipogenic commitment of the marrow-derived stromal cells and perhaps may find application during the chemo or radiotherapy to improve hematopoietic recovery. In vivo studies to examine this possibility are in progress.

Future Work

BMP4 is reported to be secreted by osteoblasts and since we find that it commits the mesenchymal stem cells towards adipocytic lineage, it becomes imperative to identify the stimuli that lead to an increased expression of this molecule. Such studies may lead to the development of a strategy for down regulation of its expression so that the marrow adipogenesis can be controlled.

In the studies carried out so far we have observed that the pharmacological intervention with statins, PTH, T00 etc was able to inhibit the BMP4-mediated adipogenesis, and therefore, we propose to examine whether such approach is also feasible in vivo as well. Such intervention may help in increasing the efficacy of the HSC transplantation by a reduction in the post-myeloablation adipogenesis.

We also propose to generate stromal cell lines stably and constitutively expressing the hematopoietic regulators like BMP4, Wnt5a, Wnt10b, HIF-1α, HIF-2α, eNOS, N-Cadherin, MSX-2, PPAR-gamma and Runx-2 to examine the effect of the signaling mechanisms evoked by these regulators on the HSC fate.

Studies on expansion, cryopreservation and differentiation of hematopoietic stem cells

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Background

In vitro expansion and cryopreservation of hematopoietic stem cells:

Umbilical cord blood (UCB) is a promising source for hematopoietic stem cell transplantations. The limitation of cell dose associated with this source has prompted the ex vivo expansion of stem cells and committed progenitors. However, the expansion procedure is known to exhaust the stem cell compartment causing cellular defects that promote apoptosis and disrupt marrow homing. The role of apoptotic machinery in the regulation of stem cell compartment has been speculated in mouse hematopoietic and embryonic systems. We have consistently observed an increase in apoptosis after cytokine culture of cord blood derived CD34+ cells compared to their freshly isolated counterpart. The present study was done to assess whether pharmacological inhibition of apoptosis could improve the outcome of expansion.

Megakaryocyte generation

In vitro generation of Megakaryocytes (MKs) from hematopoietic stem cells (HSCs) have applications both in basic research, to study the biology of these rare hematopoietic cells and in the clinics, to treat thrombocytopenic patients. We have optimized culture conditions for generation of MKs from cord blood derived CD34+ cells. It was found that serum free medium was better than serum containing medium and out of the various cytokine combinations tested in our study a combination of SCF and TPO emerged out to be the best in our hands. We have also examined the effect of nutraceuticals like Omega-3 i.e. DHA and Omega-6 i.e. AA as a supplement in the serum free medium for generation of megakaryocytes. We found that DHA and AA not only increased the number of megakaryocytes but also improved their functionality which was judged by various morphological, phenotypic and functional assays.

Dendritic cell generation

Advances in last two decades in dendritic cell biology paved way to exploit them as a promising tool in cancer immunotherapy. The prerequisite for DC vaccine preparations is large scale in vitro generations of homogeneous, mature and functional DCs. Frequent improvements are being made in the existing in vitro DC production protocols to achieve this goal. In our previous

study we reported a large scale generation of mature, functional DCs from UCB CD34+ cells. Here we report that this method can be used for the efficient generation of DCs from UCB MNCs and thus the HSC isolation step is not essential.

Aims and Objectives

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

Work Achieved

Expansion of haematopoietic stem cells

Earlier we had shown that the two anti apoptotic compounds viz ZVADFMK and ZLLYFMK enhanced the cytokine mediated expansion of freshly isolated CD34+ cells from the CB samples. To validate the clinical utility of

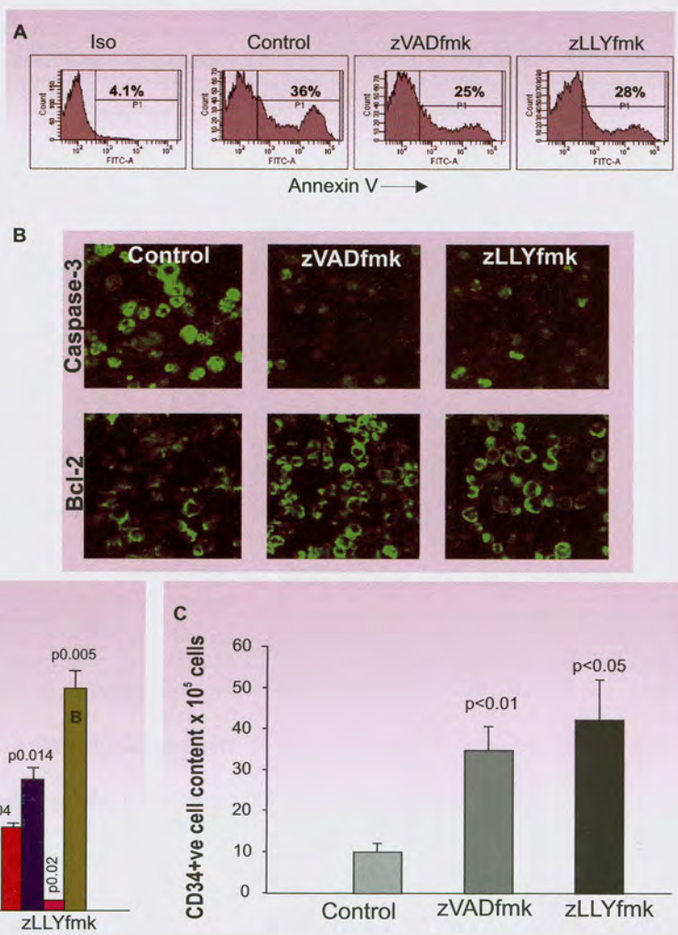


Fig.1. Antiapoptotic agents are beneficial for expansion of frozen CD34+ cells as well: Frozen CD34+ cells were expanded with cytokines in presence or absence of ZVADFMK / ZLLYFMK .There was reduction in annexin positive cells **(A)** and Caspase 3 expression (B) but increase in BCL2 expressing cells **(B)** as well as in CD34 content **(C)** and progenitor cells **(D)** in the sets cultured with the additives.

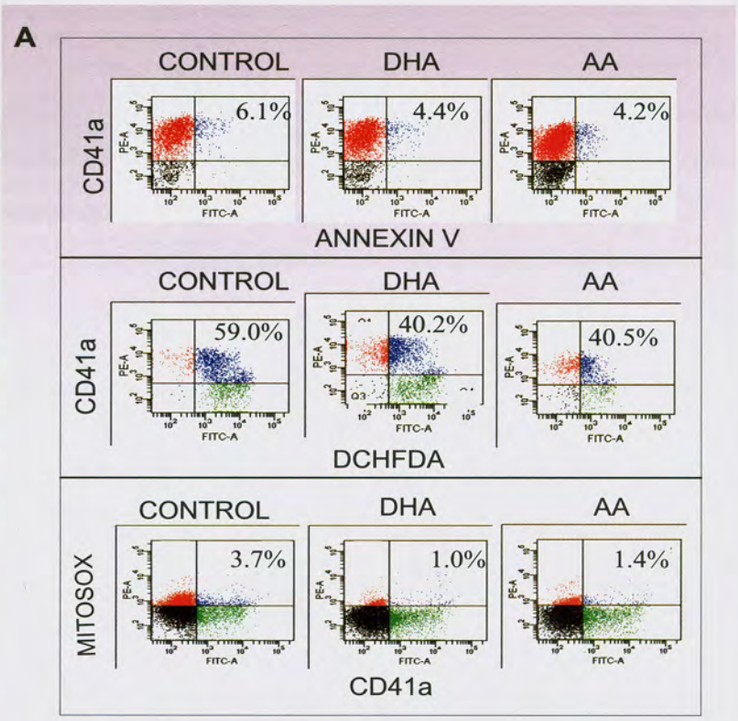
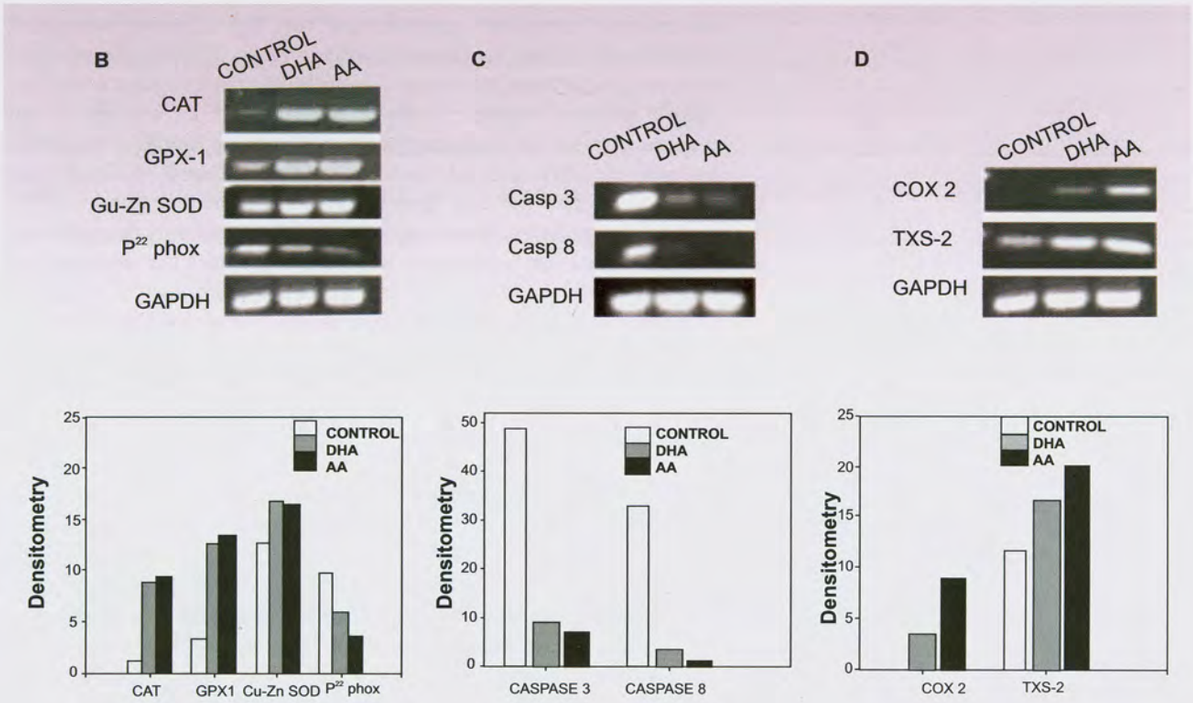


Fig.2. Nutraceuticals exert their beneficial effect by modulating apoptotic and redox pathways: Harvested cells were double stained with CD41a PE / CD41a FITC and Annexin FITC/DCHFDA/ MITOSOX. The percentage of double positive cells (upper right hand quadrant) was estimated on a flow cytometer. It is seen that in cells expanded in presence of nutraceuticals, there is reduction in Apoptosis, Total ROS generation and Mitochondrial ROS levels **(A)** in the test sets . An increase in mRNA levels of antioxidant enzymes **(B)** metabolic enzymes **(D)** And decrease in oxidant **(B)** and apoptotic pathway enzymes **(C)** is seen in test sets in comparison to control.



these compounds, we extended our study to the CD34+ cells which were previously frozen. As shown in Fig. 1A (n=3) the annexin V+ cells was significantly reduced in the CD34+ cells expanded in the presence of either zVADfmk / zLLYfmk compared to the control. There was a down regulation in the active caspase 3 expression with a concomitant upregulation of anti apoptotic bcl-2 (Fig.1B, n=3).The CD34+ cell content increased upto 4 fold in the zVADfmk/zLLYfmk sets compared to the control (Fig.1C, n=5).The clonogenic potential revealed a significantly higher functionality of these expanded cells upon protease inhibition (Fig.1D, n=4). Taken together, our findings reveal that caspase inhibitor zVADfmk and calpain inhibitor zLLYfmk are equally effective in cytokine mediated expansion of fresh as well as frozen thawed CD34+ cells.

Megakaryocyte generation

In the present study we have attempted to delineate the mechanism involved in promotion of megakaryopoiesis by addition of nutraceuticals. There are reports in literature that polyunsaturated fatty acids (PUFAs) modulate apoptotic and oxidative pathways .These processes are also associated with megakaryopoiesis and thrombopoiesis. On similar lines, we studied the levels of apoptosis, total cellular ROS and mitochondrial ROS in the cultured cells by staining them with annexin V, DCHFDA and MITOSOX, respectively. There was reduction in their levels in the CD 41+cells of test sets compared with the control set. The FACS profile of one representative experiment is depicted in Fig. 2 A. We next studied the effect of DHA and AA on gene expression of some of the key enzymes controlling the oxidative stress and apoptotic pathway .This included antioxidant enzymes such as catalase, Glutathione peroxidase-1 (GPx-1), copper- Zinc containing superoxide dismutase (Cu-Zn SOD), oxidant enzyme such as NADPH oxidase, apoptotic pathway enzymes such as caspase 3 and caspase 8. Next we analysed the gene expression levels of metabolic enzymes of DHA and AA such as cyclooxygenase-2 (COX-2) and thromboxane synthase-1(Tx synthase). We found an increase in mRNA levels of antioxidant enzymes and reduction in oxidant enzyme expression (Fig. 2 B) in test sets.There was a decrease in apoptotic pathway enzymes Caspase 3 and 8 (Fig. 2 C) and increased expression of metabolic enzymes (Fig. 2D), in test set in comparison to control. Taken together, the data suggested that the beneficial effect of nutraceuticals in our system may be due to reduction in apoptosis as well as in free radical generation .

DC generation

MNCs or CD34+ cells isolated from the same cord blood samples were used for the generation of DCs by the adherence method developed in our lab. DCs were characterised for morphology, phenotype and functional assays including antigen uptake, chemotaxis, and MLR. As shown in Fig. 3 A the phenotype of DCs from the two sources was comparable.The Dextran FITC antigen uptake (Fig.3B) and migration towards CCL9 (Fig.3C) was also equivalent irrespective of the source. In conclusion , CD34 isolation step can be eliminated to generate DCs from UCB samples.

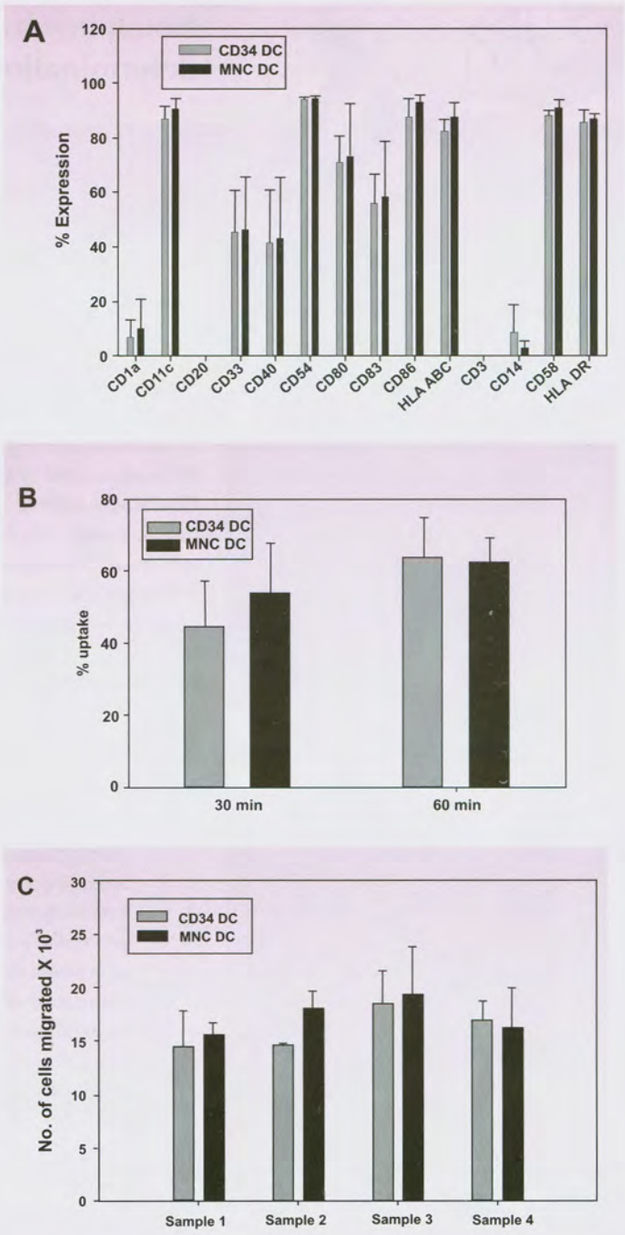


Fig.3. DCs generated from CD34+cells / MNCs of the same UCB sample are comparable in nature : **A.** Surface Phenotype, **B.** Receptor-mediated uptake ; **C.** Chemotaxis toward CCL-19 (n = 3/4).

Future Work

1. Study the engraftment potential of expanded cells in NOD / SCID mice
2. Effect of cryopreservation on expanded MKs and expansion of MKs from frozen HSCs
3. CTL generatin from DCs

Notch involvement during cell fate determination and Neurogenesis

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Background

Notch, a transmembrane protein, involved in cell-cell communication during development influences cell fate determination, proliferation, differentiation and survival. Upon ligand binding it undergoes cleavage by γ-secretase leading to its intracellular domain release that directly enters the nucleus and regulates transcriptional activity of several target genes. However, a little is known regarding its temporal influence during early development. We have used embryonic stem cells (ESCs) as a model system to investigate the differential ligand response and temporal influence of Notch signalling during ESCs maintenance, lineage commitment and differentiation.

Aims and Objectives

The major focus of our group has been,

1. The maintenance of ES cells in undifferentiated state.
2. The differentiation of ES cells into various lineages such as neural, cardiac etc. and understanding the underlying molecular basis of lineage commitment and specification.
3. Establishment of stable transgenic ES cell clones and promoter/enhancer mediated cell trapping to demarcate the cells of interest during differentiation of ES cells and their subsequent characterizations.
4. Manipulation of extrinsic factors for the efficient generation of functional cardiomyocytes, proliferative neural progenitors and differentiated neurons with special reference to the dopaminergic neuronal subtypes from ES cells in vitro.
5. Exploration of the efficacy of in vitro generated cells in cell replacement therapy using animal models.

Work Achieved

To assess the influence of Notch signalling on ESCs, it was inhibited under the +LIF and -LIF conditions. No apparent alteration in cellular morphology and characteristics, rather a marginal increment in cell number was observed with sustained inhibition under the +LIF condition. Conversely, inhibition under the -LIF condition influenced formation of morphologically large, flattened cells with decreased pluripotency markers

expression. Hence, ESCs during maintenance remained impervious to Notch inhibition. Incidentally, undifferentiated ESCs displayed nuclear NICD, which could be attenuated by γ-secretase inhibitor. This default occurrence of Notch signalling in ESCs despite no apparent role in their maintenance led us to investigate its influence on ESCs upon Notch activation. Interestingly, Notch activation resulted in the differentiation of ESCs in a dose dependent manner even when grown in presence of LIF. Cells lost their characteristic morphology accompanied by a significant decrease in the pluripotency associated genes such as Oct4 and Nanog expressions. Thus, ESCs' maintenance entailed an optimum level of Notch signalling; reduction in which did not affect its state, while its augmentation certainly led to differentiation.

Further, expression of early commitment genes was analyzed for determining the differentiation status of ESCs upon Notch activation. While the primitive ectoderm marker, Fgf5, that was negligible in ESCs, showed decreased expression with Dll4, it increased with Jag1 under the +LIF conditions. In fact, an interesting ligand biased lineage commitment and fate switching event between mesoderm and neuroectoderm occurred during Notch induced differentiation in ESCs. While Jag1 promoted neural commitment, Dll4 influenced the mesoderm induction. This differential action of ligands involved a combination of Notch receptors influencing expression of specific downstream target genes. Jag1 induced Notch signalling was deduced to promote neuroectodermal commitment through Hes5; while Dll4 promoted mesoderm induction through Hey1.

Further investigation was performed to assess Notch involvement during neurogenesis by carrying out either Notch activation (Jag1) or inhibition (gSI) at different stages of differentiation. The neural committed populations were identified and quantified based on Sox1 and Nes expressions on 4 and 7days of differentiation respectively. Our earlier study has demonstrated that the Nes+ population attains a peak during one-week time regimen (6-9days) and Sox1+ population by 4days and both diminishing subsequently. Quantifying the Sox1+ population on 4th day of differentiation indicated more number of neural committed populations upon Notch activation than inhibition. However, the reverse was true for Nes+ progenitors when detected on 7th day of differentiation. On 10th day, none of the treatments had significant influence on Nes+ progenitors compared to the control and Sox1 was absent by then. This demonstrated an interesting paradigm as both Notch activation and inhibition were involved in promoting progenitor generation in a temporal and context dependent manner. Accordingly, we used Nes-EGFP ESC clones to quantify simultaneously both early (ENPs) and late neural progenitors (LNPs) under Notch activation and inhibition conditions. These cells upon differentiation revealed populations with varying intensity of EGFP. Both ENPs and LNPs could be discriminated on the basis of their EGFP intensity by flowcytometry. While ENPs expressed low EGFP (range 101-102), LNPs displayed brighter EGFP (range >102). The total EGFP population increased significantly with gSI than Jag1 treatment. As far the ENPs and LNPs were concerned, EGFPlow populations increased with Jag1, while EGFPhigh ones increased in gSI group. Thus, Notch resided at a critical

juncture of neural development where activation promoted commitment, proliferation and maintenance of ENPs while inhibition paved way for their further differentiation into LNPs. The knowledge gained would facilitate chalking out strategies for the enrichment of stage and subtype specific neural populations from ESCs' in vitro for implications in transplantation studies.

Notch involvement during neural progenitor generation led us to investigate further the development of mature neural populations. Accordingly, Notch signalling was inhibited during neurogenesis at different time intervals and the neural maturation was quantified on the basis of MAP2+ neurons and GFAP+ astrocytes populations during 14-16days of differentiation. Notch inhibition at the initial time point attenuated the overall neural differentiation. However, at other stages it specifically promoted neuronal at the expense of glial differentiation. In parallel, neurogenesis was studied by activating Notch along these time intervals and there was no appreciable influence seen on the generation of mature neural populations. However, a concerted action of Notch and CNTF was demonstrated to be involved in promoting gliogenesis from ESCs. The schematic representation of neural hierarchy with stage specific role played by Notch has been represented in Fig. 1. Thus, our investigation has underscored a multifaceted role of Notch demonstrating the interdependency of ligand usage and lineage specification and Notch acting as a master switch during neurogenesis having stage specific influence.

Future Work

The knowledge gained would be utilized further to investigate the temporal influence of Notch during mesoderm commitments and its downstream

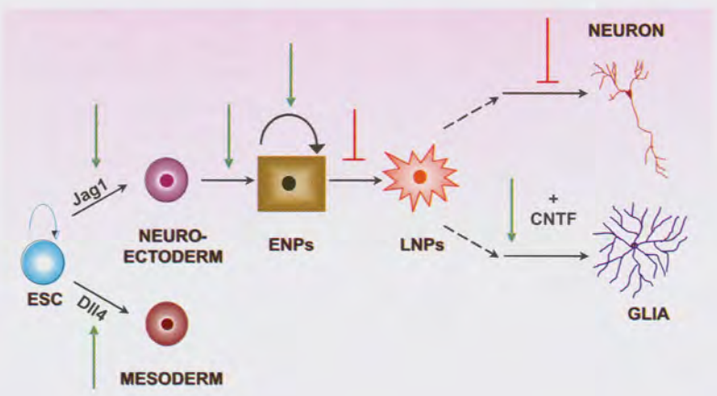

Fig.1 Schematic representation of stage specific Notch involvement (↓ : activation; ⊥ : inhibition) during lineage commitment and neurogenic proceedings from ESCs.

Translational Regulation of Insulin mRNA

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Background

Pancreatic β cells regulate insulin production to control blood glucose levels. These cells contain a large pool of cytoplasmic insulin mRNA (10–15% total mRNA), which is translationally quiescent at hypoglycemic (<3 mM glucose) concentrations. Recruitment to polysomes and activated translation of this mRNA occur in response to higher glucose levels, leading to about 50-fold increase in insulin biosynthesis within an hour. The level of insulin mRNA does not alter significantly during this period of glucose stimulation indicating the predominance of the posttranscriptional events in enhancing insulin biosynthesis. Insulin is a secreted peptide that regulates glucose homeostasis in mammals, and altered insulin production often leads to diabetes. Insulin translation is regulated by its 5'-untranslated region which is encoded by the first two exons of the insulin gene. A mouse insulin2 gene splice variant was reported with increased translation efficiency and is over expressed in diabetic mouse. We have identified a shorter mouse insulin2 splice variant which has a deletion of 12nt from the exon2 with an identical protein coding sequence (mIns2-S). This splice variant is expressed in mouse beta islets and contributes to ~75% of the total insulin2 mRNA. RNA EMSA analysis suggests the formation of a regulated RNA-protein complex with the cytoplasmic extracts. The short isoform forms a less abundant complex when compared to the long form. Reporter gene containing the novel short mIns2-S in the 5'UTR is more efficiently expressed in the cells. Our results show the existence of splice variations in insulin2 gene, which could potentially result in the altered glucose stimulated insulin biosynthesis, thus suggesting another level of regulated insulin biosynthesis.

Aims and Objectives

1. Isolation and characterization of the insulin mRNA UTR Variants.
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.

A novel insulin splice variant is expressed in mouse pancreatic islets

Introns of the mouse insulin2 RNA splice out with different efficiency. The 5' (AG/GU) as well as the 3'-splice-site (AG/CC) sequence of intron1 are not optimal for mammalian splicing. Sequence analysis of insulin2 revealed the presence of a potential 3'-splice-site at 12bp downstream to the known splice-site (Fig. 1A). RT-PCR amplification of the insulin mRNA from islets of BALB/c mice using primers in the exon1 and exon2 resulted in two specific products corresponding to the expected size of the two alternately spliced mRNA (Fig. 1B). Sequence analysis as well as restriction digestion with specific restriction enzyme (BstNI) confirmed that the shorter product (mIns2-S) corresponds to the splice variant that uses the cryptic splice-site and the longer product (mIns2) corresponds to the reported splice product (Fig. 1C). Sequence analysis of the genomic region corresponding to mouse insulin2 gene revealed that there was no alteration in the splice-site consensus sequence at the genomic level. Thus, the expression of mIns2-S is not due to mutations in the genomic DNA, but rather due to alternate splicing. Further, this novel short isoform was also expressed in βTC6 and MIN6 cell lines (mouse insulinoma cell lines).

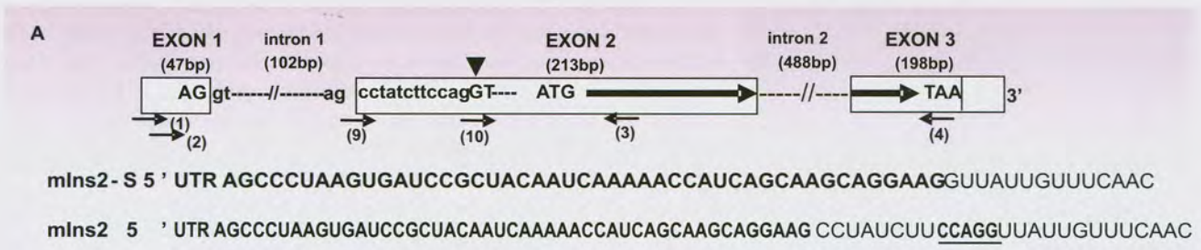
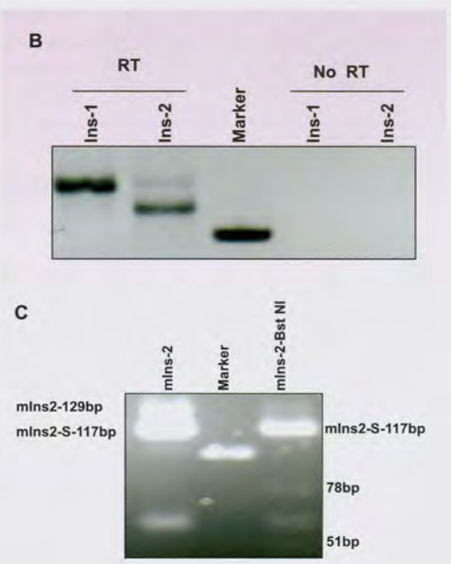
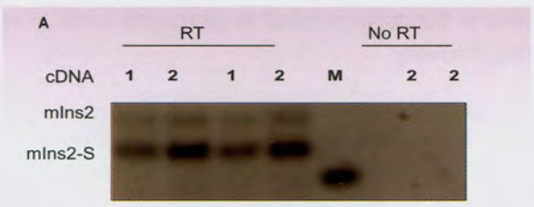


Fig.1 Mouse Insulin2 splice variant in pancreatic islets. **A.** Schematic representation of the mouse proinsulin2 gene. Insulin2 gene consists of three exons (boxes), and two introns, (dotted lines). The translation start site is in exon 2 and the stop codon in exon 3. The 5'UTR includes exon 1 and part of exon 2; the arrow head shows the new 3'splice-site junction. The position of the primers used for RT-PCR is indicated with numbers. **B.** Mouse islet cDNA was used for PCR of insulin1 with primers 5 & 6, and insulin2 with the primers 2 & 3. PCR reactions were resolved on agarose gel and samples are as indicated. **C.** Deletion of 12 bases in the mIns2-S isoforms remove the BstNI recognition sequence (Underlined in A), hence only the mIns2 isoform will be digested and 78bp and 51bp fragments will be released.





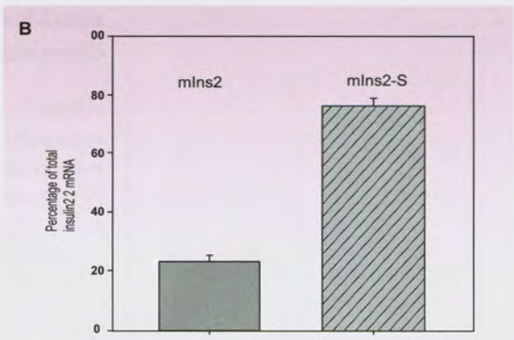
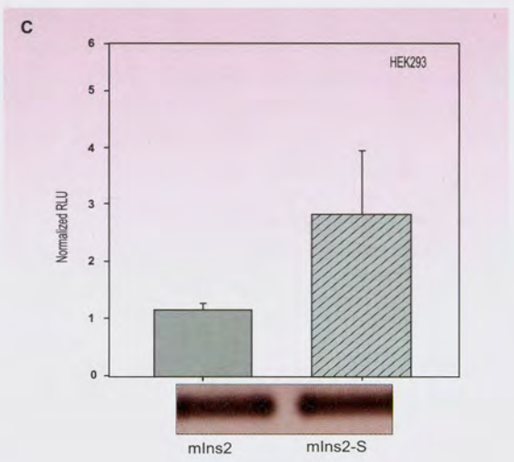
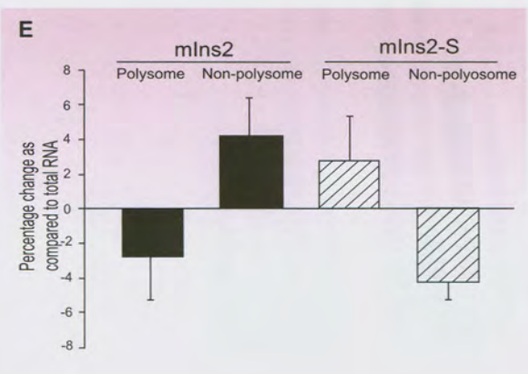
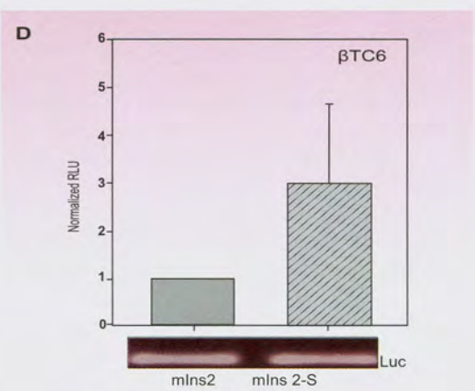


Fig. 2 Quantification of Ins2 isoforms and their translation efficiency. **A.** RT-PCR of both the isoforms was performed in the same reaction using specific primer for two different cDNA preparations, with the amount of cDNA used indicated above the lane. The top and bottom bands correspond to mIns2 and mIns2-S isoforms respectively. **B.** These two bands were quantitated and are presented as percentage of total mouse insulin2 mRNA in pancreatic islets. mIns2 or mIns2-S 5'UTR-luc pCDNA3 were transfected along with Renilla-luciferase plasmid in **C.** HEK293 or **D.** βTC6 cells. Translation of luciferase was normalized with renilla levels and expressed as fraction of translation of the Ins2 5'UTR containing plasmid. The lower panel indicates the amount of luciferase RNA at the end of transfection as detected by RT-PCR. **E.** RNA isolated from the polysome fractions was quantitated for the two splice isoforms of the insulin2 RNA. The results are expressed as percentage change from their expected percentage in the total RNA pool (75% for mIns2-S and 25% for mIns2). The graphs represent the average of three independent experiments and the error bar indicates the standard error of mean.

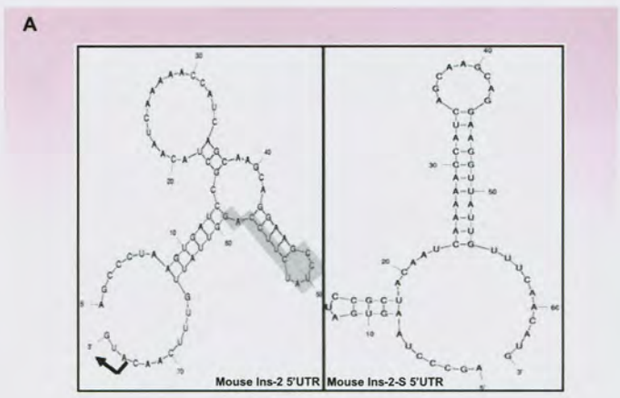






mIns2-S splice variant is the major product in pancreatic islets with differential translation efficiency

mIns2-S splice variant uses the 3'-splice-site that is similar to the consensus mammalian sequence and should be spliced very efficiently. Expression of the two splice isoforms was measured in pancreatic islets using quantitative PCR. Primers that amplify both the splice isoforms were used to perform RT-PCR with varying amounts of the cDNA. mIns2-S splice variant was found to be the predominant form (~75% of total mouse insulin2 mRNA) in pancreatic islets (Fig. 2A and 2B). These results were further confirmed by RT-PCR analysis using isoform specific primers.



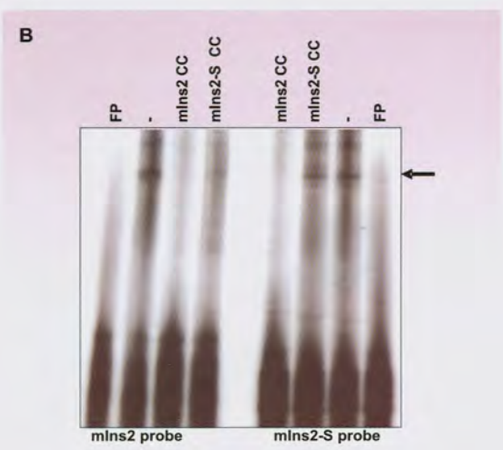


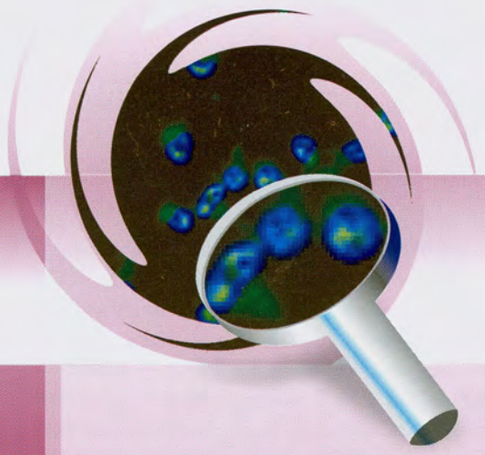
Fig. 3 A. Secondary structure of mIns2 and mIns2-S 5'UTRs. Bent arrows indicate the start of the open reading frame. The shaded area in the left panel corresponds to the 12 nucleotide sequence that is spliced out in mIns2-S. The splice junction is indicated by a straight arrow. **B.** RNA-EMSA using the radio labeled mIns2 and mIns2-S RNA as probes and extracts from HEK293 cells. The specific cold competitor RNA (CC) used is indicated. Arrow indicates the specific RNA-protein complex

We have shown previously that translation of insulin is regulated by the 5'UTR. To analyze the translation efficiency of the mIns2-S and mIns2, HEK 293 and βTC6 cells were transfected with plasmid encoding the firefly luciferase with mIns2-S or mIns2 5'UTR. The relative luciferase activity revealed that the expression level of the mIns2-S isoform is 300% greater than that of the mIns2 isoform without significant change in the RNA levels (Fig. 2C and 2D). These results suggest that the basal translation rate of the mIns2-S isoform is higher compared to the mIns2 isoform. The glucose stimulated translation of these two splice isoforms is not altered in insulin producing βTC6 cells. Actively translating RNA is associated with polysomes and the amount of polysome-associated RNA can be correlated with translation efficiency. Insulin2 RNA splice variants associated with different fractions were measured by quantitative RT-PCR. Association of mIns2-S isoform with polysomes was ~5% higher than what was expected from their abundance in the total RNA, while the mIns2 association with polysome is ~7% lower (Fig. 2E). These results are consistent with the in vivo translation assay.

Translation of specific mRNA is often controlled by its cis-acting elements with specific secondary structure in the 5'UTR. The loss of 12 bases at the 5'UTR results in dramatic alteration to the predicted secondary structure of the 5'UTR (Fig. 3A), which could probably result in loss/gain of recognition by specific cellular factors that may contribute to the differential translation of the splice isoforms. Binding of specific factors to the 5'UTR of insulin2 RNA was analyzed using RNA-EMSA. A specific RNA-protein complex was detected using either of the splice variant UTR as probes (Fig. 3B). In competitive EMSA, mIns2 was able to compete very effectively as compared to the mIns2-S, suggesting a higher binding affinity/stability of the mIns2 complex.

Future Work

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



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

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Background

In the bone microenvironment, interactions between T cells, osteoclasts, and osteoblasts play crucial roles in determining bone homeostasis. Important bone diseases such as osteoporosis, rheumatoid arthritis, Paget's disease and bone metastasis of breast and prostate cancers are caused by increased osteoclast number and activity. Osteoclasts, the multinucleated cells, differentiate from hemopoietic precursors of monocyte/macrophage lineage. The differentiation and activation of osteoclasts are under the aegis of a variety of cytokines secreted by activated T lymphocytes. 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 fully delineated. In previous studies we have shown that IL-3 irreversibly inhibits human osteoclast differentiation. We also demonstrated that IL-3 inhibits human osteoclast differentiation by inhibiting expression of c-Fms on osteoclast precursors. In further studies we characterized the cells in presence of IL-3 and also investigated the role of IL-3 on osteoclast differentiation from hemopoietic precursors of osteoporotic individuals.

Aims and Objectives

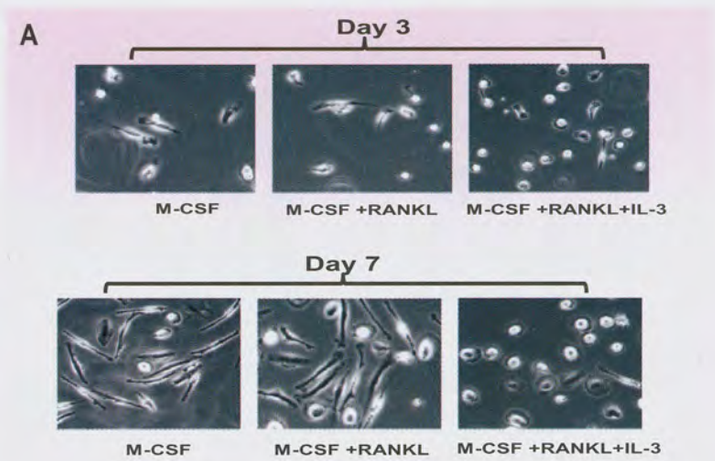
1. To elucidate the mechanism(s) of IL-3 action on human osteoclast differentiation and bone resorption.
2. To investigate the role of IL-3 in regulation of human osteoclast differentiation and activation from hemopoietic precursors of osteoporotic patients.

Work Achieved

Characterization of cells induced by IL-3

Since inhibitory effect of IL-3 on bone resorption was irreversible, and IL-3 treated cells showed expression of RANK we characterized the phenotype of these cells. We found no difference in morphology of cells treated with or without IL-3 on day 3 (Fig. 1A). However, on day 7 cells incubated with M-CSF and RANKL were mononuclear and elongated in appearance and multi-nucleation of osteoclast precursors was not seen, whereas IL-3 treated cells were mononuclear and round in shape. These cells were further characterized for macrophage/dendritic cell antigens such as

CD205, CD86, HLA-DR, CD1a, CD80, CD11c, CD14, CD83 and CD44. Cells treated with IL-3 for 7 days showed increased expression of CD1a, and CD80, whereas no effect on expression of CD11c, CD86, CD44, and HLA-DR was seen (Fig. 1B). Interestingly, IL-3 significantly down-regulated the expression of macrophage/monocyte marker CD14. Thus, increased expression of dendritic cells markers and low expression of macrophage specific marker in IL-3 treated population suggests that IL-3 diverts the cells towards dendritic cell lineage. Expression of CD205 and CD83 was absent suggesting that cells are of immature dendritic cell phenotype.



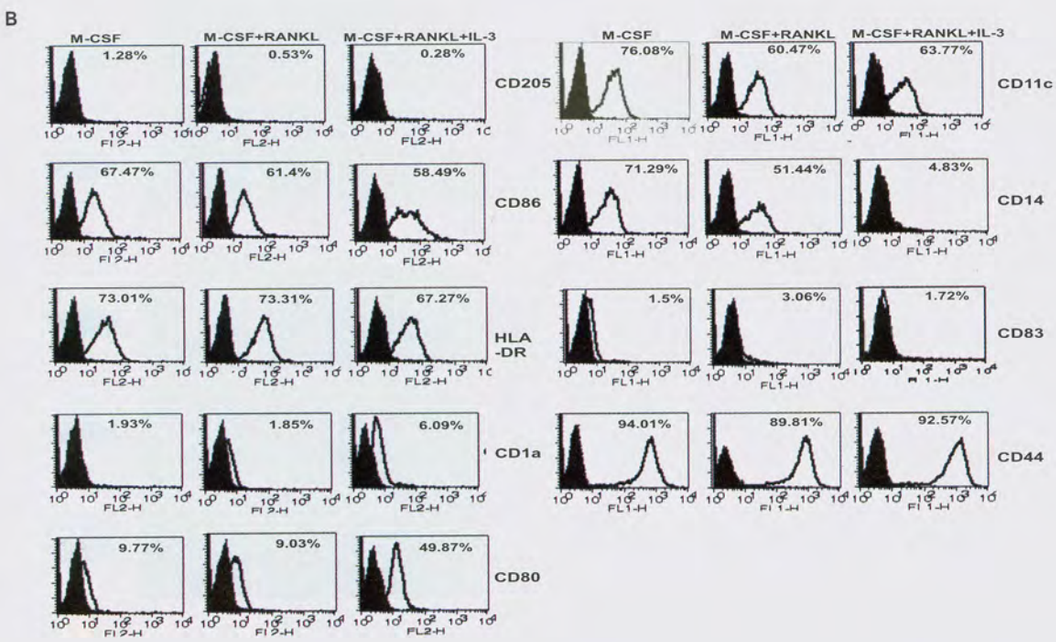


Fig. 1. Characterization of cells treated with IL-3. Osteoclast precursors were incubated for 3 and 7 days with M-CSF and RANKL in the absence or presence of IL-3 (100 ng/ml). **(A)** Phase contrast images of cells treated with IL-3. Magnification, X 10. **(B)** FACS analysis for cell surface expression of various dendritic and macrophage markers on day 7. Results are representatives of 3 individual experiments using different donors.

Effect of IL-3 on transcription factors involved in osteoclast differentiation

Since c-Fms and CD14 were down-regulated in IL-3 treated cells and PU.1 is involved in the transcription of these genes, we next analyzed the effect of IL-3 on early transcription factors PU.1 and c-Fos involved in osteoclastogenesis. The osteoclast precursors were incubated with M-CSF with or without RANKL in the absence or presence of IL-3, and expression of PU.1 and c-Fos was analyzed on days 3 and 7 by quantitative real-time PCR. IL-3 showed no significant effect on PU.1 and c-Fos expression on day 3. However, IL-3 down-regulated expression of both c-Fos and PU.1 on

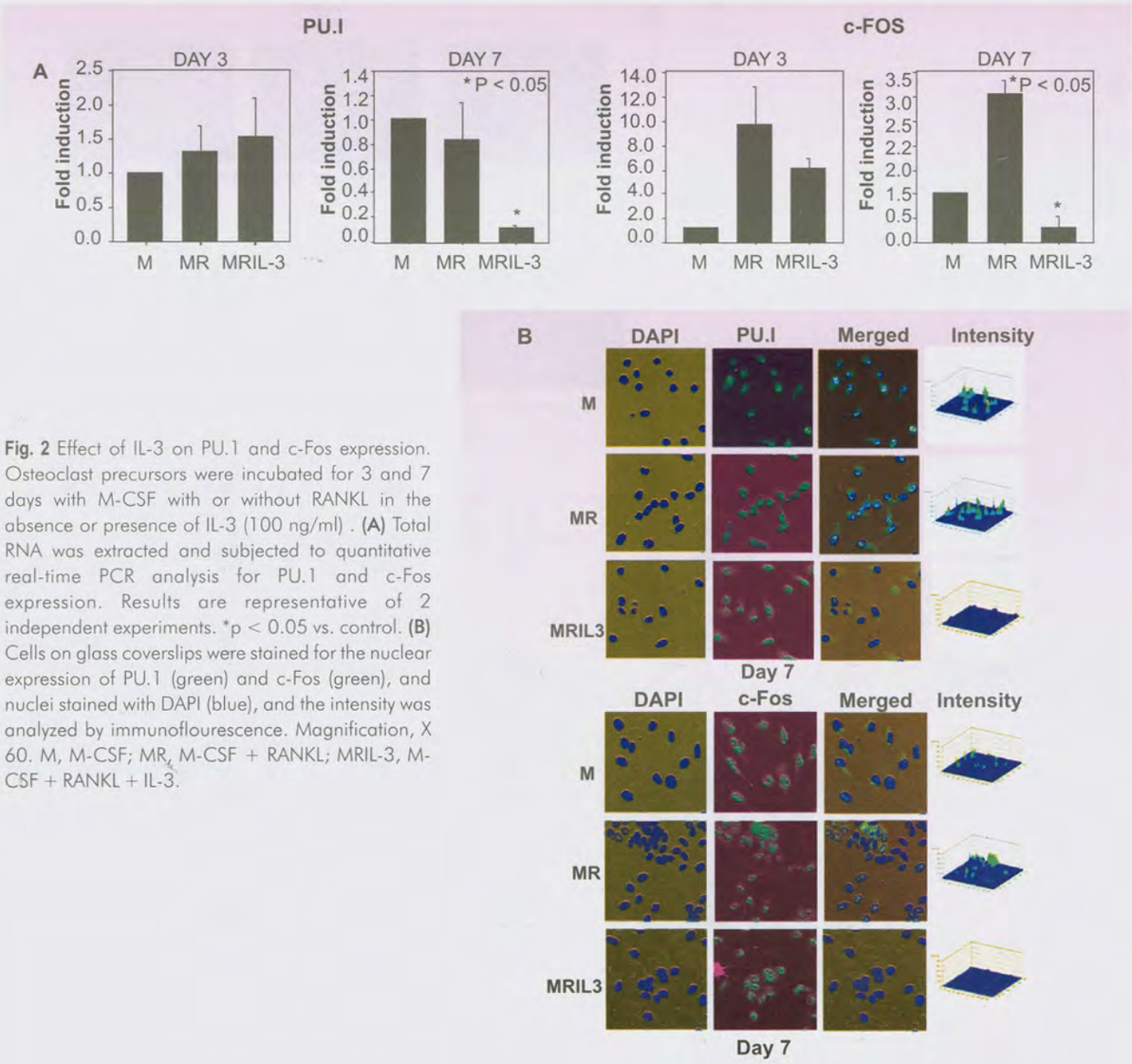


Fig. 2 Effect of IL-3 on PU.1 and c-Fos expression. Osteoclast precursors were incubated for 3 and 7 days with M-CSF with or without RANKL in the absence or presence of IL-3 (100 ng/ml) . **(A)** Total RNA was extracted and subjected to quantitative real-time PCR analysis for PU.1 and c-Fos expression. Results are representative of 2 independent experiments. *p < 0.05 vs. control. **(B)** Cells on glass coverslips were stained for the nuclear expression of PU.1 (green) and c-Fos (green), and nuclei stained with DAPI (blue), and the intensity was analyzed by immunoflourescence. Magnification, X 60. M, M-CSF; MR, M-CSF + RANKL; MRIL-3, M-CSF + RANKL + IL-3.

day 7 (Fig. 2A). Effect of IL-3 on nuclear expression of c-Fos and PU.1 was further analyzed by immunoflourescence on day 7. IL-3 down-regulated

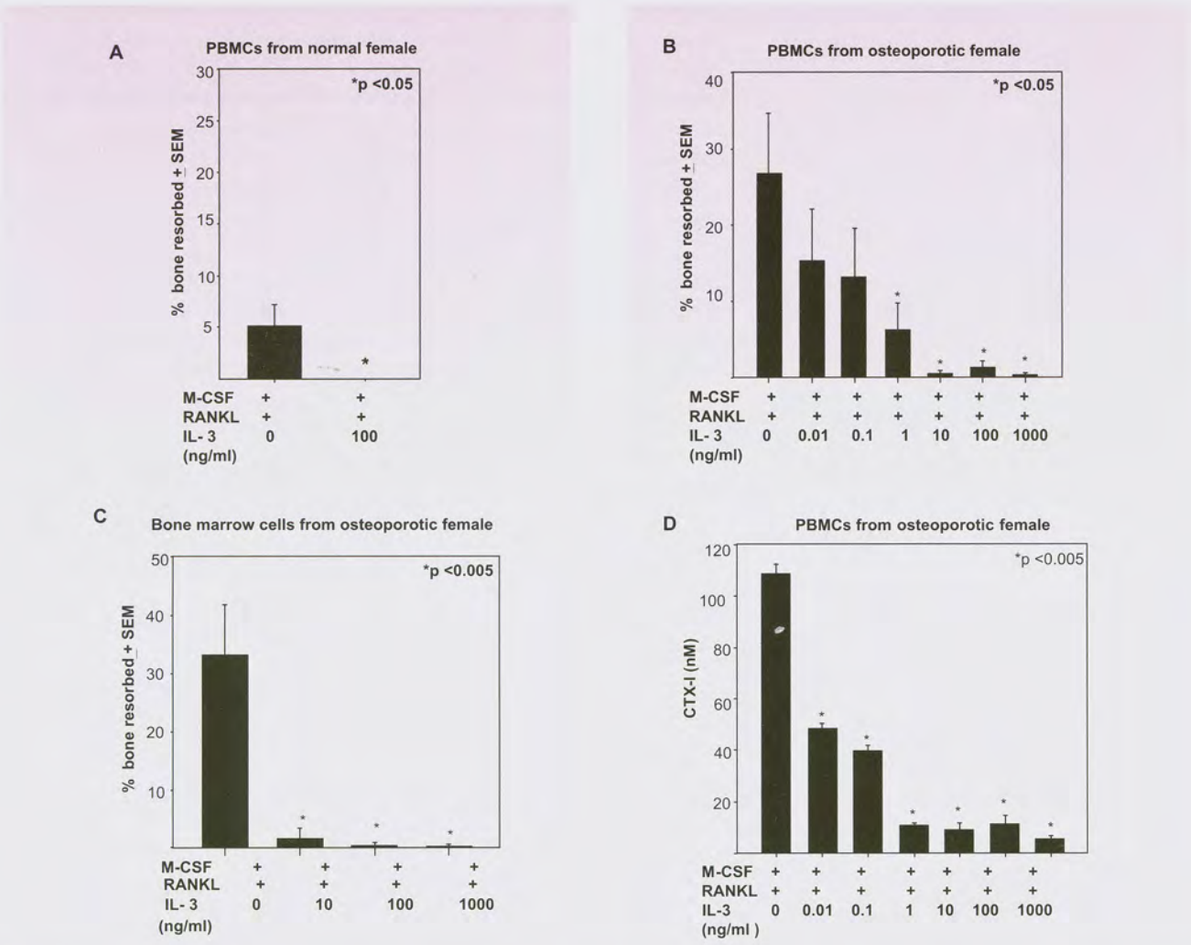
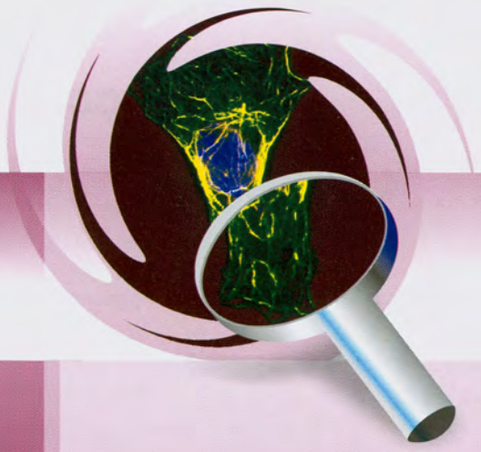


Fig. 3. IL-3 dose-dependently inhibits bone resorption and CTX-I in osteoclast precursors derived from osteoporotic patients. **(A)** Osteoclast precursors from normal females were incubated on bone slices with M-CSF and RANKL in the absence or presence of IL-3 (100 ng/ml). At day 21 bone resorption was quantified. Results are expressed as mean + SEM of 8 cultures per variable. *$p < 0.05$ vs. control. **(B)** Osteoclast precursors (5 x 10^5cells/well) from postmenopausal osteoporotic females were incubated on bone slices with M-CSF and RANKL in the absence or presence of different concentrations of IL-3. At day 21 bone resorption was quantified. Results are expressed as mean + SEM of 8 cultures per variable. * $p < 0.05$ vs. control using different donors. **(C)** Non-adherent bone marrow cells from osteoporotic female patient were plated on bone slices (2 x 10^5 cells/well) with M-CSF and RANKL in the absence or presence of IL-3. Results are expressed as mean + SEM of 6 cultures per variable.* $p < 0.005$ vs. control. **(D)** Collagen degradation product, CTX-I released in osteoporotic female PBMCs culture supernatants was estimated by CrossLaps ELISA on day 21. * $p < 0.005$ vs. control.

Osteoclast precursors obtained from normal and post-menopausal osteoporotic females were used to analyze the effect of IL-3 on bone resorption. Few bone resorption pits were observed in M-CSF and RANKL (~ 5%) treated cells from normal females, and no bone resorption was seen in IL-3 treated cells (Fig. 3A). Monocytes obtained from normal females do not give very high bone resorption values as compared to males or post menopausal osteoporotic females because of increased secretion of TNF-α and IL-1 by monocytes of postmenopausal osteoporotic females.In osteoclast precursors of post-menopausal osteoporotic females there was about 35% bone resorption in control, and IL-3 dose-dependently inhibited bone resorption, and significant inhibition of bone resorption was seen from 1 ng/ml (Fig. 3B). We also evaluated the effect of IL-3 on bone resorption using osteoclast precursors isolated from bone marrow of osteoporotic females. In control cultures there was about 45% bone resorption and, IL-3 dose-dependently inhibited bone resorption in this culture system also and significant effect was seen from 10 ng/ml (Fig. 3C). This inhibitory effect of IL-3 on bone resorption was further confirmed by assessing its effect on clinical marker of bone resorption, CTX-I. IL-3 decreases CTX-I levels in a dose-dependent manner in osteoporotic PBMCs and significant effect was seen even at 0.01 ng/ml (Fig. 4D). All these results suggest that IL-3 is a potent inhibitor of bone resorption in clinical conditions also, and may serve as a potential antiosteolytic agent to treat osteoporosis and other diseases of skeletal system.

Future Work

In future studies we plan to investigate the role of IL-3 on human osteoblast differentiation of mesenchymal stem cells.



Non-canonical Wnt signaling regulates directional cell migration through Nup358 and Dvl

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Background

Establishment of molecular asymmetry is the fundamental principle governing cell polarity, a process that is instrumental in epithelial cell organization, directed cell migration, asymmetric zygotic and stem cell divisions, and neuronal differentiation. The most striking aspect of polarity is the revelation that the same set of evolutionarily conserved proteins is being used in bringing about molecular asymmetry in diverse cellular contexts. For example, Par proteins, which were discovered in 1988 during a screen for Caenorhabditis elegans mutants that failed to undergo asymmetric zygotic divisions, are key players in epithelial, neuronal and front-rear polarity as well. In other words, it appears that variations of a common mechanistic theme result in unique shapes, functions and asymmetries that characterize polarized cells. Given the importance of cell polarization in almost all aspects of growth, development and homeostasis of metazoans, it is not surprising that many developmental disorders and diseases are caused due to defective functioning of this process . Moreover, many polarity proteins are also identified to be tumour suppressors. However, the molecular insight into the functioning of the polarity proteins is rather limiting.

Many signaling pathways have been shown to modulate the activity of Par polarity complex (Par3, Par6 and atypical protein kinase C–aPKC. Recently, non-canonical Wnt signaling is implicated to have a critical role in activating Par complex and regulating front-rear polarity of fibroblasts and neuronal polarity of cultured hippocampal neurons . The pathway involves Dishevelled (Dvl) and adenomatous polyposis coli (APC), and the current model suggests a role for Dvl in binding to Par complex and activating aPKC.

Our recent studies demonstrated the involvement of a nucleoporin (Nup358) in cell polarity. Nup358 is a large protein that resides on the cytoplasmic face of the nuclear pore complex and has been implicated to have a role in mRNA export in Drosophila and mammalian cells. We showed that Nup358 interacts with APC and is required to establish front-rear polarity during directed cell migration, at least in part, by regulating the localization of APC to the leading edge Molecular details of Nup358 function in cell polarization will be investigated by focusing on the following objectives.

Aims and Objectives

1. To characterize the interactions of Nup358 with components of Wnt5a/Dvl polarity signaling
2. To understand the molecular details of how Nup358 functions in Wnt5a/Dvl polarity signaling.

Work Achieved

To assess the involvement of Nup358 in Wnt5a mediated polarization, NIH3T3 cells were subjected to control or Nup358 RNAi and assayed for centrosome polarization (Fig. 1). The results clearly show that Nup358 plays a role in Wnt5a polarity pathway.

Further we tested if Nup358 interacts with other components of Wnt signaling. Using immunoprecipitation assays, we found that Nup358 interacts with Dvl, through its N-terminal leucine-rich-region. Further analysis suggested that the interaction required the middle PDZ domain of Dvl. Moreover, we identified a novel PDZ-dependent interaction between the N-terminal region of APC and Dvl. Consistent with the in vivo physical interaction data, transfection of the N-terminal region of Nup358 (BPN) showed co-localization with ectopically expressed Dvl2 (Fig. 3). The domain structures of Nup358 and Dvl are depicted (Fig. 2).

Put together, these data suggest that Nup358 plays a role in Wnt5a-mediated cell polarization and interacts with down stream players of Wnt signaling such as Dvl and APC. Future investigation would test the hypothesis that Nup358, Dvl and APC may cooperate to mediate non-canonical Wnt-mediated front-rear polarity during directed cell migration.

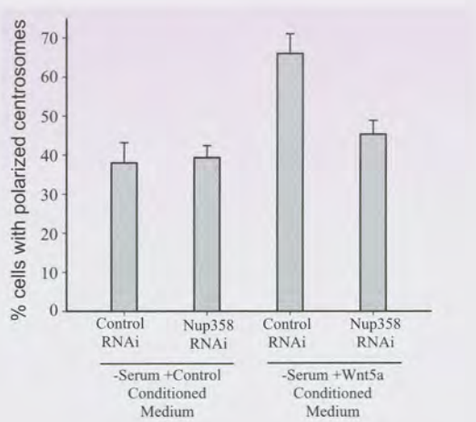

Fig.1 Wnt5a-mediated polarization during cell migration requires Nup358. Control or Nup358 siRNA treated NIH3T3 cells were serum starved, scratches were made using pipette tip and treated with Control conditioned (no serum) or Wnt5a conditioned medium. Cells were fixed and analyzed for the orientation of centrosomes using mouse anti-g-tubulin antibody. Polarization was scored positive if the centrosomes were present in a 120° sector in front of the nuclei of those cells migrating towards the wound. The results are representative of three independent experiments. Error bars indicate standard deviations.

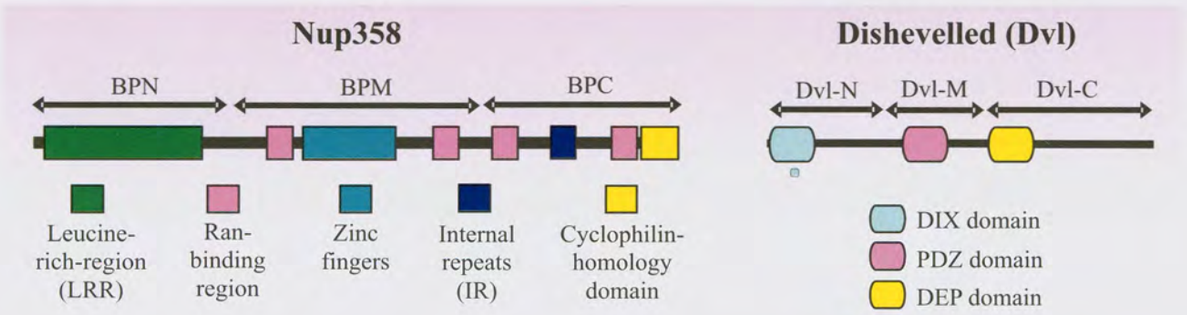

Fig. 2 Domain structures of Nup358 and Dvl proteins. Double-edged arrows indicate the fragments used in this study

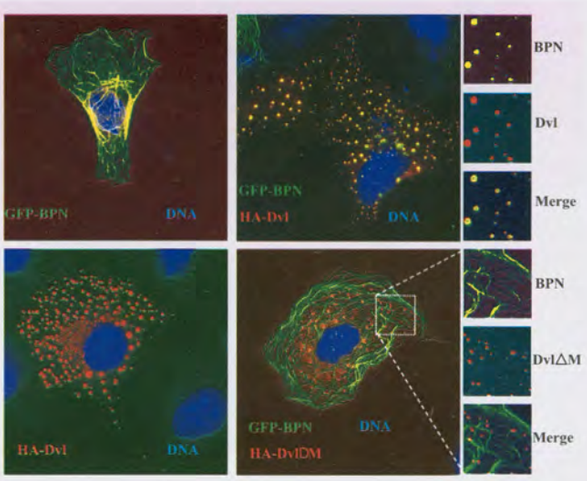

Fig. 3 BPN requires the middle region of Dvl for co-localization. COS-7 cells transfected with either GFP-BPN alone (top-left, green), HA-Dvl alone (bottom-left, red) or double transfected with GFP-BPN and HA-Dvl (top-right) or HA-Dvl DM (bottom-right) and analyzed for their localization by microscopy. DNA is stained in blue. Note that upon co-transfection with Dvl but not with DvlDM, GFP-BPN loses the microtubule association and gets recruited to Dvl puncta.

Future Work

1. Investigate the functional relevance of interaction between Nup358 and Dvl
2. Understand the molecular mechanism by which Nup358 functions in non-canonical Wnt signaling

Research Reports

Cancer Biology

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Gene Expression: Protein Interaction Systems Network Modeling Identifies Transformation-Associated Molecules and Pathways in Ovarian Cancer

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Background

Therapeutic decisions in oncology are based on correlations between tumor characteristics and possibility of disease relapse. Limitations of such approaches however, are now leading to the development of therapies considering individual-specific genetic defects. The resolution of breast cancer into four molecular gene expression-based classes represents a successful outcome of such approaches. Besides suggesting distinct cell origins, these classes correlate well with histological grading and clinical characteristics. Over the last decade, ovarian cancer is realized to represent a group of histologically distinct diseases that correlate with different origins; hence require appropriation with individual 'molecular signatures'. Gene-expression profiling based identification of prognostic and/or predictive biomarkers towards improving disease and therapy risk assessment along with a mechanistic understanding of gene interactions in pathways, networks and/or complexes is desirable to unravel the biological behavior of tumors. Unfortunately, most studies are restricted by limited sample size and a minimal commonality of genes between different analyses. We present here, an alternative analysis of gene expression data to extract a "signature" of commonly modulated genes derived from three independent datasets of serous ovarian carcinoma. Further, resolution of expression-based interaction networks of these genes and known protein-protein interactions confirmed existing markers with predictive/prognostic value, and 'discovered' others. More importantly, the predicted network based interactions and known functionality of the identified gene set provide novel insights towards a mechanistic understanding of cellular transformation processes.

Aims and objectives

1. To identify and validate the gene signatures in serous ovarian adenocarcinoma (SeOvCa).
2. To identify the different nature of associations between these SeOvCa genes.
3. Deciphering the epigenetic regulation governing the expression of identified SeOvCa gene signature through genome wide screening.
4. To identify protein interaction networks of the SeOvCa gene signature.

Work Achieved

Derivation and validation of a serous ovarian adenocarcinoma gene signature

Genes likely to be important in serous ovarian carcinoma were identified using three differential gene expression datasets– (i) A4 *in vitro* ovarian carcinoma progression cell model established earlier from a patient presenting with advanced Grade IV serous adenocarcinoma that presents a pliable cell system for validating top-down data-driven analyses, (ii) TCGA gene expression database for ovarian serous cystadenocarcinomas, and (iii) individual gene profiling in the IST serous adenocarcinoma database. Analyses of these datasets led to the derivation of a prioritised list of 30 commonly regulated genes that we together termed the SeOvCa signature. SeOvCa also validated in an independent expression dataset (GSE3149), associated with different grades of ovarian tumors. Curating published data from research literature relating to SeOvCa genes further revealed the association of 11 genes with ovarian cancer (MAL, MCM2, MMP9, RRM2, SOX17, SYNCRIP, DAB2, FBN1, HNMT, KLF2, SMARCA2), 12 with cellular transformation events in other cancers (ATAD2, BCAT1, CDCA4, EXO1, LAMA5, MEST, SLC39A4, FEMP1, LHFP, SGK1, PAPSS2, PTGIS), and 7 with no pre-identified association with cancer (Discovery group-TM7SF2, TNNT1, DIXDC1, GNB5, LRRC17,PROS1,RNASE4).

Systems Networks Prediction of SeOvCa Interactions from Gene Expression Data

Associations between SeOvCa genes were identified using a previously described algorithm – ARACNe to the TCGA microarray datasets. ARACNe identifies statistically significant gene-gene co-regulation by mutual information, an information-theoretic measure of relatedness that integrates data processing inequality to eliminate indirect relationships. The final reconstructed network effectively removes bias from partially known functional similarities between genes to reveal relationships with highest probability of direct interactions. This allows independent prediction of regulatory phenomena within a defined context even for genes without pre-established functionalities. Interactions were identified at three levels –

1. **Node-Node interactions**- Strikingly, these were restricted to downregulated nodes (Fig.1A), indicating repression to be more co-regulated than activation.
2. **Node-Linker-Node interactions** involving 23 nodes and 99 connecting linker genes that generate a network (Fig.1B). Node-linker networks can identify co-regulatory modules, an example is the FBN1-LHFP node-node interaction supported by a large number of linkers, which is thus predicted as being strongly co-regulated than with either PAPSS2 or LRRC17 (Fig.1A).

3. **Node-interactors networks** that encompass all possible interactions in the gene expression data. The resulting network has three types of nodes (Fig.1C) - (i) Isolated Nodes (MAL,GNB5,TM7SF2) that do not associate with any gene in the dataset, (ii) Stand-alone Nodes (SLC39A4,MEST,LAMA5,MMP9) that though distanced from the main network, maintain their influence on specific genes to form isolated or "Stand-alone" hubs, (iii) Social nodes – A majority of SeOvCa genes interact with other nodes, linkers and exclusive interacting genes (interactors) to generate a complex network in which up- and down-regulated nodes segregate distinctly. Edge analysis within this network revealed mutual exclusivity with positively correlating node-partner gene interactions being more frequent than negative correlations.

SeOvCa Protein Interactions

We further scanned the Human Protein Atlas for SeOvCa protein expression and identified positive correlation in ovarian cancer of ATAD2, MCM2, SYNCRIP, TNNT1, FBN1, PROS1, PTGIS, RNASE4, marginal correlation of MEST, DAB2, SGK1; while MMP9 was an outlier. Literature search additionally supported the involvement of MAL, HNMT, SMARCA2 proteins in ovarian cancer. Further exploration of known PINA Networks led to the delineation of three types of protein-protein interactions between SeOvCa proteins -

1. **Node-Node PPIs** between MCM2 and GNB5.
2. **Node-Linker-Node PPIs** - 12 nodes and 15 connecting linkers generate a network analogous to the ARACNe generated Interface Network.
3. **Node-interactors networks** involve all possible interactions within SeOvCa to identify - (i) Independent Proteins with no known

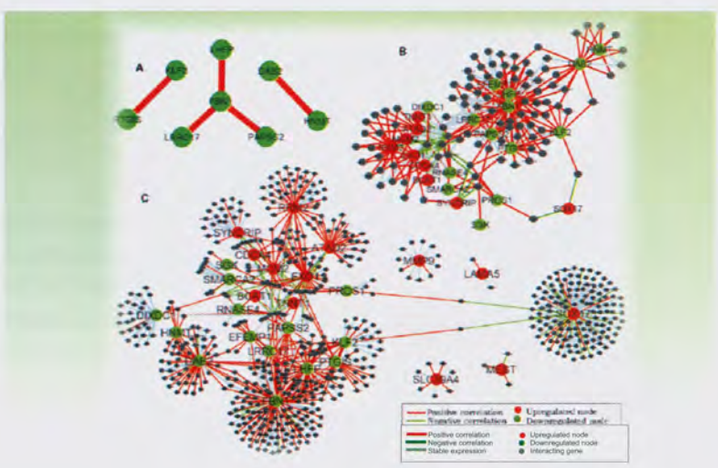


Fig. 1 ARACNe generated SeOvCa interaction networks for **(A)** node-node, **(B)** node-linker-node, and **(C)** node-interacting gene interactions. Red and green circles represent upregulated and downregulated nodes respectively; dark gray nodes, interacting partners predicted by ARACNe. Red and green edges, positively and negatively correlating interacting partners; light blue edges, interacting partners that maintain a stable expression.

interactions (MAL, SLC39A4, EFEMP1, HNMT, LHFP, LRRC17, PTGIS, RNASE4), (ii) Stand-alone Protein Network hubs (BCAT1, CDCA4, MEST, SOX17, TM7SF2, KLF2, PROS1, PAPSS2) that maintain their influence on specific genes to form isolated hubs, (iii) Social nodes – SeOvCa proteins that have exclusive PPIs with other nodes or indirect ones through linkers to generate a complex network.

Integration of Interaction Networks suggests c-Myc transformation supported by altered p53 and retinoblastoma (Rb) signaling

Differences between PINA generated networks and the contemporary ones in ARACNe exist due to comparisons at different levels of gene regulation i.e., experimentally established PPIs vs. gene expression profile based interactions. Thus, molecules common to both networks (6 nodes,9 interactors) would be strongly associated with ovarian cancer (Fig.2A). The MCM2 hub (CDC7, MCM3, MCM4, MCM7) involved early in DNA replication, cell cycle progression, p53 inactivation and interacts with the RRM2 hub to link DNA mismatch repair and replication. Significantly, Cdc7 kinase is a predictive marker in ovarian cancer, maintains cell viability during replication stress and is required for loading the MCM2-MCM7 complex onto chromatin. FBN1(DCN) and DAB2(TGFBR2) together with another SeOvCa gene SGK1 are linked to TGFβ signaling and are critical in controlling its apoptotic effects, microsatellite instability and DNA mismatch repair. All these effects are highly probable in the current situation wherein we identified extended networks and functional pathways of each hub. The information along with published SeOvCa associations, led us to derive a regulatory network involving altered retinoblastoma (Rb) signaling, c-Myc activation and p53/cell cycle/DNA damage repair pathways (Figs.2B).

RB pathway alterations primarily involve inactivation of its function in senescence that depends on the transient recruitment of SMARCA2 into RB/HDAC1 mega-complexes. SMARCA2 downregulation abrogates G1-S growth arrest through modulation of E2F, Cdk4/6–cyclin D, Cdk2-cyclin E and Cip/Kip/Ink4a which are downstream effectors of Rb. Such evasion of stasis/senescence barriers may be considered as a first step towards immortalization, a recognized hallmark of cancer. ATAD2 is a physiologic target of pRB/E2F and functions as a coactivator for the transcription factors ERα, AR and c-myc by recruiting CBP to target E2F1,Cyclin D1,c-MYC and BIRC5 (survivin) through a positive feedback loop. SGK1 (AR target) downregulation suggests irrelevance of AR signaling in ovarian cancer. 8q chromosomal region amplifications of ATAD2 and c-Myc, together with BCAT1 (c–myc target) upregulation contribute to tumor development and progression. SYNCRIP associates with IGF-II mRNA binding protein 1 (IGF2BP1) to limit the transfer translation-coupled decay of c-myc RNA that enhances its stability. The PI3K mediated interaction of c-myc with the pre-replicative minichromosome maintenance complex MCM2-MCM7 leads to its localization to early sites of DNA synthesis and

replication initiation. MYCN activation by E2F1 enhances transcription of MCM2-MCM7 members and downregulate p27 that together with BMI1 further mediates stem cell self-renewal.

Myc deregulation generates DNA damage, replication stress and genomic instability through inactivation of the p53-mediated DNA damage response (DDR) involving ATM-ATR-CHK1-CHK2 checkpoints. CDCA4, an E2F1 target regulates E2F and p53 transcription, cellular proliferation and cell fate determination. ATR phosphorylates MCM2 resulting in aberrant loading of the MCM complex onto chromatin and cell cycle progression. EXO1 mediates DNA mismatch repair, suppresses replication fork instability and enhances resistance to DNA-damaging agents. RRM2, another downstream target of the p53-ATM-ATR-CHK1 axis mediates DNA repair and co-operates with MCM2 towards cell proliferation. RRM2 also enhances invasion through NF-kappaB-dependent MMP-9 activation, and angiogenesis through decreased thrombspondin-1 and increased VEGF production. De-repression of CXCR4 through KLF2 downregulation further

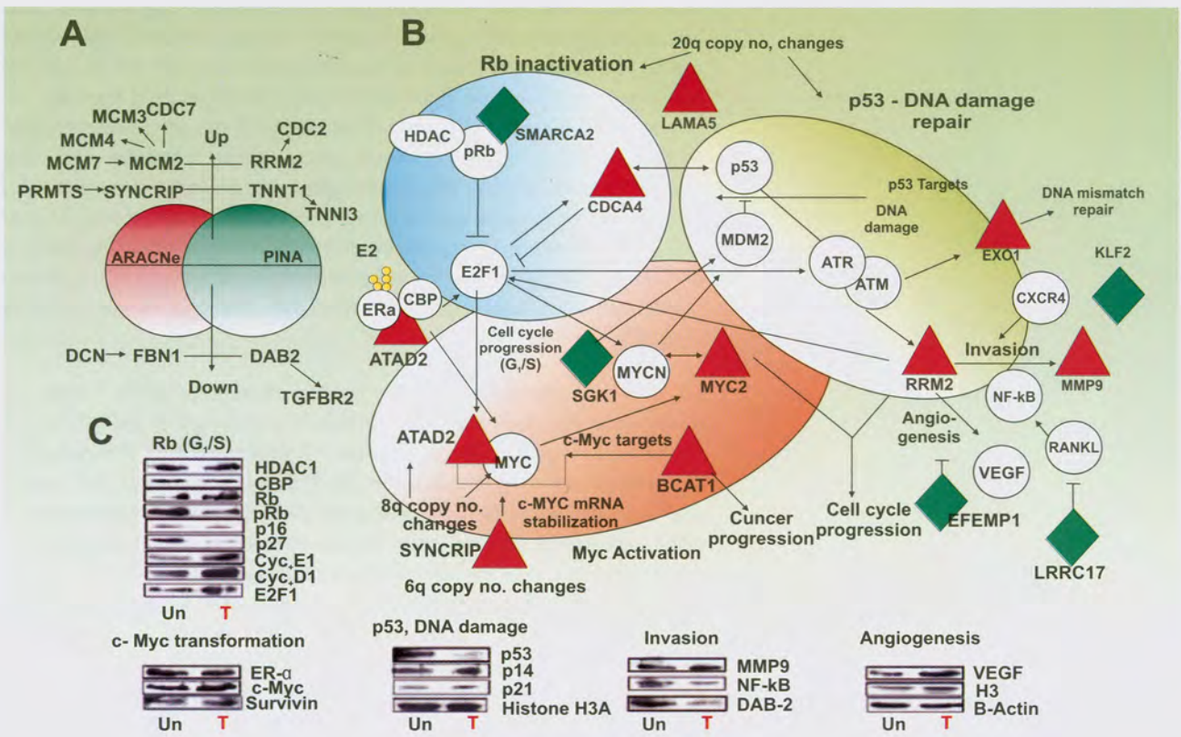
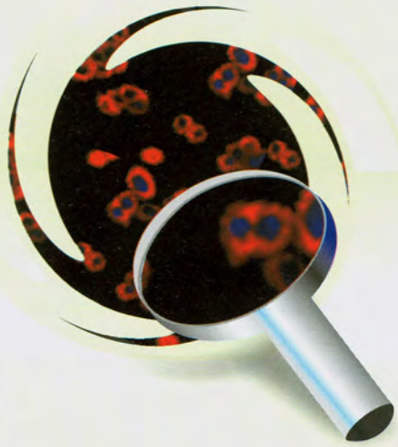


Fig.2 Derivation of functional modules. **A** overlapping nodes and interactors in the ARACNe and PINA networks. **B** Schematic representation of the three functional modules derived to be significant in serous ovarian carcinoma. **C** Western blots validating some of the proteins and pathways implicated in the predicted functional modules (Un, untransformed; T, transformed A4 cells).

supportes migrating cancer cells. Loss of LRRC17 leads to enhanced interactions between RANKL and its ligand NF-kB; while EFEMP1 downregulation signifies loss of anti-angiogenesis activity in transformed cells. LAMA5-associated 20q13.3 amplifications may involve upregulation of CAS and ZNF217 (a putative oncogene) and correlate with Cyclin D1, Rb, and p53 alterations. A part of our predicted model (p53-Rb inactivation) in mouse OSE has been shown to lead to formation of neoplasms comparable to high-grade human serous ovarian carcinomas. Finally, since model systems are essential to validate any set of interactions and regulations, we used the A4 cell system that has wild-type p53 to identify some of the partners implicated in predicted pathway dysregulation (Fig.2C).

Future Work

1. Evaluate the biomarker potential of SeOvCa genes in serous ovarian carcinoma.
2. Elucidation of SeOvCa profiles in Cancer stem cell hierarchies within tumors.



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

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Background

There is a medical need for advances in cancer treatment since surgery, radiotherapy and conventional cytotoxic chemotherapy have made only a modest overall impact on mortality. Thus, the significance of discovering new targets, pathways and strategies for therapeutic intervention in cancer is extremely 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.

Heat shock proteins were first discovered as a cohort of proteins whose expression is induced by heat shock and various other stresses. The induction of heat shock proteins is an adaptive response by the cell, which confers resistance to further stresses. The expression levels of several members of the Hsp family are significantly elevated in many cancers, and in case of some tumor types this is linked with poor prognosis. The correlation between Hsp over expression and chemoresistance in many tumor types is likely due to the ability of Hsps to inhibit apoptosis. For that reason, the pharmacological manipulation of Hsp levels likely represents an underexploited opportunity either to render tumor cells susceptible to the induction of apoptosis by chemotherapeutics and/or radiation or, alternatively, to directly and selectively disrupt their survival. Hsp-targeted pharmacological strategies may portent the advent of a novel approach to rendering tumor cells sensitive to conventional chemotherapies. Also, hyperthermia is being developed as an anticancer modality in combination with chemotherapy. To achieve success a basic understanding of the biology of heating procedures as well as the understanding of the development of thermotolerance that ensue, will be required and hence provide clear answers with regard to optimal recovery times and suitable intervals between heat treatments and that of optimum conditions for hyperthermochemotherapy.

We analyzed the consequences of altered expression (upregulation or downregulation) of heat shock proteins on survival of the cells exposed to drugs. The study reveals functional involvement of alterations in the expression of Hsp27 and Hsp40 following 5-FU or carboplatin exposure, on survival of hepatoma cells. Data suggests that stress augmented expression of Hsp27 and Hsp40 facilitates survival of cells whereas, prior

inhibition of Hsp27 and Hsp40 expression followed by drug exposure significantly reduces cell survival in Hep3B and HepG2 cells. The observations of the present study, brings into light the effectiveness of Hsps inhibitors in combating the survival of drug-exposed cells, which may prove to be clinically relevant in a long run. We also explored the interplay of HSF1 and p53 in regulating the expression of Hsp70 upon heat stress and its role in conferring carboplatin resistance in hepatoma cells. The data suggests that in HepG2 cells harboring wild type p53 and Hep3B cells harboring a p53 mutant, heat shock induced classical increase in Hsp70 protein expression in HepG2 cells whereas; no such increase in Hsp70 expression was observed in Hep3B cells. The study reveals that endogenously induced Hsp70 under regulation by p53/HSF1 is responsible for resistance to carboplatin exposure following heat stress.

Aims and objectives

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

Work achieved

I. Alteration in the expression of specific heat shock proteins on exposure to chemotherapeutic drugs, 5-FU or carboplatin.

Diverse molecular and cellular processes are activated in response to stress stimuli on cells, DNA damage being one of them. 5-FU and carboplatin are important chemotherapeutic agents used widely for the treatment of various cancers. Few reports document chemotherapeutic drug induced expression of Hsps in cancer cells. Alteration in the expression of Hsps due to exposure to chemotherapeutic drugs has been reported in some tumor types; however most of the studies have dealt with biopsy samples. An attempt was thus made to study the changes in the expression of major heat shock proteins on treatment with DNA damaging agents, 5-FU or carboplatin in vitro on hepatoma cells, Hep3B and HepG2. In order to investigate the time course of Hsps induction, cells were exposed to drugs for definite time periods before processing for western blots. Fig. 1A and 1B show an induced expression of Hsp27 and Hsp40 in a time dependent manner in Hep3B and HepG2 cells respectively. In Hep3B cells (Fig. 1A), as shown on exposure to 5-FU, Hsp27 and Hsp40 are induced significantly after 4 h of treatment and on carboplatin exposure the two molecules are induced after 8 h of treatment. In HepG2 cells (Fig. 1B), both the drugs induce the expression of Hsp27 and Hsp40 only after 12 h of treatment.

Inhibition of Hsp27 and Hsp40 endow a better outcome to chemotherapeutic drugs in hepatoma cells.

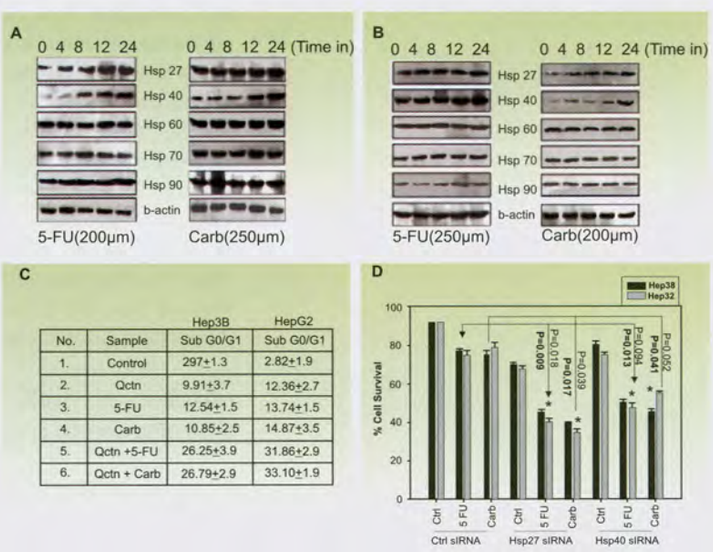
To observe the effect of inhibition of Hsp27 and Hsp40 on drug induced cell killing, biochemical inhibitor of Hsp's quercetin (Qctn) was utilized. Fig. 1C corresponds to FACS analysis carried out in Hep3B and HepG2 cells. The

data represented demonstrates significant changes in the proportion of cells in sub-G0/G1 phase indicating that Qctn and drugs together effect cell survival more callously. Knockdown of Hsp27 and Hsp40 using specific siRNA in both Hep3B and HepG2 cells was also performed. Quantitative MTT assay (Fig. 1D) carried out utilizing siRNA against Hsp27 and Hsp40 revealed that the inhibition of Hsp27 and Hsp40 potentiates the action of 5-FU or carboplatin as observed from the decreased cell survival where siRNA mediated knockdown preceded drugs exposure.

The study highlights that the use of inhibitors of Hsp27/Hsp40 or knockdown of Hsp27/Hsp40 in combination with chemotherapeutic drugs may be a rational treatment approach for HCC in cases where main objective is to prevent the progression or recurrence of the disease as summarized in Fig. 2.

II. Differential heat shock response in Hep3B and HepG2.

In recent times, hyperthermia has become an attractive co-adjuvant in the treatment of cancer for clinicians. Therefore, the endeavor to probe into effect of heat stress on carboplatin mediated cell death in hepatoma cell lines, Hep3B and HepG2 was sought after. The results (Fig. 3A) suggest that Hep3B and HepG2 differ in the responses to carboplatin treatment after exposure to elevated temperature/hyperthermia; Hep3B being sensitive in comparison to HepG2. A probable difference in the heat shock response was explored to clarify the differential retort of Hep3B and HepG2 to hyperthermia. Observations revealed that heat induces Hsp70 in HepG2 but not in Hep3B as shown by western blot analysis (Fig. 3B) and confocal analysis (Fig. 3C). Heat activated p53 (Fig. 3D) and HSF1 (Fig. 3E) in HepG2 only as revealed by CAT assay and gel shift assay respectively. It was also observed that p53 and HSF1 regulated the heat induced Hsp70 by



No.	Sample	Hep3B Sub G0/G1	HepG2 Sub G0/G1
1.	Control	297±1.3	2.82±1.9
2.	Qctn	9.91±3.7	12.36±2.7
3.	5-FU	12.54±1.5	13.74±1.5
4.	Carb	10.85±2.5	14.87±3.5
5.	Qctn +5-FU	26.25±3.9	31.86±2.9
6.	Qctn + Carb	26.79±2.9	33.10±1.9

Fig. 1. 5-FU and carboplatin exposure augments the expression of Hsp27 and Hsp40 specifically to promote cell survival and inhibition of their expression enhances drug induced cell killing. Hep3B **(A)** and HepG2 **(B)** cells were treated with either 5-FU or carboplatin as indicated and processed for western blotting to probe against major Hsps. **(C)** Hep3B and HepG2 cells were either treated with drugs or quercetin (Qctn) alone or in combination and processed for FACS analysis. **(D)** Hep3B and HepG2 cells were transfected with control siRNA or siRNA against Hsp27 and Hsp40 followed by drugs exposure and processed for MTT assay.

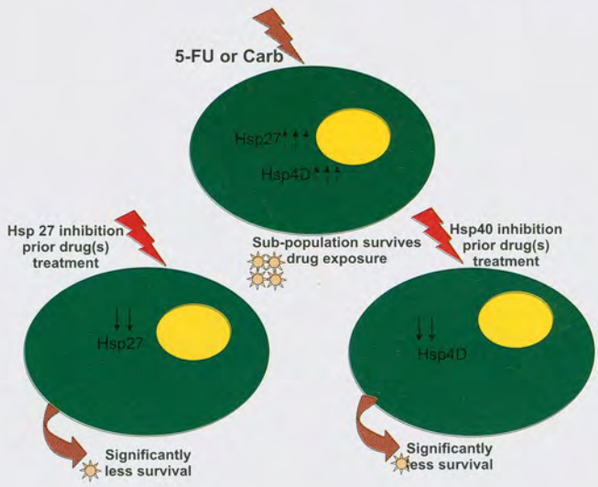


Fig. 2. Proposed model of the study

utilizing their respective inhibitors, PFTα (Fig. 3D) and Qctn (Fig. 3E). Further, the study disclosed that heat induced HSF1 is under regulation of p53 (Fig. 3F) as depicted by inhibitor and siRNA knockdown experiments.

p53/HSF1 downregulation increases receptiveness of HepG2 cells to carboplatin treatment following heat shock.

To strengthen the fact that endogenously induced Hsp70 acts as a survival protein in HepG2 communicating resistance and that targeting p53/HSF1 will reverse the resistance, the cells were exposed to p53, HSF1 or Hsp70 siRNA followed by heat shock and subsequently carboplatin and thereafter MTT assay was performed following incubation period of 36 h (Fig. 4A).

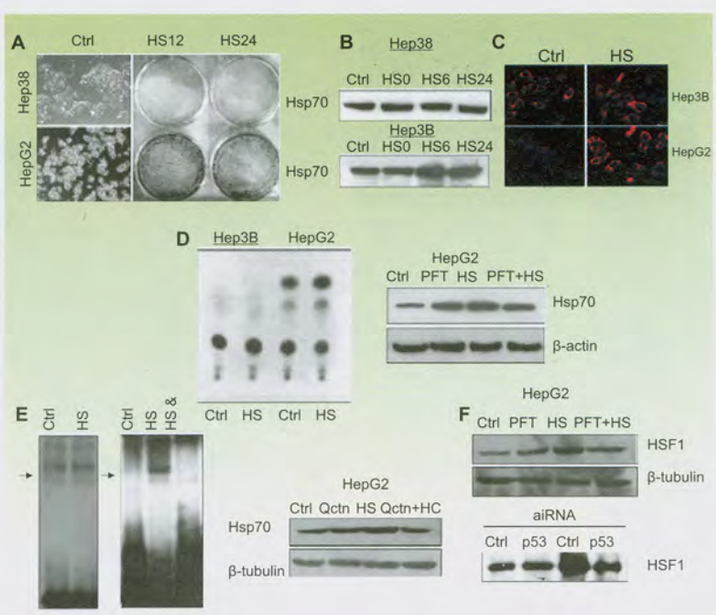


Fig. 3. Differential heat shock response in Hep3B and HepG2. Drug survival assay **(A)** Hep3B and HepG2 cells were heat stressed and then exposed to carboplatin were consequently plated for colony formation. **(B)** Hep3B and HepG2 cells were heat stressed and probed for Hsp70 or **(C)** confocal analysis. **(D)** Cells were heat stressed and processed for CAT assay. HepG2 cells were heat stressed with or without PFTα and probed for Hsp70. **(E)** Cells were heat stresses and processed for EMSA. HepG2 cells were heat stressed with or without Qctn and probed for Hsp70. **(F)** HepG2 cells were heat stressed with or without PFTα or p53 siRNA and probed for Hsp70.

To confirm that induced Hsp70 acts as survival factor in spite of the fact that p53 is activated, co-immunoprecipitation was performed. Upon heat stress, Hsp70 seem to complex with p53 in vitro thereby preventing the p53 induced death cascade in HepG2 cells (Fig. 4B). The study thus puts forth the notion of targeting p53/HSF1 to effectively achieve success of using hytperthermia in cancer therapy. It reveals p53/HSF1 as targets to overcome the predicament of utilization of hyperthermia in cancer treatment.

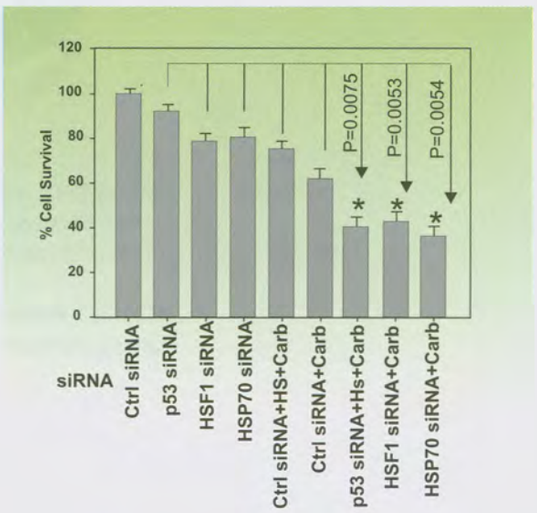
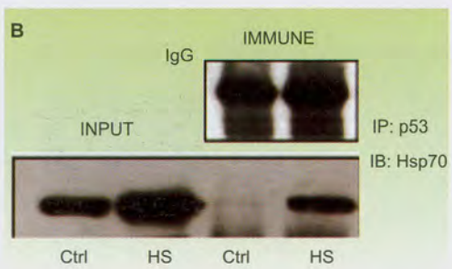
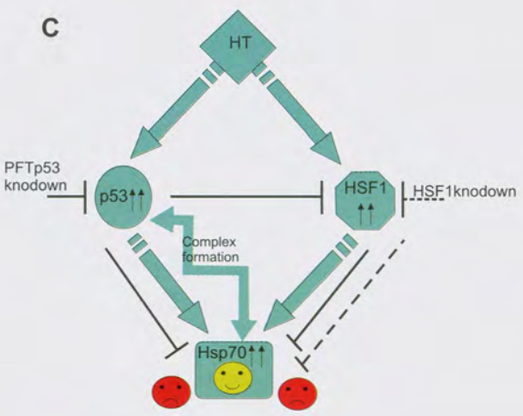


Fig.4. p53/HSF1 downregulation increases receptiveness of HepG2 cells to carboplatin treatment following heat shock. **(A)** HepG2 cells were. HepG2 cells plated in triplicate in 96 well plates were exposed to carboplatin (200 μM) for 36 h with or without heat stress following transfection with control siRNA, p53 siRNA, HSF1 siRNA or Hsp70 siRNA. **(B)** Untreated and heat stressed HepG2 cells were processed for co-immunoprecipitation analysis with anti-p53 antibody and probing with anti-Hsp70 antibody. **(C)** Proposed model of study.





Future work

Effects of chemotherapeutic drugs on cell lines derived from various human solid tumors will continued to be investigated. Also, the involvement of metabolic malfunctioning on the cancer progression and efficacy of chemotherapeutic drugs outcome will be investigated.

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

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Background

Glioblastoma multiforme (GBM) is the most common and highly aggressive type of primary brain tumor. Tumor-associated macrophages (TAMs) are frequently found in glioblastomas and a high degree of macrophage infiltration is associated with a poor prognosis for glioblastoma patients. 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-kB and PI3K/Akt. Malignant gliomas constitutively express high levels of activated Akt and NF-kB which correlate with the aggressive nature and resistance of these tumors. The mammalian Target of Rapamycin (mTOR) network functions downstream of PI3K/Akt pathway to regulate cell growth, proliferation and survival. mTOR exists in two distinct complexes -mTORC1 and mTORC2 that differ in their components and sensitivity to rapamycin. the mTORC2 component, is important for phosphorylation of Akt (S473) that is constitutively activated in gliomas and is involved in tumor progression.

Aims and Objectives

1. Identification of the downstream targets of Akt and NF-kB pathways in TNF-α mediated response 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.

Work achieved

The mTOR 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-mediated signaling during TNF-α -mediated response.

We investigated the role of Rictor, a core component of mTORC2, in proliferation, survival and invasion of gliomas. For this purpose, human glioma cell lines, LN-18 and LN-229 cells were transfected with pool of 4

different siRNA sequences against Rictor and stimulated with TNF-α. Interestingly, Rictor ablation significantly increased the cellular MMP-9 expression in cell lines as determined by immunofluorescence (Fig 1). MMP-9 activity measured by gelatin zymography was higher in siRNA transfected cells, with more significant increase in LN18 cells. There was no significant difference in MMP-9 activity on stimulation with TNF-α between control and Rictor-silenced cells (Fig 2). Neither silencing nor exposure to TNF-α affected the levels of MMP-2 in the cell lines. These results suggested that Rictor functioned as a negative regulator of MMP-9, but might not have a role in TNF-α-induced MMP-9 secretion.

Further experiments with in vitro matrigel invasion assay revealed that Rictor-silenced cells were highly invasive through the matrigel membrane with 100% and 38% increase in invasion compared to the control transfected LN18 and LN229 cells respectively. Stimulation with TNF-α enhanced the invasion of control transfected and siRNA transfected cells. Monoclonal blocking antibody to MMP-9 inhibited invasion by ~75% in the cell lines, in the presence or absence of TNF-α treatment (Fig. 3A, B). Blocking antibody to MMP-9 showed a compelling correlation between the extent of MMP-9 activity and matrigel invasion by these cells. Preliminary studies indicate the activation of Raf1-MEK-ERK pathway that results in the increased MMP-9 expression and invasion. Rictor ablation did not affect

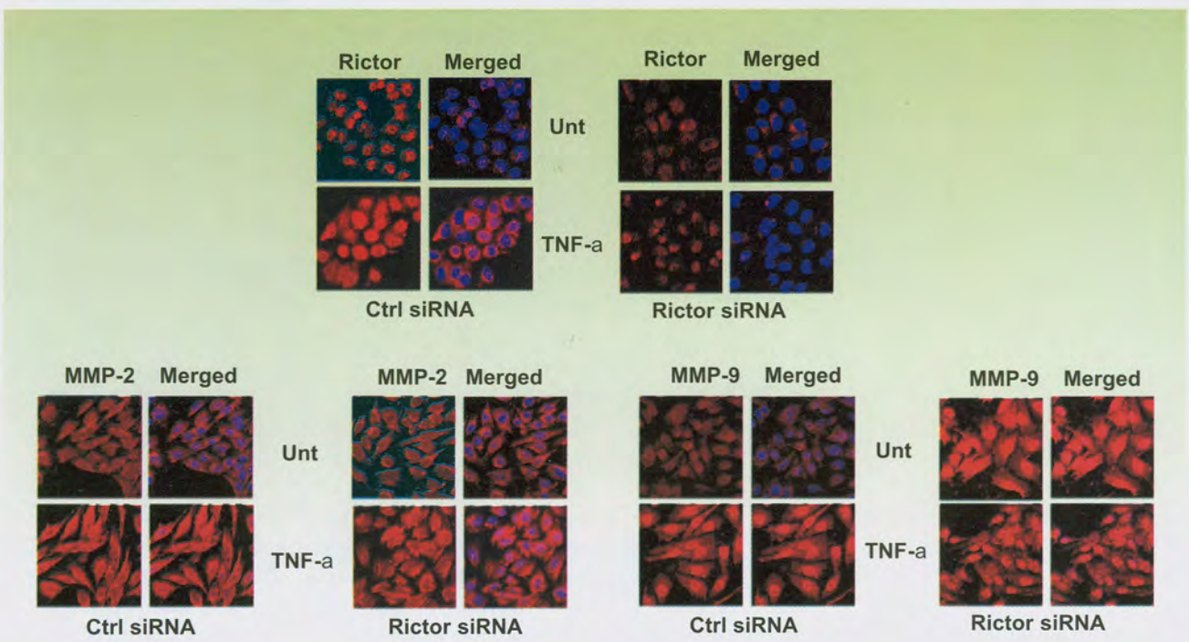

Fig.1. Rictor silencing enhances the expression of MMP-9. LN18 cells transfected with control (ctrl) and rictor siRNA were treated with TNF-α (10mg/ml) for 12h and processed for immunostaining. The cells were fixed with paraformaldehyde and incubated with Rictor, MMP-2 and MMP-9 antibodies followed by cy-3-conjugated secondary antibody. DAPI was used to stain the nucleus (Magnification 63X).

Fig. 2. Rictor silencing enhances MMP-9 but not MMP-2. Conditioned medium of LN18 and LN229 cells transfected with control (ctrl) and Rictor siRNA were treated with TNF-α (10ng/ml) for 12h was collected and subjected to gelatin zymography analysis for MMP-9 and MMP-2 levels. The fold changes of MMP-9 activity are in comparison with untreated ctrl transfected cells. The zymogram is representative of three independent experiments.

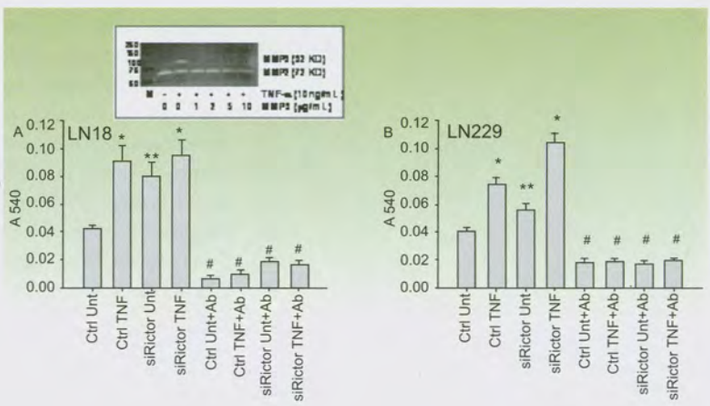

Fig. 3. Rictor regulates invasion through MMP-9. LN18 **(A)** and LN229 **(B)** cells transfected with control (ctrl) or rictor siRNA were incubated with or without MMP-9 antibody (Ab) and seeded onto matrigel-coated invasion chambers for 24h. The cells that invaded were stained with crystal violet, eluted and absorbance measured. *$p<0.05$ control vs rictor siRNA transfected, **$p<0.05$ untreated vs TNF-α treated cells, #$p<0.05$ in absence vs presence of MMP-9 antibody. The efficacy of the antibody to block MMP-9 was checked in LN18 cells by zymography (inset). M-MW marker.

TNF-α-induced MMP-9 activity and invasiveness, suggesting that TNF-α in the microenvironment of tumor might overrule the its function as negative regulator of MMP-9.

Conclusions- Our findings suggest a novel role for rictor in bridging two major pathways – Akt (PKB)/mTOR and Raf 1-MEK-ERK in regulating MMP-9 activity and invasion of glioma tumor cells.

Future work

To extend the findings in primary cultures derived from human glioma samples and examine whether an interaction exists between the mTORC1 and mTORC2 pathways in responses to TNF-α and IL-1beta to influence tumor progression and invasive potential of gliomas.

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

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Background

It is now well-established that most of the human genome is transcribed into non-coding RNA sequences than into protein-coding sequences. Whilst, only about 1% of human genome is protein-encoding, most of the genome is transcribed into stable non-protein-coding RNA transcripts. Many of the identified RNAs are long non-coding RNAs like Evf-2, XIST, Air and HOTAIR and are subjected to splicing, poly-adenylation, and other post-transcriptional modifications and their loci tend to be associated with chromatin. The regulatory control of transcription and splicing patterns, evolutionary conservation, and presence of predicted secondary structures suggest that the transcriptional products from each locus are functional. Furthermore, their spatiotemporal expression patterns suggest that these ncRNA loci might contribute to regulation of cell proliferation and differentiation programs. The other class of RNAs are the small RNAs that bind to Argonaute (Ago) proteins and play important role in regulation of gene expression. The effector RNA molecules include the 21–25 nucleotide long miRNAs and the small interfering RNAs (siRNAs), which direct sequence specific target mRNA silencing. The Argonaute proteins are further classified into Ago and Piwi clade proteins. While, siRNAs and miRNAs associate with the Ago clade Argonautes, recent studies have identified a class of 24–30 nucleotide long Piwi interacting RNAs (piRNAs), which bind to Piwi clade Argonautes. The piRNAs have important roles in germ-line development in flies, fish and mice. In a screen to identify genes important in cellular signaling we have identified a 612 bp. Long non-coding RNA with predominant expression in brain. The project deals with study of the cellular and molecular signaling pathways evoked by this nc-RNA.

Aims and Objectives

1. To characterize role of Sense and Anti-sense transcripts of Ginir and Giniras in cell proliferation.
2. To understand the molecular mechanisms and signaling pathways elicited by this pair of Ginir anad Giniras transcripts in cellular homeostasis.

Work Achieved

Our lab has focused on studying the role of a novel pair of non-coding,

sense (Ginir) and anti-sense (Giniras) RNAs in cellular growth control. Canonical checkpoint regulatory proteins monitor error–free S-phase and G2-M transitions in cycling cells to prevent cancer by coordinating the density and the sequence of firing of replication origins to avoid replicative stress and by disallowing chromosome segregation before the DNA-replication is completed. Our results establish that homeostasis of a pair of naturally expressed, non-coding and overlapping sense and anti-sense transcripts (SAST) is critical to normal cell cycle progression. Further, we provide evidence about the role of sense transcript – Ginir as a genomic instability-inducing RNA (Ginir), promoting oncogenic transformation, while its anti-sense counterpart (Giniras) acts as its negative regulator. Further, using luciferase reporter system we found that Ginir function was mediated through an RNAi response that targeted the tumour suppressor protein fhit, followed by induction of replicative stress leading to activation of several proteins of checkpoint pathway comprising ATR, Chk1, ATM, H2ax, tp53 and p21cip/waf. By contrast, over expression of Giniras was non-oncogenic in normal cells but interfered with Ginir function as demonstrated by its ability to retard in vivo tumour growth induced by Ginir-transformed cells. We confirmed this effect in both mouse – NIH3T3 and human – Sk-N-MC cell systems (Fig.1). Consistent with this data, we investigated the potential of Ginir as a promoter of cell cycle progression, wherein we observed that the serum starved NIH-Ginir cells exhibited a disproportionately greater number of Ki67 positive cells indicating the ability of Ginir to induce a more rapid S-phase and G2-M progressions compared to NIH cells overexpressing Giniras or empty vector sequences . Similar effects on cell cycle kinetics were evident in Ginir cells on treatment with microtubule de-polymerizing agent nocadazole but not in Giniras and other control cells. Also, Ginir induced increase in level of check-point proteins that are closely involved in monitoring fidelity of DNA replication and chromosome segregation such as Ataxia telangiectasia mutated (ATM), ATM-Rad52-related protein (ATR), check point kinase1 (Chk-1) and check point kinase 2 (Chk-2). The NIH-Ginir cells but not the NIH-EV cells consistently demonstrated a greater density of stalled replication forks,

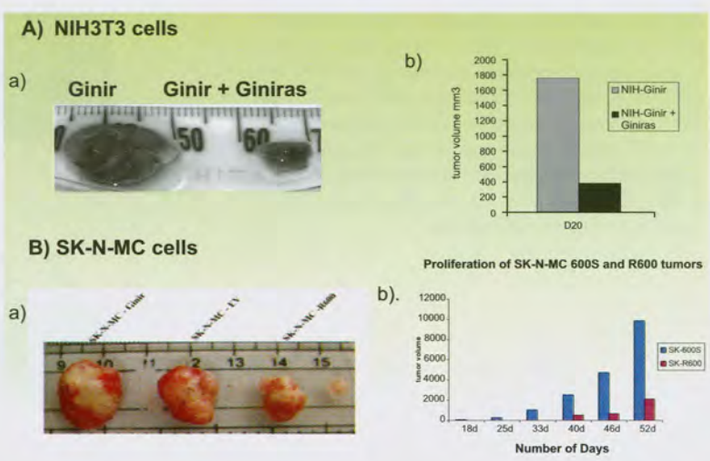

Fig.1. Giniras dominantly inhibits tumour growth induced by Ginir in NIH3T3 and Sk-N-MC cells as analysed from size of excised tumors (a) and tumor growth kinetics (b).

demonstrating that Ginir over expression resulted in an inappropriate G1-S transition and/or progression through the S-phase. As a consequence of the stalling of replication forks was a greater replicative stress response was evident in NIH-Ginir cells leading to activation of intra S-phase check point kinases ATM and ATR that phosphorylated many of their downstream cellular substrates which included H2ax, Chk1 and p53. Activation of ATM and ATR led to phosphorylation of Serine 15 in p53 that prevented its proteasomal degradation and promoted nuclear entry, activating the transcription and translation of its downstream target CDKN1A (also known as p21cip1, Waf1). Remarkably, the over-expression of CDKN1 protein in Ginir cells was inconsistent with its role of a suppressor of cell growth and an inducer of cell senescence. We resolved this puzzle by showing that due to an activation of Akt1 by ATM, the CDKN1 protein was phosphorylated at Thr-145 residue expelling it from the nucleus to the cytoplasm that impaired its growth inhibitory functions. Secondly, we found that CDKN1 in cytoplasm was responsible for an increase in the nuclear levels of CyclinD1 by acting as assembly factors of CDK4-CyclinD1 complexes to promote their nuclear import to induce cell cycle progression. Thirdly, a part of the nuclear CDKN1 collaborated with proliferating cell nuclear antigen (PCNA) to stabilize the integrity of the stalled replication forks against collapse. Because prolonged stabilization of stalled replication forks were highly recombinogenic, it led to increased somatic recombinations induced by the activation of DNA-repair mechanisms causing a high level of genomic

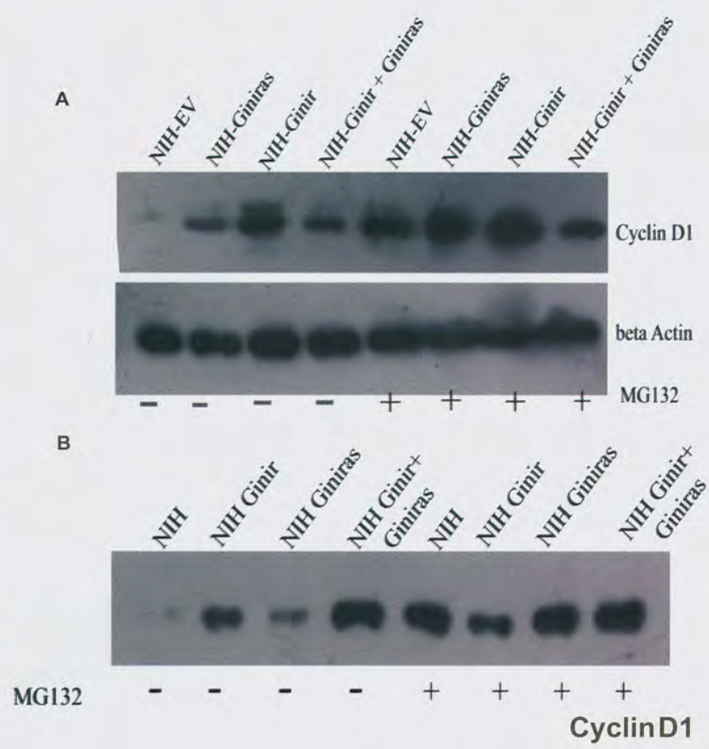

Fig. 2. (A) Western Blot of Cyclin D1 showing its overproduction and survival to degradation in NIH-3T3 Ginir cell line as compared to other cell lines. MG132 treatment (20 μM , 6 hours) does not lead to an excess accumulation of cyclin D1 in NIH-Ginir cells as compared to other cells. **(B)** Fbxo-31 and Cyclin D1 complexes formed during degradation of cyclin D1 were immuno-precipitated. Accumulation of Cyclin D1 in all other cell lines took place whereas in NIH- Ginir cells it did not occur, indicating that it remains resistant to degradation.

instability. Significantly, the enhanced expression of Cyclin-D1 in Ginir cells but not in Giniras cells was followed by high levels of phosphorylation of down-stream target pRb. Recent studies have indicated that Cyclin D1 protein expression is regulated by multiple mechanisms including its own proteasomal degradation. Cyclin D1 is a known substrate of poly-ubiquitination complex SKP1/CUL1/F-box (SCF) and Cyclin D1 is targeted for degradation by the SKP1/CUL1/F-box (SCF) ubiquitin ligase during the cell cycle and in response to DNA damage. We found that treatment of cells with the proteasome inhibitor MG132 stabilized the endogenous cyclin D1 protein ≈ 4- fold in NIH-Control and NIH-Giniras cells and about ≈ 2- fold in NIH –Ginir+Giniras cells (Fig. 2). However, in Ginir cells, the presence MG-132 did not show accumulation of Cyclin D1 suggesting that Ginir interferes with formation of the proteasomal degradation complex of SKP1/CUL1/F-box (SCF) thereby leading to a continued maintenance of high levels of Cyclin D1 in cells. In conclusion, our studies indicate that the high level of GI induced by Ginir which predisposes the cells towards tumorigenicity is an outcome of an over-activated DNA damage response pathway and a faulty proteasomal degradation machinery leading to inhibition of G0 growth arrest, erroneous and faster cell cycle progressions, inhibition of differentiation, loss of genomic integrity all culminating towards transformation and tumorigenicty.

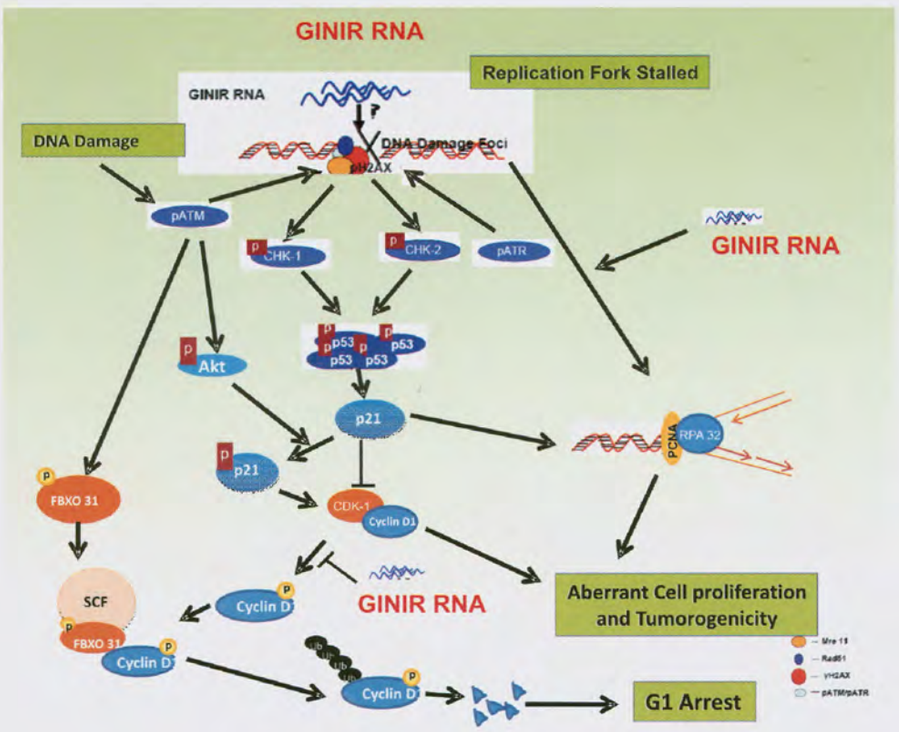

Fig. 3. Mechanism of action of GINIR

Collectively, our data demonstrate 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 as depicted in Fig. 3. We propose that Ginir and Giniras represent a pair of novel ncRNA regulators impacting on normal cell cycle progression, tumour suppression and cancer. 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 Ginir in gene promoter DNA hypermethylation and study its role with respect to chromatin regulation of gene expression .
2. Characterize the role of Giniras as a tumor suppressor in various experimental systems and explore it's potential as a therapeutic target in Cancer.

Research Reports

Signal Transduction

Gopal Kundu	52
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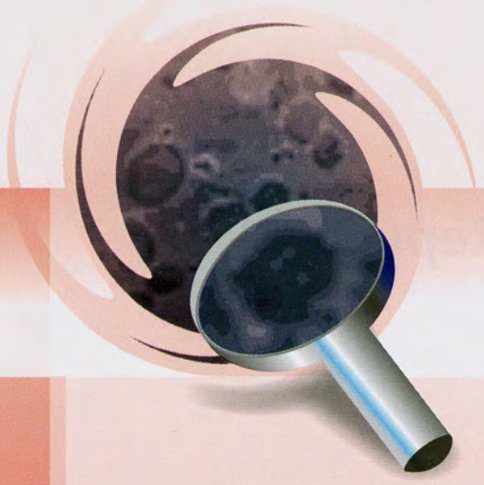

Osteopontin, a potentially important therapeutic target and diagnostic marker in cervical and breast cancers: Attenuation of tumor progression and angiogenesis by Semaphorin 3A in melanoma model

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Background

Cervical cancer is one of the leading causes of death among women in the developing countries and around the world. Therefore, there is an urgent need to identify signaling mechanism regulating cervical cancer progression which may lead to the advances in therapeutics. p38 kinase is the member of mitogen activated protein kinase (MAPK) family of serine/threonine kinase. p38 MAPK is induced by various stimuli including inflammatory cytokines, growth factors as well as physical and chemical stresses resulting in cellular proliferation, differentiation, migration and apoptosis. The activation of p38 is mediated by MAP kinase kinase 3 (MKK3) and MKK6 in a selective manner within Thr-Gly-Tyr motif present in kinase domain. The increased activation of p38 has been reported in breast and bladder carcinoma. However, the role of p38 in regulating cervical cancer progression is not well defined.

Osteopontin (OPN) is a member of SIBLING family of chemokine like phosphoglycoprotein. The N-terminal region of OPN binds to integrins while C-terminal region interacts with CD44 receptor. Previous reports have shown that enhanced expression of OPN correlates with cervical cancer progression. Upon interacting with integrins OPN triggers series of signaling events leading to expressions of matrix metalloproteinase (MMP)-2, MMP-9, cyclooxygenase-2, vascular endothelial growth factor, ICAM-1 and cyclin D1 and Bcl2 ultimately inducing cell migration, angiogenesis and tumor progression. However, the role of OPN in regulating cervical cancer progression and the mechanism underlying this process is not studied well.

Understanding the mechanisms of various tumor suppressor molecules in regulation cancer progression and their possible role in cancer therapeutics is under intense investigation. Semaphorins has been originally known as a large family of evolutionary conserved axonal guidance molecules. However, involvement of semaphorins in various physiological as well as pathophysiological processes including cell migration, regulation of immune response, angiogenesis and cancer have also been described. Among various semaphorins, some members of semaphorin 3 (SEMA3) family, known to involve in inhibition of tumor progression and has been considered as potent tumor suppressor. Loss of expression of SEMA3B and SEMA3F gene had been shown to associate with lung cancer progression. Moreover, Semaphorin 3A (SEMA3A),

shown to inhibit angiogenesis may also act as tumor suppressor. Knockdown of endogenous SEMA3A significantly induces in vitro migration of human breast cancer cells which indicated that SEMA3A may act as a potent tumor suppressor. Furthermore, overexpression of SEMA3A significantly attenuates in vivo breast tumor growth in mouse xenograft model. However, the role of SEMA3A on melanoma progression is not clearly understood.

Aims and Objectives

(a) To investigate whether OPN induces CD44 mediated p38 MAPK activation leading to NF-kB activation and NF-kB dependent furin/uPA expression in cervical cancer cells.

(b) To study whether OPN-induced p38 MAPK plays any role in furin-dependent cervical cancer growth in NOD-SCID mice model.

(c) To examine whether SEMA3A regulates tumor growth and angiogenesis in melanoma model and to delineate the mechanism underlying this process.

Work Achieved

In this study, we provide evidences that OPN regulates CD44-mediated MKK3/6 and p38 dependent NF-kB activation leading to furin expression which ultimately augments cervical cancer cell migration (Fig. 1 & 2). Our in vivo data revealed that p38 and furin played crucial role in OPN-induced cervical tumor growth in mice xenograft model. The clinical specimen analysis established the strong correlation between enhanced expressions of OPN, p-NF-kB p65 and furin with cervical cancer progression. In

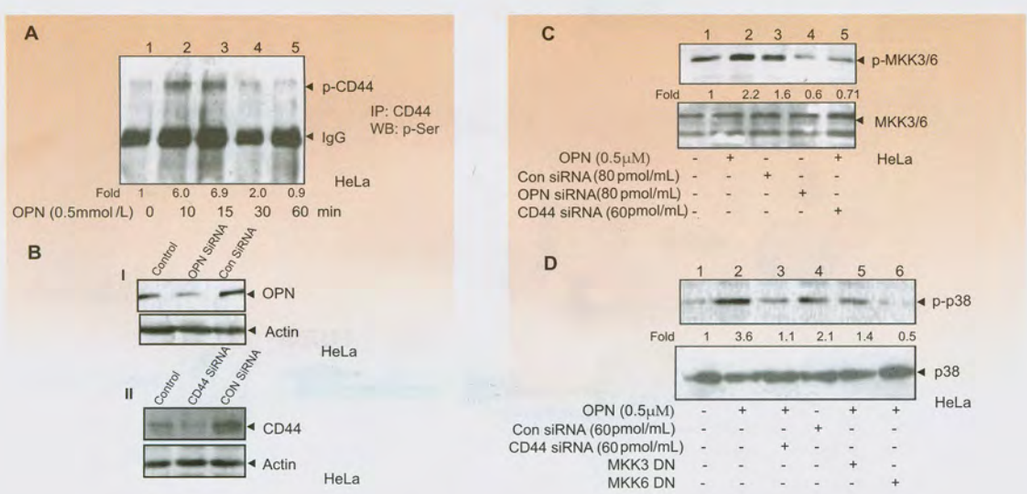

Fig.1 Osteopontin stimulates CD44-mediated MKK3/6 and p38 phosphorylation in HeLa cells

summary, these data suggested that targeting OPN and its downstream p38 signaling might provide a novel therapeutic strategy for the treatment of cervical cancer.

Current studies have shown that axonal sprouting inhibitor semaphorin 3A (SEMA3A) acts as a potent suppressor of tumor angiogenesis in various cancer models. Therefore, understanding the molecular mechanism underlying SEMA3A mediated regulation of tumor metastasis and angiogenesis is yet to be a field of intense investigation. In this study, using multiple in vitro and in vivo approaches we have shown that SEMA3A acts as a potent tumor suppressor in mice melanoma model. Melanoma cells overexpressed with SEMA3A showed significant reduction in motility, invasiveness and proliferation as well as in vivo tumor growth, metastasis and angiogenesis (Fig. 3). Moreover we have observed that melanoma clones overexpressing SEMA3A showed increased sensitivity towards anti cancer agent like curcumin as compared to parental cells. Our results demonstrate, at least in part, the functional approach and molecular mechanism(s) underlying SEMA-3A mediated inhibition of tumorigenesis and angiogenesis and a clear understanding of such mechanism(s) may facilitate the development of novel therapeutic approaches for the management of malignant melanoma.

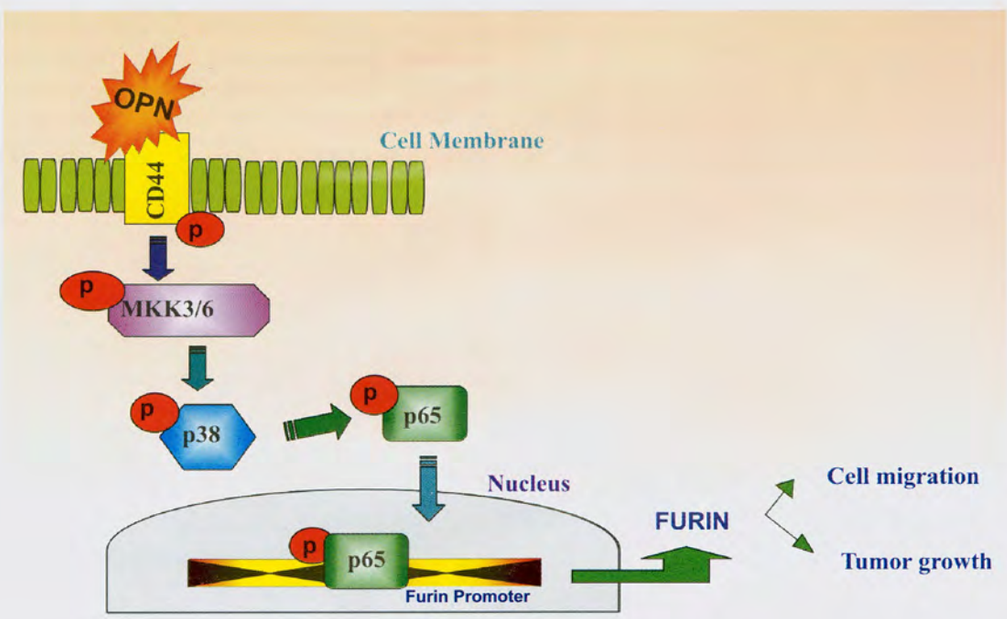

Fig.2. Schematic representation of osteopontin-induced CD44-mediated MKK3/6 and p38 dependent NF-kB activation and furin expression leading to cell migration and tumor growth in cervical cancer

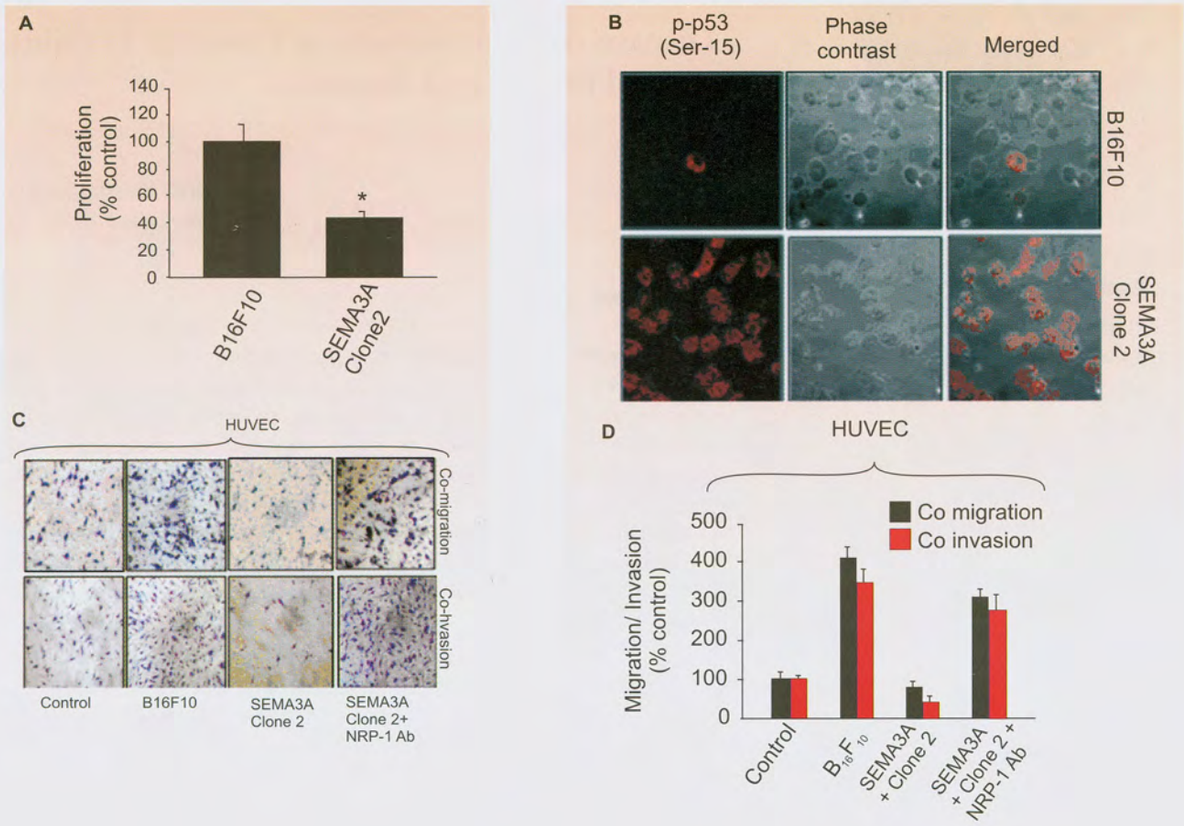

Fig.3. Overexpression of SEMA3A inhibits proliferation and tumor-endothelial interaction through induction of phosphorylation of p53 in melanoma cells

Future Work

The therapeutic and diagnostic significances of osteopontin and therapeutic potential of SEMA3A in regulation of tumor growth and angiogenesis in various cancer models will be further studied.

Disease relevant mutants of Caveolin-1exhibit normal folding and dynamics

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Background

Caveolae were observed as little-caves or micro-domains of 50 to 100 nm size on the plasma membrane of most mammalian cells enriched with cholesterol and sphingolipids. Caveolae are involved in several important physiological functions such as signal transduction, endocytosis and intracellular cholesterol transport. They bud directly from the plasma membrane to form free transport vesicles for directed docking and fusion to mediate the endocytosis and transcytosis of macromolecules. The only marker protein of caveolae was identified as VIP21 or Caveolin-1 (Cav-1). Caveolins are a family of 21 to 25 kDa integral membrane proteins that have been implicated in various cellular functions. Three Caveolin genes exist in mammals that code for five different isoforms of the protein. Cav-1 is localized to plasma membrane in caveolae as well as in intracellular compartments such as endoplasmic reticulum and trans-golgi network to fulfill its role in the dynamic trafficking of caveolae between its origin and targeting or functional sites. Cav-1 is an oligomeric protein, associated with membrane via a central 33-amino acid hydrophobic domain in which both N- and C- termini are towards the cytoplasm leaving no extra cellular segments. Cav-1 plays an important role in cholesterol trafficking, lipid metabolism, signal transduction, cell survival, membrane damage and repair. The Cav-1 gene co-localizes to 7q31.1 region, near D7S522 locus that is deleted in most human cancer forms including breast cancer and also Cav-1 has been detected as one of the 26 genes that were down-regulated in many breast cancer cell lines, tumor samples and solid tumors. Cav-1 knockout mice have helped in understanding the role of Cav-1 in the pathogenesis of mammary epithelial cell hyperplasia, tumorigenesis and metastasis in vivo. Interestingly, screening of human breast cancer samples by a Japanese group identified a prominent mutation in the Cav-1 gene at amino acid position 132 i.e. Proline at 132 was mutated to Leucine (P132L) in ~16% of cancer patients. The expression of recombinant P132L in NIH-3T3 was found to result in cellular transformation accompanied by activation of p42/44 MAPK cascade. A similar result was revealed by anti-sense mediated silencing of Cav-1 expression. In addition, P132L was shown to get retained at the golgi, and exhibited dominant negative character causing intracellular retention of wild-type Cav-1 and has misfolded oligomerization. Although considerable knowledge about the assembly of caveolae vesicles and its major marker protein, Cav-1, is known at functional level, the overall

nature of the dynamics of the caveolae vesicles is just emerging. This is because the caveolae not only exist as static platforms at the cell membrane but also exhibit fission and fusion dynamics with the plasma membrane. Hence, it is intriguing to note the survival of the cells expressing the misfolded Cav-1 as it is anticipated that the presence/ accumulation of misfolded oligomers of P132L in ER/golgi should have triggered an unfolded protein response (UPR) pathway and elimination of the cells harboring such mutations through apoptosis. In another example, a mutant form of Cav-3, which has high homology to Cav-1, was found to contain a deletion mutation i.e. a deletion of 91-93 amino acids. These amino acids are part of scaffolding domain of Cav-1. The 91-93del mutation does exhibit ring like structures but deficient in filament formation seen for the wild-type Cav-1 protein. Hence, the structural, functional and dynamic features of P132L, 91-93 deletion mutants and the nature of their dominant negative phenotype or otherwise need a better understanding to help us dwell into various modes of survival of cells, particularly in breast cancer scenario where the P132L mutation has been discovered.

Aims and objectives

1. At molecular level, understand the folding and dynamics of Cav-1 with the help of mutants of Cav-1 seen in human cancer as well as in limb-griddle muscular dystrophy, probably the only other disease where mutations in Caveolin protein were observed.
2. Correlate the nature of dynamics of caveolae with the observed UPR pathways of cells expressing Cav-1 mutants to obtain clues, if any, regarding the role of caveolae in suppression of UPR activation and/or its sustainability.

Work Achieved

The Cav-1 plays diverse roles in mammalian cells and several processes begin with changes in its dynamics. The Cav-1 gene is located near a known fragile site that is deleted in several cancer forms with down-regulation of Cav-1 expression. Studies till date concluded that the P132L mutant Cav-1 was retained in Golgi and appeared to exist as a misfolded oligomer that exhibited dominant negative character. Considering the dynamic nature of Cav-1, our aim is to understand the folding and dynamics of Cav-1 especially its mutant forms and their responses to external stimuli.

In order to achieve the above goals, we have constructed the following mutants viz., P132L, C133R (observed in breast cancer). The C133R mutation, however, was not found independently but present along with P132L in some of the 16% breast cancer patients with caveolin mutations. A single palmitoylation deficient mutant, C143A has been known to target the caveolar membrane normally like the wild-type Cav-1 but its dynamics are not known. The 91-93 amino acid deletion (the amino acids 'T-F-T' deleted in case of limb-girdle muscular dystrophy; referred as 91-93del). A

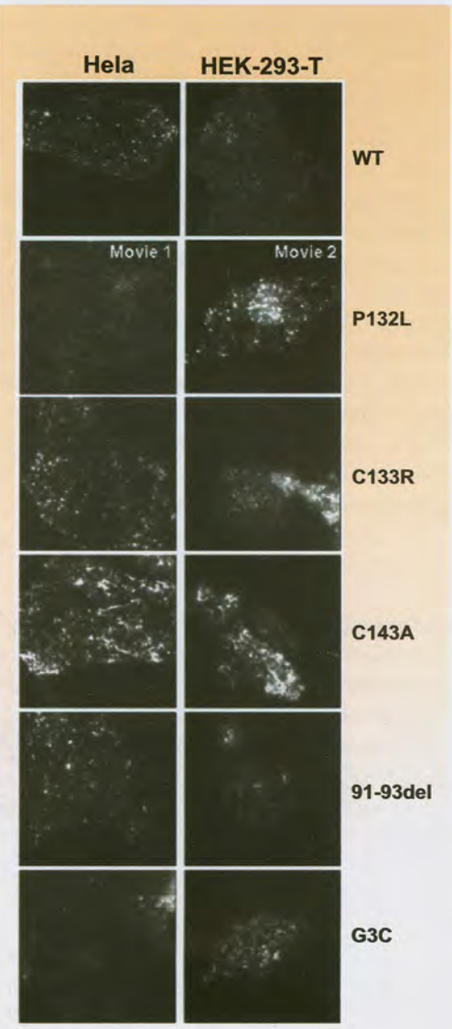

Fig.1. Membrane localization of Cav-1 and its mutants by GFP expression in HeLa and HEK-293-T cells by TIRFM. HeLa and HEK-293-T cells were seeded on cover-glasses and transfected with Cav-1-GFP or its mutants.The coverslips were processed for TIRFM visualization as described in methods section. Various panels represent the still TIRFM images representing the membrane localization of Cav-1-GFP or its mutants in both the cells. Supplementary material movies 1 and 2 are marked within the panel. All the videos were collected at 25 frames per second and the microscopic magnification factor is 105 nm per pixel.

new mutation referred as G3C was also created in anticipation to induce some degree of deliberate misfolding through improper disulfide bond formation in the Cav-1 protein to study the folding and dynamics.The dynamics of caveolae were studied by employing total internal reflection fluorescence microscopy (TIRFM). TIRFM is a very sensitive technique that provides a direct, quantifiable visualization of dynamic events occurring at the cell membrane within 200 nm which is superior to the resolution seen in case of laser confocal microscopy. Also, TIRFM is more appropriate for study of Cav-1 like proteins as they can be visualized without the interference of background fluorescence due to the staining of intra-cellular proteins. It has been shown that each caveoale vesicle consists of a fixed number of Cav-1 molecules (144 + 39) assembled to form caveolar coat irrespective of the presence or absence of endogenous Cav-1. In order to enhance our understanding further, we have used two stimulants viz. TGFα and Taxol. Both these stimulants induce changes in the dynamics of Cav-1 that are well characterized, while the former does it by binding to the epidermal growth factor receptor (EGFR), the latter modulates it by binding to microtubules.

The confocal and TIRF microscopic data obtained by us on cellular localization of P132L, shown in Fig. 1, is in excellent agreement with earlier studies. An important outcome of the present study is the presence of P132L at the membrane of HeLa and HEK-293-T cells. The P132L is capable of targeting to the membrane both in the presence of endogenous Cav-1, as well as on its own (Fig. 2). This observation unambiguously proves that the misfolded P132L itself, irrespective of the endogenous Cav-1 can assemble into the caveolae vesicles which exhibit the characteristic 'kiss and run' dynamics with the cell membrane of HeLa and HEK-293-T cells. The visibility of P132L at membrane surface is an indication of reasonable or near normal folding. Moreover, our confocal and FRET observations also show that the P132L is capable of forming functional complex with wild-type Cav-1 and carry it along to the cell surface (Fig. 3). It must be noted that our FRET data is at single caveolae vesicle level which means the caveolae vesicle (composed of about ~140 molecules or so) has both wild-type Cav-1 and P132L whose dimension is about 50 to 100 nm, thus, providing an unambiguous proof of hetero-oligomerization under live conditions. Therefore, the P132L may not seem to behave in a dominant negative manner as it did not completely trap the wild-type Cav-1 in golgi. All the other mutants studied here have shown the cellular localization patterns and dynamics identical to the wild-type Cav-1.

Among all the mutants of Cav-1 examined in this study, the responses of P132L and 91-93del mutants to taxol and TGFα, although elicited subtle differences with respect to wild-type Cav-1, their dynamics indicate normal assembly and function in HEK-293-T cells. In addition, the absence of palmitoylation has also not affected the docking sites in case of C133R and C143A mutants for taxol, as there was transient recruitment as well as internalization of both the mutants upon taxol treatment. While, the homo-oligomers of 91-93del mutant in HEK-293-T showed immediate cell

surface recruitment and further internalization with time upon taxol treatment, the hetero-oligomers expressed in HeLa showed defective responses. It is reasonable to infer that the hetero-oligomers of P132L and 91-93del mutants have erroneous dynamics towards taxol and TGFα but not their homo-oligomers as they both exhibited normal dynamics to same stimuli.

We also examined the unfolded protein response pathway due to the accumulation of various mutant forms of Cav-1. Importantly, while the P132L did not exhibit any noticeable difference in the status of molecules

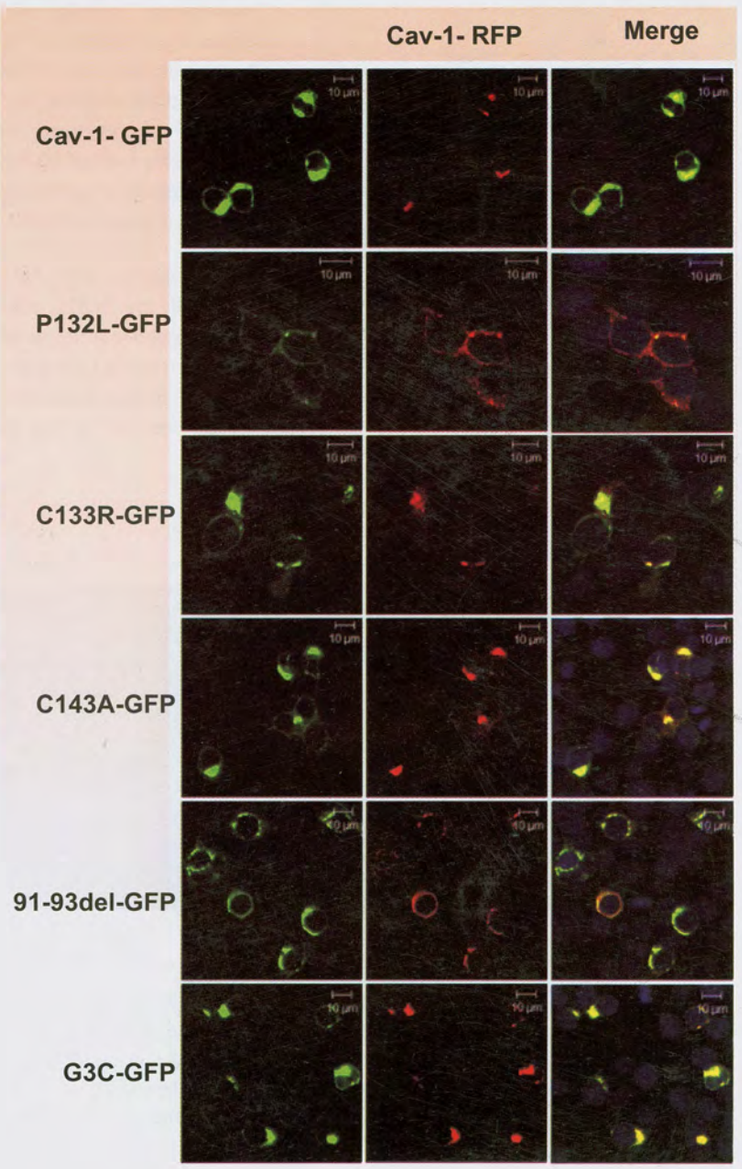

Fig. 2. Hetero-oligomerization status of Cav-1 mutants on co-transfection with Cav-1-RFP in HeLa and HEK-293-T cells by confocal microscopy: HeLa cells were seeded on confocal cover-glasses and co-transfected with Cav-1-RFP and Cav-1-GFP or its mutants for confocal microscopy. Green represents the GFP signal of Cav-1 wild-type or its mutants. Red panel shows the RFP signal of wild-type Cav-1. Merged panel shows the co-localization of Cav-1 mutants with wild-type Cav-1.

involved in the UPR, the 91-93del and G3C showed mild UPR activation i.e. an increase in expression of BiP/GRP-78 (Fig. 4), there was a divergence of UPR from pro-apoptotic pathway to cell survival due to the short life of GADD-153. We have observed the down-regulation of GADD-153 at later time-points, despite of persistent up-regulation of GRP-78. Thereafter, cells might have adapted to survival either due to improved folding and/or partial fulfillment of essential functions by mutant caveolae vesicles and suppression of downstream molecule, GADD-34. These set of observations suggest a plausible role for caveolae vesicles for sustained activation of the UPR pathway, which may need further attention. Interestingly, among the kinases that have been shown to block the dynamics of caveolae, the KIAA0999 and MAP3K2 kinases were able to block the dynamics of P132L mutant at cell surface. While the silencing has neither disassembled the P132L (formation of lesser intense spots) nor resulted in large cluster formation of P132L at cell surface, indicating that the vesicles formed by P132L are very much akin to the wild-type Cav-1 both in terms of self assembly and function. Thus, the P132L appears to function in a normal fashion very much like the wild-type Cav-1.

In summary, our data, for the first time, suggests that the mutants of Cav-1, especially the P132L, although shown to have misfolded oligomerization and retained in golgi, exited the ER/golgi network. This could be one of the reasons why the UPR activation was unsuccessful as the cells have adapted for survival. This argument is also consistent with the physical control of the P132L vesicles by the KIAA0999 and MAP3K2 kinases. The role for

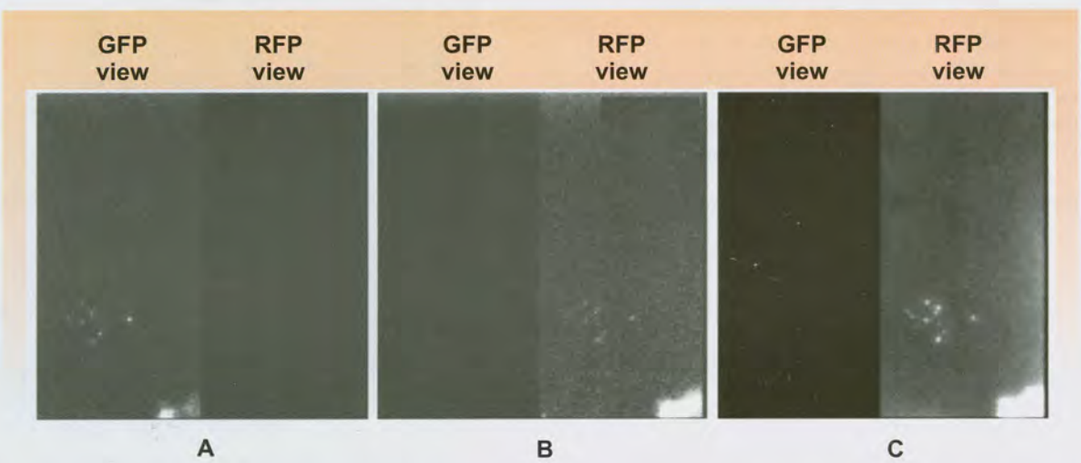

Fig. 3. Hetero-oligomerization of P132L with wild-type Cav-1 in HEK-293-T by TIRFM. HEK-293-T cells were seeded on TIRFM cover-glasses and co-transfected with Cav-1-RFP wild-type and P132L-GFP mutant and processed after 24 hours of transfection for TIRFM recordings. A beam splitter is used to split the GFP and RFP signals for simultaneous visualization. The left and right panes respectively represent GFP and RFP signals. Controls used for this visualization are shown in Panel A was obtained with GFP excitation which shows no signal in the RFP field. Panel C was obtained with RFP excitation which shows the presence of Cav-1-RFP protein in the cell. All the videos were collected at 25 frames per second using 60X objective.

Future Work

We will be investigating the kinases involved in regulating the dynamics of caveolae with the help of the external docking proteins and stimulants to know the role of caveolae vesicles in membrane repair pathway initiation.

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

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Background

High glucose-induced cardiac apoptosis plays an important role in diabetic complications. However, the molecular mechanisms of cardiac damage by glucose are incompletely understood. Perhaps, the cytotoxic actions of glucose are mediated, in part, through oxidative stress and intracellular Ca^{2+} overload. These two insults are linked to each other, viz., the oxidative stress induces an increase in $[Ca^{2+}]i$ while the increase in $[Ca^{2+}]i$ increases oxidative stress. Furthermore, mitochondria being a storehouse for intracellular calcium, a source of reactive oxygen species (ROS), and a sensor of oxidative stress, play a key role in regulation of apoptosis under a variety of pathological conditions including diabetes. However, the sequence of cellular events that initiate Ca^{2+} influx leading to Ca^{2+} overload and oxidative stress ultimately leading to cardiac cell death due to high glucose remain unknown. It is suggested that Ca^{2+} overload can cause cytotoxicity and trigger apoptotic cell death by activating two pathways, the mitochondria-dependent and -independent pathway. The mitochondria-dependent pathway involves generation of Reactive Oxygen Species (ROS), opening of permeability transition pores, loss of mitochondrial membrane potential, release of cytochrome c from mitochondria and activation of caspase-9 and -3. The mitochondria-independent pathway involves calpain, a Ca^{2+}-dependent cysteine protease that activates caspase-12 localized on the cytoplasmic side of ER. We studied the precise mechanism(s) of high glucose mediated cardiac cell death.

Aims and Objectives

1. To study the precise mechanism(s) of high glucose induced calcium mobilization, mitochondrial calcium overload and subsequently mitochondria-dependent and -independent cell death cascade in cardiac myoblast H9c2 cells.
2. To elucidate the role of Ca^{2+} in calpain activation and caspase deployment in high glucose mediated cell death.

Work achieved

During the last year we had studied and reported 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 cardiac fatigue. It also causes increased intracellular calcium levels, mitochondrial dysfunction, and oxidative and

nitrosative stress in H9c2 cells, producing a dose-dependent growth inhibition. This work provided valuable insight into CMZ- induced cardiotoxicity.

Further, we also studied the precise mechanism(s) of high glucose induced apoptosis in cardiac myoblast H9c2 cells. In the present study, we investigated the role of mitochondrial Ca^{2+} accumulation in initiation of mitochondria-dependent and -independent apoptotic pathways involving calpain activation and caspase recruitment in high-glucose treated rat ventricular myoblast H9c2 cells. Compared with 5.5mM glucose, the cells treated with 33 mM glucose for 96h revealed an increase in the percentage of apoptotic cells in high glucose treated cells (Fig. 1A). Cardiomyocyte death exhibited several classical markers of apoptosis including increased population of cells with sub-G0/G1 DNA content (Fig. 1B), appearance of annexin V on the outer plasma membrane, DNA condensation, and subsequent activation of caspases and cleavage of PARP (Fig. 3E). A

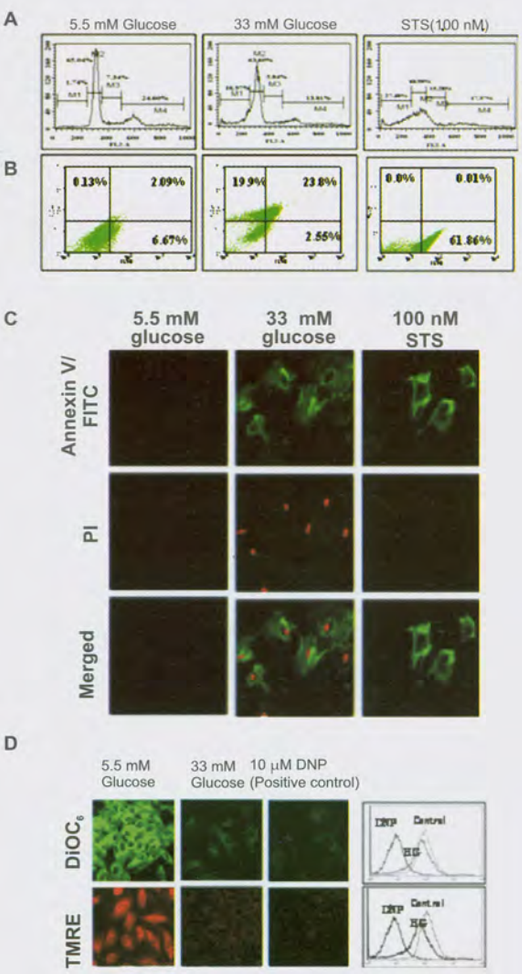
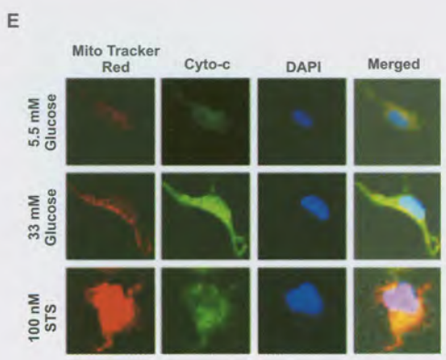


Fig.1. Effect of high glucose on H9c2 cells. **(A)** Quantitative determination of cell cycle by PI staining on FL-2A channel of flow cytometer. H9c2 cells were treated with 5.5 and 33 mM glucose for 72 h and various phases of cell cycle were quantified. **(B)** Quantitative estimation of high glucose induced apoptosis for 72 h. H9c2 cells were treated 5.5 mM and 33 mM glucose for 72 h. **(C)**Qualitative annexin V-FITC/PI staining and bright field imaging of H9c2 cells treated with 5.5 and 33 mM glucose.**(D)** Detection of loss of mitochondrial membrane potential on H9c2 cells treated with high glucose. Confocal laser scanning images and flow cytometry overlay of cells stained with DiOC6 and TMRE. **(E)** Cytochrome c immunostaining after treatment of H9c2 cells with high glucose for 48 h. Nuclear condensation visualized with DAPI staining. Staurosporine served as positive control for the experiment. Representative of 25-30 cells each in 3 separate experiments



positive control treatment with 100 nM staurosporine for 20 min also caused significant increase in apoptotic population (Fig. 1A), with substantial changes in H9c2 cell morphology with the appearance of cell body shrinkage observed after 72 h exposure. Substantial phosphatidylserine externalization was also detected in cells exposed to 33 mM D-glucose and staurosporine using annexin V (Fig. 1C). However, cells exposed to 33 mM mannitol did not show increased numbers of apoptotic cells.

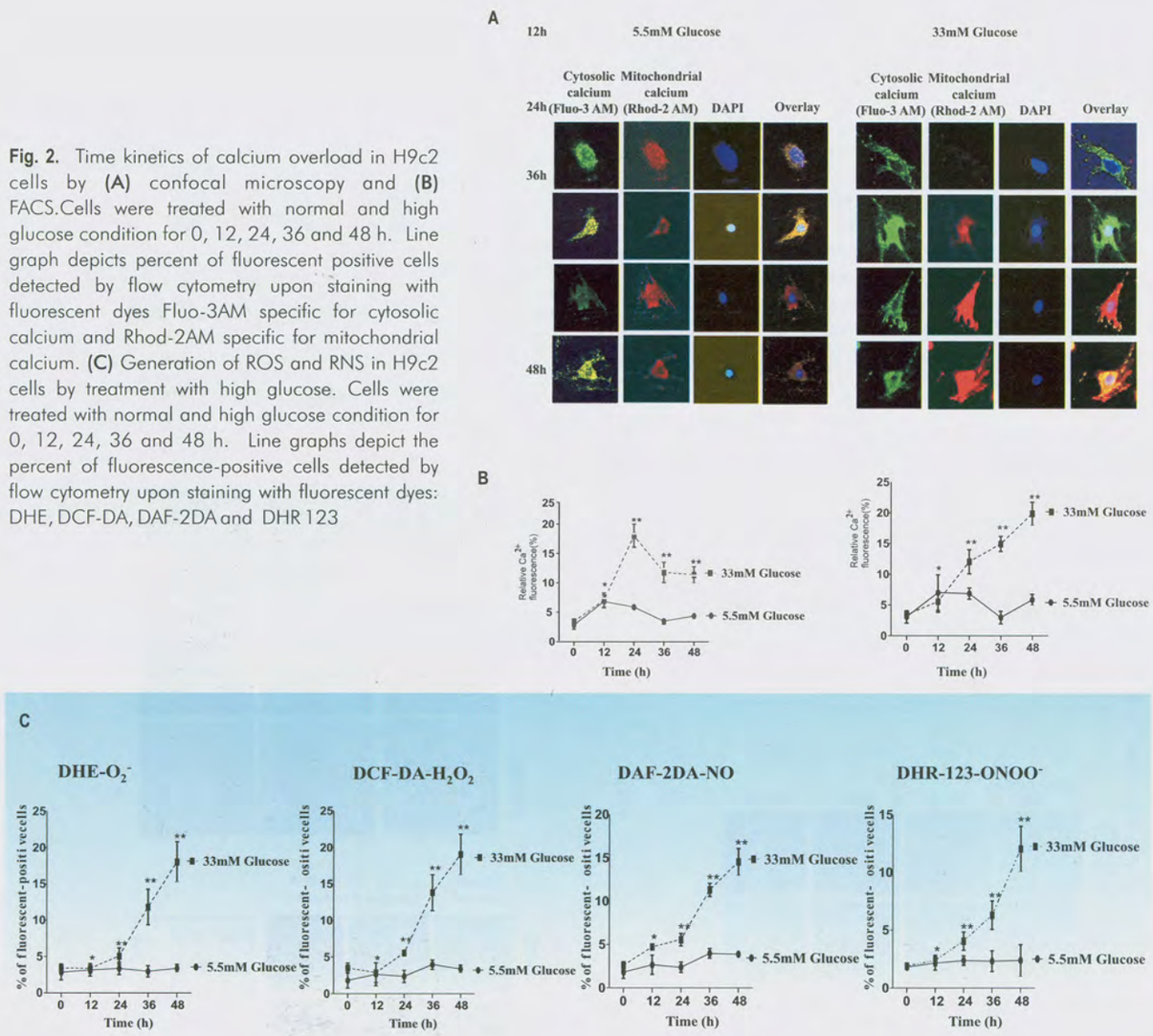


Fig. 2. Time kinetics of calcium overload in H9c2 cells by **(A)** confocal microscopy and **(B)** FACS.Cells were treated with normal and high glucose condition for 0, 12, 24, 36 and 48 h. Line graph depicts percent of fluorescent positive cells detected by flow cytometry upon staining with fluorescent dyes Fluo-3AM specific for cytosolic calcium and Rhod-2AM specific for mitochondrial calcium. **(C)** Generation of ROS and RNS in H9c2 cells by treatment with high glucose. Cells were treated with normal and high glucose condition for 0, 12, 24, 36 and 48 h. Line graphs depict the percent of fluorescence-positive cells detected by flow cytometry upon staining with fluorescent dyes: DHE, DCF-DA, DAF-2DA and DHR 123

While studying the molecular mechanisms of cardiac apoptosis by glucose, it was observed that the earliest biochemical event subsequent to treatment with high glucose was rise in the intracellular calcium and its further accumulation into the mitochondria. Our confocal and FACS results using two specific probes Fluo-3 (for cytosolic Ca^{2+}) and Rhod-2AM (for mitochondrial Ca^{2+}) revealed a time-dependent rise in intracellular Ca^{2+} for up to 24h, after that a rise in the accumulation of mitochondrial Ca^{2+} was observed for up to 48 h in H9c2 cells exposed to 33 mM glucose (Fig. 2A and B). Concomitant to this was an increase in the generation of reactive oxygen species (ROS- i.e.O_2^- and H_2O_2, detected using the membrane-permeative fluorescent dyes DHE and DCF-DA.) and reactive nitrogen species (RNS-- i.e.NO and $ONOO^-$, detected using fluorescent dyes DAF-2DA and DHR-123) (Fig. 2C) further leading to decrease in the mitochondrial membrane potential ($\Delta\Psi m$), detected using $DiOC_6$ and TMRE (Fig. 1D) and release of cytochrome c into the cytosol (Fig. 1E).

The study further evinced the mechanism of calcium-dependent cysteine protease calpain-1, which triggers non conventional caspase cascade as alternate mode of cell death.

It is suggested that Ca^{2+} overload can cause cytotoxicity and triggers apoptotic cell death by activating calpains. It is also shown that 33 mM glucose induces apoptosis in cardiomyocytes via activation of calpain-1 but not calpain-2. Our western blot results indicate that increasing concentrations of calcium (10 nM, 1 μM) for 1 h led to proteolytic cleavage of caplain-1. This proteolytic activity of calpain-1 was also observed by 1 h treatment of H9c2 with THG (2 μM), a known calcium influx promoter. The proteolytic activity of the extracellular Ca^{2+} and THG was inhibited by the calcium chelator EGTA (10 μM). These results indicate that intracellular calcium accumulation is required for activation of calpain-1 (Fig. 3A).

It is also demonstrated that, H9c2 cells treated with 5.5 mM glucose did not show activation of calpain-1, however, treatment of the cells with high glucose for 72 h induced activation of calpain-1. This activation of calpain-1 was also preventable by EGTA indicating that activation of calpain-1 is a result of high glucose induced Ca2+ (Fig 3A). Further, the calpain-1 specific inhibitor (ALLN) also prevented proteolysis activity under 33 mM glucose environment indicating that proteolytic activation of calpain-1, which is an important event in cardiac cell death, is indeed because of the 33 mM glucose treatment (Fig 3B). Fig. 5 shows representative proteolytically cleaved calpain-1 fragment. β-actin was used as loading control for the experiment.

Previous studies have shown activation of calpain-1 by high glucose, and it is also known that calpain activates the cytosolic effector caspase-12. Therefore, we hypothesized that high glucose induced activation of Calpain-1 in our study may lead to the cleavage of procaspase-12 into active caspase-12 affecting its downstream targets. The results of our study showed that 33 mM glucose cause activation of caspase cascade via activating calpain-1. Western blot results indicated that H9c2 cells treated with high glucose for 72

h caused cleavage of procaspase-12 and procaspase-9 while normal glucose neither showed activation of effector caspases (caspase-12 and caspase-9). Treatment of H9c2 with Ca^{2+} (1 μM) and THG (2 μM) showed cleavage of caspase-12 and caspase-9. This cleavage was however inhibited when the cells were treated with EGTA (Fig. 3C).

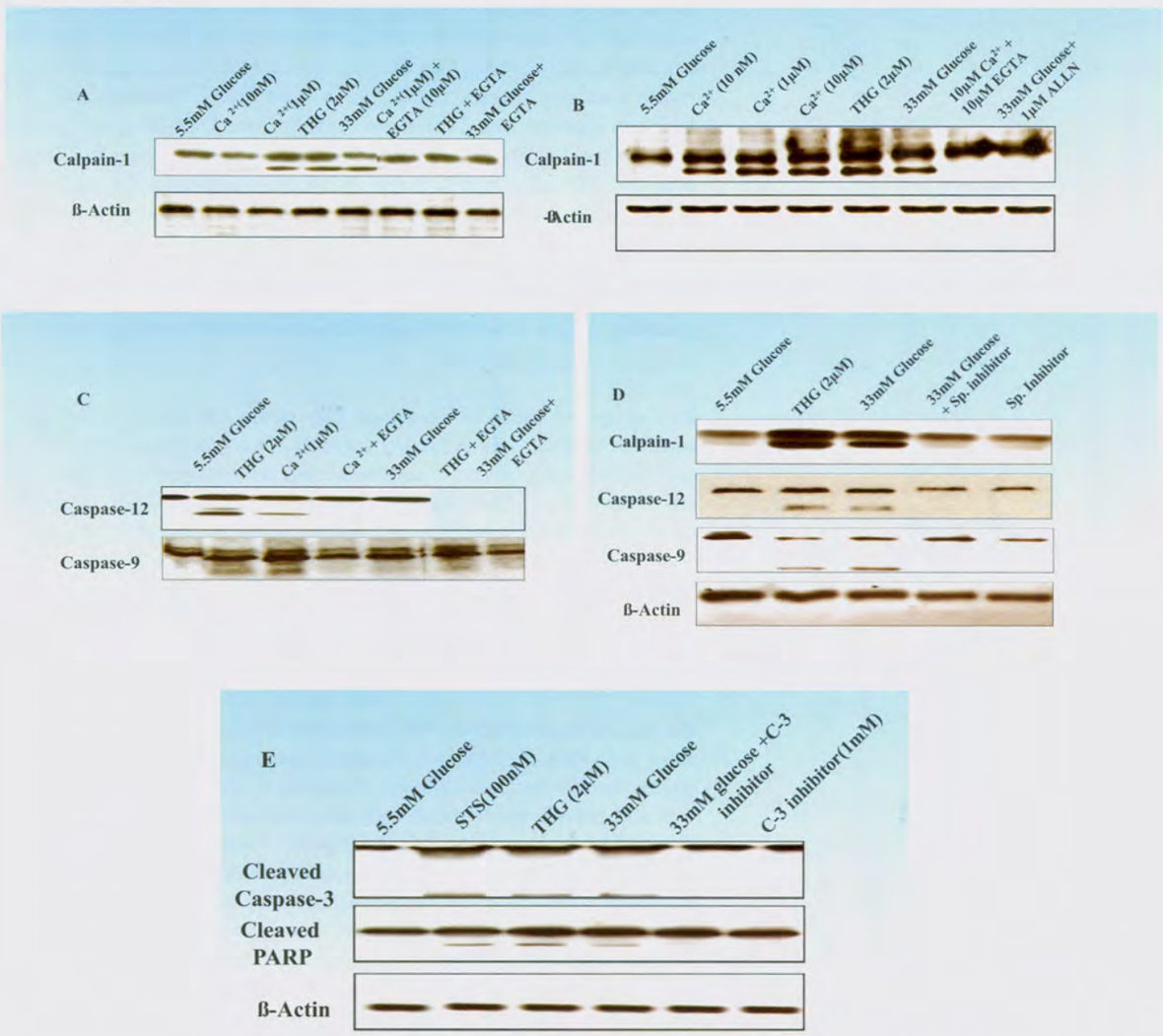


Fig. 3. Effect of calcium and high glucose on **(A)** proteolysis of calpain-1. H9c2 cells were treated with 5.5mM glucose **(B)** Effect of calcium overload and high glucose in proteolysis of calpain-1. **(C)** Activation of caspase 12 and caspase 9 by high glucose. **(D)** Activation of ancillary caspase cascade by high glucose via calpain proteolysis. **(E)** High glucose induces apoptosis via caspase 3 activation and PARP cleavage. Data representative of best of three separate experiments. Actin served as loading control.

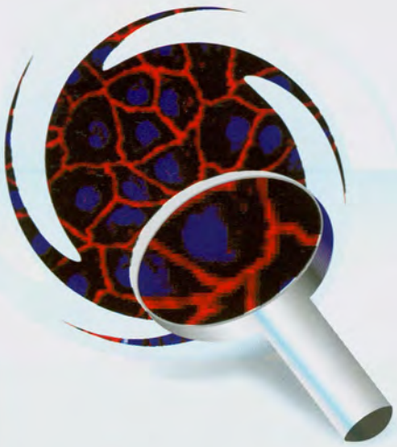
Next, we examined the possible involvement of effector caspases in high glucose-induced apoptosis. It was observed that calcium overload caused the activation of caspase-12 (Fig. 3C). Western blot results indicated that the cells treated with 5.5 mM glucose neither showed activation of calpain-1 nor activation of effector caspases (caspase-12) while a 72 h treatment with 33 mM glucose induced activation of calpain-1 along with cleavage of procaspase-12 in H9c2 cells. This cleavage of procaspase-12 was prevented by the pancaspase inhibitor (Z-VAD-FMK). Thus, 33 mM glucose caused activation of caspase cascade via activating calpain-1. Further we observed that both THG as well as 33 mM glucose activated caspase-9. Interestingly, cleavage of procaspase-9 was prevented by pancaspase inhibitor in 33 mM glucose condition. Apparently, activated caspase-9 was not observed in 5.5 mM glucose treatment (Fig. 3D).

Western blot results for expression of caspase-3 and PARP showed cleaved fragment in cells treated with staurosporine (100 nM), THG (2 μM) for 1 h and high glucose (33 mM) for 72 h (Fig. 3E). However, pretreatment of cells with caspase-3 inhibitor (1 mM Z-DEVD-FMK) prior to 33 mM glucose treatment prevented cleavage of procaspase-3, thus indicating that caspase-3 activation via calpain-1 plays crucial role in high glucose induced apoptosis in H9c2 cells.

Taken together the data elaborates for the first time that high glucose induces apoptosis by both mitochondria-dependent and independent pathways via concomitant rise in intracellular calcium $(Ca^{2+})i$. This information increases the understanding of cardiac cell death under hyperglycemic condition and can possibly be extended for designing new therapeutic strategies for treatment of diabetic cardiomyopathy.

Future work

1. Study the effect of calcium channel blockers on oxidative and nitrosative stress in diabetic rat.
2. Regulation of cytosolic and mitochondrial ROS: A cross talk between calcium and ROS in diabetic cardiomyopathy and therapeutic efficacy of Cu-Zn-SOD and Mn-SOD mimetics.



Endothelial cell disorders in diabetes

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Background

Coronary artery disease is the most prevalent heart disease that occurs because of stenosis / narrowing and blockage of coronary arteries, restricting the blood flow to myocardium (heart muscles). Although several treatment strategies for coronary artery disease are in use, it is known to be the cause of over 7 million deaths/year worldwide. These treatments include medical management (Statins, antihypertensive, smoking cessation as well as blood glucose control in diabetic conditions), percutaneous coronary interventions (PCI: balloon angioplasty, coronary stents, drug eluting stents and other devices to treat chronic total occlusions) and coronary artery bypass graft (CABG) surgery. CABG involves surgical removal of blood vessels (usually saphenous vein) from patient's body and grafting this to the coronary arteries so as to bypass the atherosclerotic narrowing in order to improve blood supply to the myocardium.

Autologous grafts are normally used in such surgeries but in case of 30-40% of patients, these vessels are unsuitable for surgery. With this limited availability of autologous blood vessels, synthetic grafts have gained popularity and have been in regular use as the only alternative. The materials of choice are ePTFE (expanded poly tetra fluoro ethylene), Dacron (PET- poly ethylene tetrapthalate), and polycarbonate polyurethane (PU). However, though these vessels are biocompatible, they lack endothelial cell lining, which results in poor patency of such synthetic vascular grafts.

Endothelial cells provide a physical interface between blood and surrounding tissues and also maintain a haemostatic-thrombotic balance that regulates inflammation and angiogenesis. It is proposed that endothelialization of artificial prosthesis using autologous vascular endothelial cells would help in improving the patency rates of these grafts. There is therefore a need to change the surface properties of synthetic small caliber vascular grafts to avoid platelet aggregation along with better endothelialization. The other limitation is to achieve large numbers of transplantable autologous human endothelial cells. In this study, we report the generation of small diameter ePTFE tubings seeded with human endothelial cells. We also discuss herein our ongoing research in understanding endothelial cell dysfunction in diabetes.

Aims and Objectives

1. To achieve endothelialization of small diameter vascular ePTFE grafts using human endothelial progenitor cells
2. To assess markers of endothelial dysfunction in diabetes

Work Achieved

It is proposed that the development of an autologous hybrid vascular prosthesis (synthetic vascular grafts seeded with patient's own endothelial cells) would improve the outcome of cardiac bypass surgeries. Synthetic grafts such as ePTFE, dacron and polyurathane are durable and nonimmunogenic materials of choice. Several reports demonstrated till now have confirmed that coating of synthetic grafts with endothelial cells can reduce thrombosis and hyperplasia on engraftment. However, one of the major issues in making this possible is adhesion and growth of endothelial cells over the luminal surface of these biomaterial surfaces,

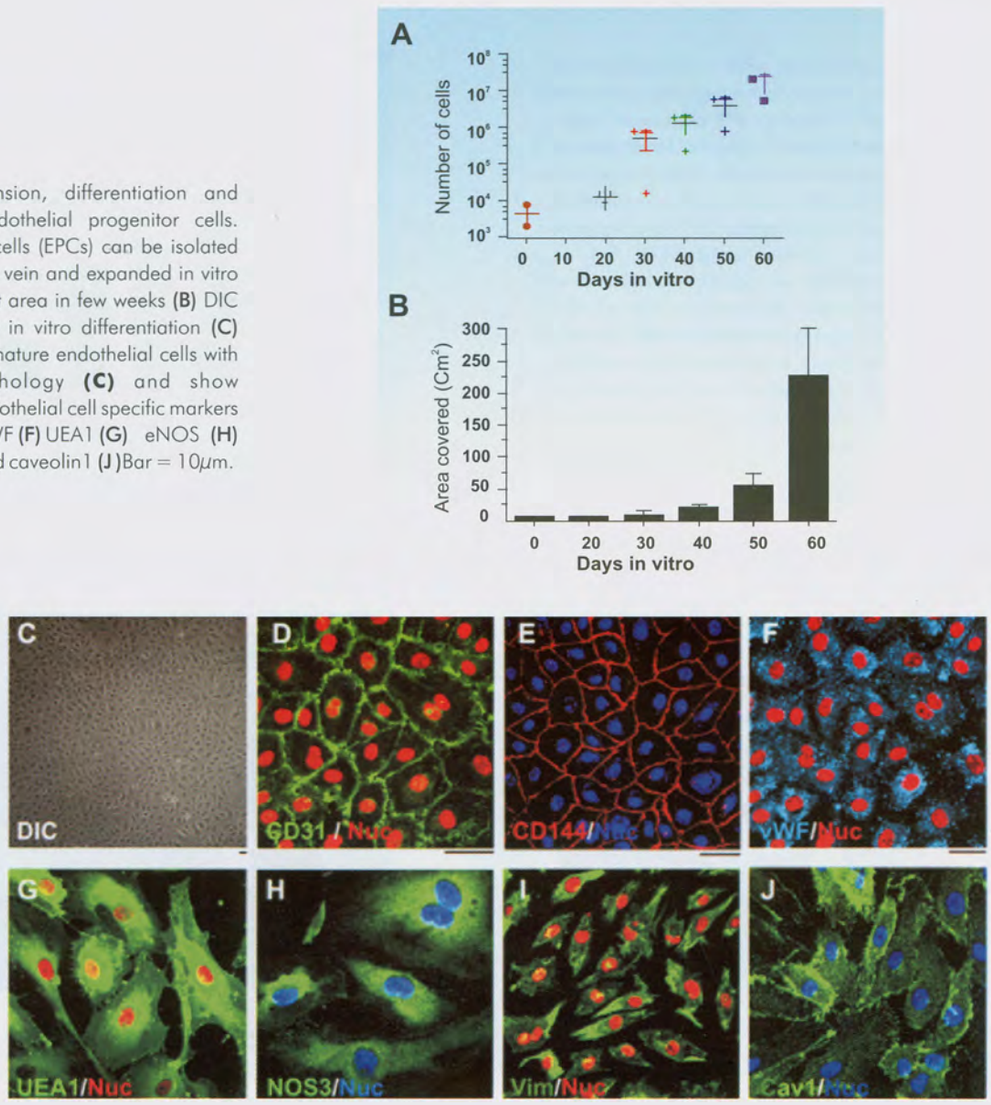
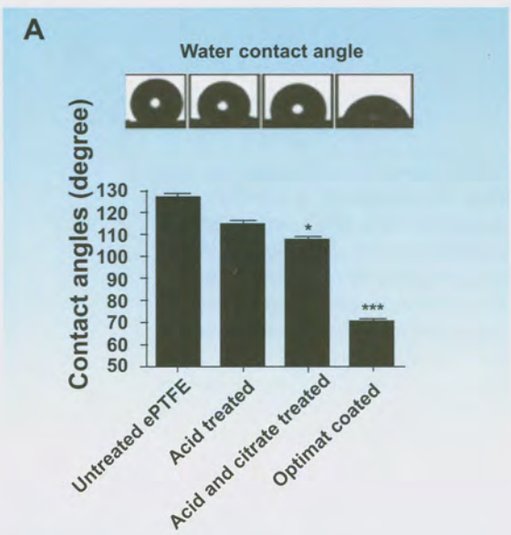
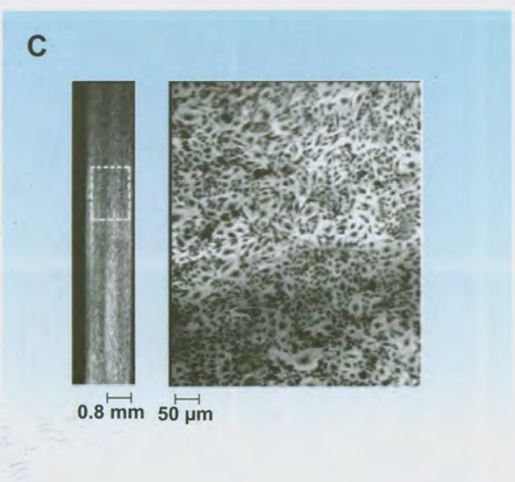


Fig. 1 In vitro expansion, differentiation and characterization of endothelial progenitor cells. Endothelial progenitor cells (EPCs) can be isolated from human saphenous vein and expanded in vitro **(A)** to occupy significant area in few weeks **(B)** DIC images of EPCs during in vitro differentiation **(C)** EPCs differentiate into mature endothelial cells with cobble stone morphology **(C)** and show immunopositivity for endothelial cell specific markers CD31 **(D)** CD144 **(E)** vWF **(F)** UEA1 **(G)** eNOS **(H)** as well as Vimentin (I) and caveolin1 **(J)** Bar = 10μm.

especially small diameter vascular grafts. Several protocols to seed endothelial cells on ePTFE surfaces have been described after coating the synthetic grafts with different proteins, peptides or metals.

We observe that human endothelial progenitor cells (EPCs) can be obtained from human saphenous vein , or carotid artery and can be used to differentiate into mature endothelial cells. We show here that EPCs can be isolated and expanded in vitro under xeno-protein-free conditions. Cells retain CD133 expression during expansion and then differentiate into mature CD144+ cells. These cells can be expanded around a million-fold in human AB serum supplemented medium (Fig. 1). We then observed that





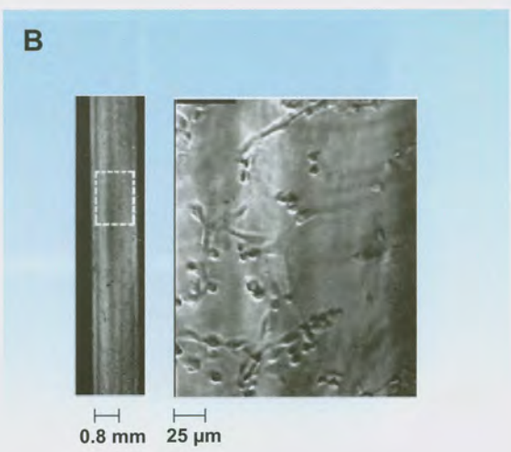


Fig. 2. Optimat coated ePTFE tubings show better hydrophilicity. Water contact angle measurements of untreated ePTFE tubings and after each stage of treatment are shown in **(A)** Acid-treatment and OptiMat-coating reduced the contact angle to as low as 70° indicating conversion of hydrophobic surface (water contact angle 125°) to a hydrophilic surface (water contact angle 70°). For easy visualization of cells, we used plastic capillaries with similar hydrophobic properties to that of ePTFE tubings. We observed that endothelial cells show minimal attachment to untreated surfaces **(B)** but better adhesion and growth over acid-treated and OptiMat-coated surfaces **(C)** White dotted boxes in capillary images on left side in panels B and C are enlarged to visualize cell growth (shown on right side) in each capillary.

the hydrophobic properties of ePTFE can be modified to render them hydrophilic following acid-treatment and coating with a defined combination ("OptiMat") of ECM components (Fig. 2). This combination was seen to be more potent in achieving better adhesion than individual components alone. We observe that treatment of the ePTFE surface with acidified buffer facilitates binding of OptiMat components to these synthetic grafts. Coating of the ePTFE surface decreases the blood platelets adhesion and their activation (CD62P or p-selectin immuno-positivity) by at least 8-fold as compared to untreated ePTFE grafts. Further analysis of change in specific functional group(s) in ePTFE is being carried out and will help to elucidate the cause of the differential platelet adhesion after treatment and OptiMat coating.

Future Work

During diabetes, increased circulating glucose concentrations and fluctuations in blood osmolarity as well as free radicals produced from advanced glycosylated end products (AGEs), lead to endothelial damage. Although endothelial damage has been studied in other conditions the early markers of such damage to endothelium in diabetic conditions yet remains unknown. Investigation of endothelial specific soluble receptors as well as secretory molecules in hyperglycemia condition may help in early diagnosis of vascular diseases. We plan to carry out proteome analysis of endothelial cells exposed to hyperglycemia in vitro. We plan to assess differential production of a subset of these proteins that appear to be regulated in hyperglycemia by using 2 dimensional (2D) gel electrophoresis and MALDI analysis. These studies can provide potential markers for assessment of early damage to endothelial cells in diabetes.

Research Reports

Biodiversity

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Insect and Microbial Genomics

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Background

Microbial Genomics

Microorganisms represent about half of the global biomass as they are ubiquitous and form predominant form of life on earth. They not only outdo the eukaryotic cells in number but 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. Initially, 16S rRNA sequence studies enabled us to have a glimpse of this diversity. In the recent years high-throughput sequencing technologies enable us to have a better understanding of uncultivable majority of microbes. Our laboratory uses these methodologies to understand community structure and function of some unique ecological niches.Insect Genomics:

Malaria is caused by mosquito mediated infection of protozoan parasites, Plasmodium and is one of the deadliest tropical diseases, affecting 200-300 million people worldwide with a mortality rate of ~1 million people every year. Evolution of insecticide-resistant mosquitoes and drug-resistant parasites make the control of the disease difficult. 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. We are involved in the genome analysis of this mosquito species.

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

Study of Midgut bacteria of Aedes mosquitoes and their role in the infectivity of dengue virus

Comparative analysis of bacterial communities from midgut of three Aedes aegypti strains having differential ability to transmit dengue virus was done:

MOYO-R (Refractory strain), MOYO-S (susceptible strain), and MOYO-D (Refractory strain). Pseudomonas related clones dominated the libraries of the three strains. There was significant difference in the libraries of three strains as Pedobacter and Janthinobacterium were present only in MOYO-R strain libraries. Bacillus species were found in MOYO-S and MOYO-D strains libraries were not found in MOYO-R strain libraries and Rahnella found in MOYO-R and MOYO-D strains libraries was absent in MOYO-S libraries. Comparative analysis of midgut bacteria from virus infected and un-infected Aedes aegypti mosquitoes was performed but no significant difference was observed as Serratia and Asaia were present in both the cases.

16S rDNA clone library based comparative analysis of midgut bacterial communities from different growth stages of Aedes aegypti was carried out and Serratia, Pseudomonas, Rahnella, Asaia, Gluconobacter were detected in different stages of mosquito life cycle. A bacterial community shift from Rahnella to Asaia was observed during the development. Real time PCR based estimates showed increase in the count of Asaia.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 compare to other naturally occurring bacteria, and therefore more suitable candidate for future paratransgenesis strategies.Phylogentic profiling and molecular characterisation of microbiota associated with housefly (Musca domestica) gut

Houseflies (*Musca domestica* L.) are cosmopolitan, ubiquitous, synanthropic insects and act as mechanical or biological vector for various microbial agents. The study was aimed at identifying bacterial complexity of housefly gut by both culturable and culture independent approaches. Total 136 bacterial strains could be isolated from the gut of houseflies collected from various places such as Public park, open garbage dump area, public lavatories, hospital, residential area etc. Multifactorial identifications based on phenotypic, biochemical, molecular and phylogenetic analysis placed these isolates into 20 different genera. Majority of the isolates were affiliated to known pathogens such as Klebsiella, Aeromonas, Shigella, Morgenella, Providencia and Staphylococci. Culture independent studies using 16S rRNA gene library complemented these results. However, additional bacterial taxa such as α-Proteobacteria, δ-Proteobacteria and Bacteroidetes were seen in the16S rRNA library. We demonstrated that the housefly gut is a reservoir of previously unreported pathogenic bacteria. This is the first study wherein we identified housefly gut microbiota from diverse sampling sites using culturable as well as culture independent methods. The study has a medical significance and would direct future research addressing the role of housefly gut microbiota in relation to disease outbreaks and spread. This in turn will help in designing new therapeutic treatments for housefly borne diseases.

Two new species of the genus Ignatzschineria, namely Ignatzschineria indica sp. nov. and Ignatzschineria ureaclastica sp. nov. were described from the adult flesh fly (Diptera: Sarcophagidae)

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.

Monitoring of development of gut flora in human infants

The development of the gut flora in the normal delivered breast fed infants starting from the day 0 up to the first year of their life was monitored. It was found that colonization starts immediately after the birth. Initially with the normal environmental microbes and microbes from the mother, and it goes through rapid changes till a stable flora established, i.e., up to one year.

Effect of mode of delivery on the gut microflora of infants

Fecal microbiota of vaginally born (VB) infants was found to be distinctly different from their counterpart cesarean section born (CB) infants. The most abundant bacterial species present in VB infants were Acinetobacter sp., Bifidobacterium sp. and Staphylococcus sp. While CB infants fecal microbiota was dominated with Citrobacter sp., Escherichia coli and Clostridium difficile. An interesting finding of our study was recovery of large number of Acinetobacter sp., a noso-comial pathogen, in the feces of the VB infants. Athough none of the infants had shown any clinical symptoms of disease, this observation emphasizes the potential risk of Acinetobacter related epidemic outbreak in infants from this region. In addition variation in the microbial community structure due to genetic and environmental factors is being investigated.

Lonar Lake

Stable Isotope Probing reveals acetate-utilizing bacteria from highly alkaline lake sediment.

Acetate is an important intermediate in the decomposition of organic matter in anoxic fresh water, soda alkaline lakes. The lonar lake sediment samples were incubated in presence of [U-13C] acetate for a period of 3 days under anaerobic conditions. The sediment samples were collected from three different sites in three seasons - summer, winter and rainy, with the aim of studying the spatial and temporal variation in the microbial diversity. DNA isolated from all labeling experiments were subjected to density gradient centrifugation for the separation of labeled DNA from unlabeled DNA using Peristaltic pumps. The Gradient DNA's were purified and further used for diversity analysis by means of a single strand conformational polymorphism SSCP). The SSCP profiles generated in the pilot scale experiment showed sufficient incorporation of 13C sodium acetate.

On the basis of the results from pilot experiment; sediment samples from all three sites and seasons were pulsed with 13C and 12C sodium acetate and

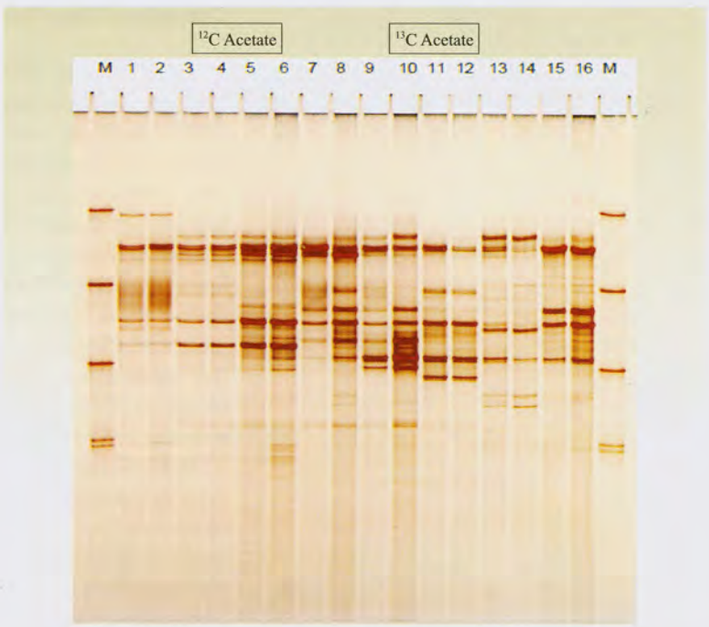

Fig. 1. SCP gel comparison of (13C sodium acetate incubation) heavy DNA fractions 6 and 7 of all sediment samples.
Lanes 1,2- Season3 Site 1,
Lanes 3,4- Season 3 Site 2,
Lanes 5,6- Season 3 Site 3,
Lanes 7,8- Season 2 Site 1,
Lanes 9,10- Season 2 Site 2,
Lanes 11,12- Season 2 Site 3,
Lanes 13,14- Season 1 Site 1,
Lanes 15- Season 1 Site 2,
Lanes 16- Season 1 Site 3.

incubated for 3days. The SSCP profiles so generated indicated that the 13C sodium acetate is being utilized at all sites. Fig. 1 depicts the comparison of representative heavy DNA fractions from all sediment incubation experiments. Stable-isotope probing (SIP) was also carried out using methane, methanol and methylamine. Denaturing gradient gel electrophoresis fingerprinting analysis was done on the heavy 13C-labelled DNA extracted from sediment to confirm the enrichment and labelling of active methylotrophic communities. Also, 16S rRNA and functional gene clone libraries were constructed.

Methylomicrobium, Methylophaga and Bacillus spp. were identified as the predominant active methylotrophs in methane, methanol and methylamine SIP microcosms, respectively. Lack of mauA gene amplification in the methylamine SIP heavy fraction also suggested that methylamine metabolism in Lonar Lake sediments may not be via methylamine dehydrogenase enzyme pathway. A large number gene sequences from this study did not show any affiliation with extant methanotrophs or methylotrophs. Thus, they may represent hitherto uncharacterized novel methylotrophs or heterotrophic organisms that may have been cross-feeding on methylotrophic metabolites or biomass. This study is an important landmark in understanding the relevance of methylotrophy in the soda lake sediments of an impact crater lake.Insect Genomics:

Characterization of the EST library

As described in the previous report, we have generated a partial EST library from 16-22 hrs Anopheles stephensi embryos. All ESTs are submitted in NCBI GenBank database with accession numbers, FL483337 to FL484476. The sequenced ESTs were classified into 6 classes based upon their putative function (Table 2): I (Energy and Metabolism); II (Cytoskeleton and cell structure); III (Intracellular trafficking and vesicular transport); IV (Protein synthesis machinery); V (Signal transduction) and VI (Conserved function). Some of the most notable transcripts in the library are, transcript coding for a Membrane-associated guanylate kinase (MAGUK) family protein (FL484404), profilin homologue (FL483965) which is implicated in the gastrulation movements and play component roles in directing filopodial protrusion and other aspects of epithelial and amnioserosa cell behavior in Drosophila and also the putative homologues of Flightless I (FL484405) and Rab6 (FL484226), both are implicated in the regulation of actin cytoskeleton. Some of the identified transcripts also belonged to components of chromatin dynamics/architecture. Transcript FL483522 shows homology to the Drosophila tautotis gene, which acts as neuronal regulator. Only 3% of the transcripts were classified as those playing role in intracellular trafficking and vesicular transport. Notable among these transcripts are those coding for GAP junction forming-Innexin family protein (FL484321) and a NIEMANN pick type C2 protein NPC2-related (FL483726) both showing homology to ENSANGP00000020577 and ENSANGP00000017003, respectively. Further characterization of these transcripts is in progress.

Characterization of putative boundary elements from the Anopheles stephensi

It is thought that changes in the regulatory elements are responsible for bringing about phenotypic diversity of animal forms. The regulatory elements in one chromatin domain exert their positive and negative effects exclusively on the promoters located with in the same domain. The chromatin domain boundary elements mark, separate and insulate adjacent domains from the influence of the regulatory elements from neighboring domains. In order to check the functional conservation of these elements, we have identified and tested putative enhancer blocking elements from the hox sequence of Anopheles stephensi. The elements were identified using an in-house designed algorithm (Dr. Rakesh Mishra group, CCMB, Hyderabad) and cloned in enhancer blocking assay vectors and tested in transgenic flies.

To check the boundary activity, embryos were stained with β-galatosidase and staining pattern were compared with controls. To compare the relative levels of staining for each transgenic line, stainings were preformed simultaneously in the same grid. Vector line 56.68 was used as positive control. All the stainings were repeated for minimum of 3 to 4 times to observe consistent pattern and then placed into a specific category.

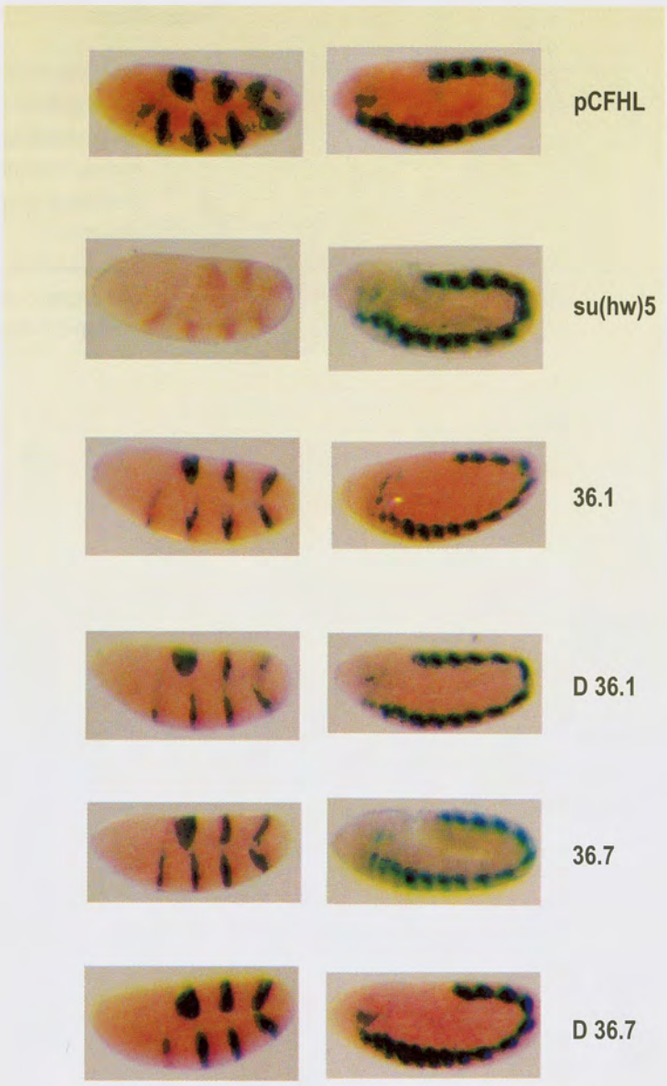

Fig. 2. Panel showing results of the boundary element assay.pCfHL : control staining. Su(hw)5 : positive control. 5 binding sites of Su(hw) enhancer.36.1: line 1 showing staining pattern. D36.1: line with excised test element. 36.7: Line 2 showing staining pattern D36.7: Line with excised

Staining pattern indicated that the elements are acting as boundary element. When compared to control line, the expression of the β-gal gene product is less in the two transgenic lines 36.1 and 36.7. The staining pattern is comparable to the control lines when the element is excised.

Future Work

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

Research Reports

Bioinformatics & Proteomics

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Coupling QM/MM approach for incorporation of peptides in lipid coated

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Background

As we are all aware domains of modern computational science are converging on complex problems in the general field of systems biology. There is now a credible possibility of modeling drug delivery vesicles (liposomes) and their properties with qualitative and quantitative insight coming from atomistic calculations. Such a work will help extend accurate quantum mechanical (QM) methods to large-scale atomistic molecular mechanics (MM) modeling. Also, it will allow coarse-grain parameterization and, further on, mathematical models which are now developing in systems biology. Starting from Density Functional Theory (DFT) methods and recent theoretical studies of the properties of phospholipid molecules, consistent QM/MM and MM methodologies, as well as their counterparts as functions of time, Born- Oppenheimer dynamics (BOMD) and molecular dynamics (MD) will be generated. These methods will be applied to understand and predict the properties of lipid layers (bilayers), in particular, fluidity, and their evolution in the presence of ions (physiological conditions) and of additional biological agents. The strategy will be to build links between QM electronic-structure calculations at the finest molecular scale and equilibrium and non-equilibrium MM simulations to address mesoscopic system properties, using error control across the different scales. This strategy has not been used up to now in the domain of biochemical simulations, which explains the discrepancies found, for lipids, between atomistic QM and MM approaches. The reason for this situation comes from the fact that most of the MM parameterizations have been established on the basis of macroscopic experimental properties, most generally giving information at a time scale much larger than the simulation time of less than 50 ns (for example, NMR time scale is between 10^{-3} to 10^{-4}s according to the field frequency). The methodology developed will thus incorporate in the MM approaches, as much as possible, the information provided by QM studies of the properties which will be explored at the mesoscopic level. Thus, understanding the formation of vesicles from phospholipids bilayers and their fluidity and permeability

properties is the basis of a large number of applications in the domain of drug delivery, in particular release of the active species according to the pH or ionic concentration changes. Prediction of structural changes (phase transition in particular) of membranes by modification of one or several constituents or addition of external molecular species may have potential therapeutic applications.

Aims and Objectives

1. To create the initial structure of the phospholipid membrane by placing the required number of lipids in a solution (water) with the appropriate force fields.
2. To allow the system (lipids and water) equilibrate by running an initial simulation which may result in the formation of the well known lipid bilayer.
3. To generate consistent QM/MM and purely MM methodologies as well as their counterparts as a function of time Born-Oppenheimer Molecular Dynamics/Classical Molecular Dynamics in order to study properties of phospholipid membrane.
4. To integrate various methodologies and associated computer codes, such as density functional theoretic deMon2K code and different types of polarizable and reactive force fields (GROMACS, NAMD, CHARMM, ReaxFF).
5. To use the above methodology to understand and predict the properties of lipid bilayers, in particular, fluidity and its variation according to the nature of the lipids, addition of molecules inside the bilayer and presence of metal ions (Na^{+}, Ca^{2+}) in the solution by modeling of the lipid bilayer properties.

Work Underway

Configuration and Parameterization

Building phospholipid bilayer

The lipid bilayer was generated using a pre-equilibrated lipid membrane followed by energetic optimization and then the equilibrium run was carried out. Water molecules were specified by TIP3P parameterization and all lipids and water bonds were constrained with the SHAKE algorithm. Force field parameters were converted to GROMACS format and validation was

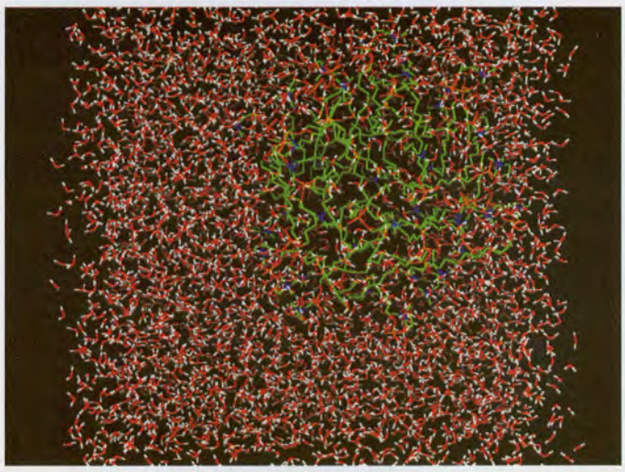

Fig.1 A snapshot of the simulated bilayer system consisting of 46 DMPC molecules and 1370 water molecules

ensured by comparison of individual energetic contributions to ensure proper porting. Equations of motion were inserted by using GROMACS simulation package.

The initial bilayer system configuration consisted of a 16X12.5X 9.8 nm3 box containing 46 DMPC molecules interspersed with 14 cholesterol molecules and solvated above and below with 1370 water molecules (Fig.1). The molecular composition of the leaflets is almost symmetric. The bilayer was parallel with the z-co-ordinate axes originating from the centre and extending 3.4nm into the aqueous phase. Periodic boundary conditions were used in all directions. vanderWalls and Coulomb cut-offs were set to 1.5nm, and the particle mesh Ewald summation was used for electrostatic interactions with the default associated parameters. Simulations were performed in the isothermal-isobaric ensemble at a temperature of a 308K and a pressure of 1 atm maintained by a Berendsen thermostat (τ=0.1ps) and Parinello- Rahman barostat (τ=1ps), respectively. Pressure coupling was applied isotropically to match the parameterization conditions. The sensitivity of the barostat scaling approach was tested by semi isotropic expansion in the xy-plane from that along the z-coordinate axis specifically designed for interface systems.

Interfacial behavior of Ergosterol and Lanosterol in mixture with DPPC and DMPC is also being executed simultaneously.

Potential of mean force calculations

To calculate the free energy values along the bilayer and to avoid local hysteresis, the system has to be minimized to eliminate bad contacts (Fig.2).

Further the system was equilibrated and calculations are being performed using constant force approach as implemented in the GROMACS pull code.

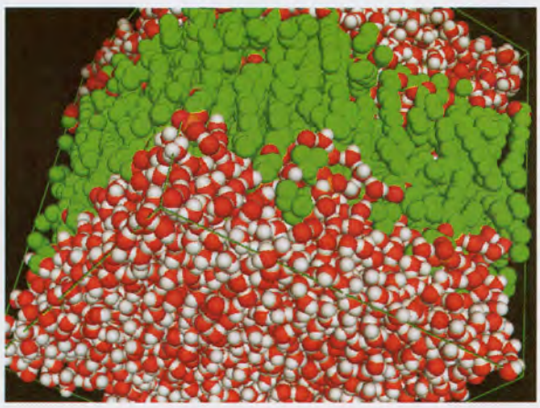

Fig. 2 Snapshot of the molecular cluster from 3:1 simulation. Solvent is omitted for clarity.

Furthermore, in order to determine molecular interactions of biomembranes, we are writing code in the Perl programming language too.

All graphics presented are generated with Gnuplot (Linux version)

MD simulation studies suggest changes in the large scale properties of the bilayer but these changes are of local nature. However, the average effect on lipid chain ordering and thermotropic behavior is small. The formation of ordered domains involves co-operative interactions in terms of complexes, which in turn form networks through hydrogen bonding pathways. The current state is therefore very encouraging as it provides deep insight into the nature of atomic scale phenomena with a level of detail missing in any experimental technique. As a consequence, there are now fascinating issues that needs to be resolved such as the issues of domains in the multicomponent bilayers. The related effects on multicomponent bilayer due to interdigitation phenomenon and others need to be explored. Work in this direction is underway.

Moreover, since the treatment of electrostatics is particularly important in lipid membrane systems, considerable attention is being paid to this issue. Two descriptions of partial charges are being employed. In the first case, we consider no charges and in the second approach, charges will be derived from ab inito quantum mechanical calculations using Gaussian with the Hartree-Fock method.

Developing new mass spectrometry based methods to study the metal binding sites of metalloproteins and protein-protein interactions

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Background

Metalloproteins play an important role in the chemistry of life processes, such as dioxygen transport, electron transfer, catalysis, etc. Metal ions in these proteins are used to perform a wide variety of specific functions related with these processes. How a particular metal ion executes these distinct functions highly depends on fine-tuning by the protein, which is accomplished by the coordination structure around the metal. The type, orientation, and number of amino acids or ligands around the metal very much influence the general reactivity of the metal center. With this in mind, an important step in understanding the chemistry of a metalloprotein is determining the coordination environment around the metal. Several techniques are available to provide this information, such as various X-ray techniques, NMR, EPR, and Raman spectroscopy, but these techniques are usually not very sensitive and often require pure samples. Because of its sensitivity and structural characterization capability, mass spectrometry (MS) has had a well-recognized impact on the analysis of peptides and proteins. The structural information that this technique has provided for metalloproteins so far, however, has not extended much to coordination structure. Metal-catalyzed oxidation (MCO) reactions generate reactive oxygen species (O^{2-} or $OH^{\cdot}$) through the redox cycle. The reactive oxygen species can interact with nearby amino acid residues and can oxidize or cleave the polypeptide at sites where the reactive oxygen species are generated. MCO reactions are caged processes in which amino acid residues at the metal binding sites are specific targets. The reactive oxygen species are hindered from diffusing into the surrounding medium because they react quickly with amino acid residues near the metal binding site. MCO reactions combined with MS provide a powerful means of characterizing the metal binding site of proteins, especially given the sensitivity of MS. The general approach of combining MCO reactions and MS involves first oxidizing the protein under the appropriate reaction conditions. The oxidatively modified sites are then identified using a combination of proteolytic enzymes and the peptide sequencing ability of MS.

Protein-protein and protein-ligand interactions are involved in most biological machineries. Characterization of these noncovalent interactions using mass spectrometry has great advantages over other technologies because of high sensitivity and potential to provide stoichiometry and

structure information for protein complexes. Electrospray ionization (ESI) MS investigations of noncovalently-bound complexes are interesting because of their relevance to solution biochemistry. ESI is a gentle ionization method, and when it is coupled with highly sensitive mass spectrometry, ESI-MS is well suited for studying noncovalent interactions in protein complexes.

Aims and Objectives

1. Identification of metal binding sites of metalloproteins using metal-catalyzed oxidation reactions and mass spectrometry

2. Obtaining information about the secondary coordination sphere around the metal in metalloproteins using detuned metal-catalyzed oxidation reactions and mass spectrometry.

3. Studies of noncovalently-bound protein-protein and protein-ligand interactions using electrospray and matrix assisted laser desorption ionization techniques.

4. Development of mass spectrometry methods for the structural characterization of peptides and proteins.

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Immunomodulation of THP-1 monocytes/macrophages by Intra-erythrocytic stages of *P. falciparum* & hemozoin

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Background

Antigen presenting cells (APC) and monocyte/macrophage (MO/MQ) readily phogocytose all stages of malaria parasites (ring stage, trophozoites & schizonts), as well as the parasites components (free merozoites & malaria pigment-hemozoin). These cells (MO/MQ, dendritic cells) play an important role in antigen presentation. The pattern of antigen presentation and the expression of various cell surface molecules including co-stimulatory molecules, is dependent upon the kind of parasite components which the cells acquire (since different stages of parasites express different proteins). Recently, it has been suggested that parasite hemozoin and beta-Hematin can be used as immuno-adjuvant to boost immune responses against malarial parasite infection.

So far, there is no report about the influence of different parasite stages and parasite components on the expression of macrophages (APC) genes. The signaling pathway i.e., TLR, STAT, nFkB etc, in the macrophages, which acquires the plasmodium components are modulated/influenced by these antigens leading to differential expression of various surface molecules, secreted cytokines. These differential expression results in a varied antigen presentation and modulating the interaction of these cells with other immune system components and contributing to the final out come of the immune responses to the parasite infection. The LPS stimulation induced internal signaling pathway is very well known, and we have studied the malaria factors induced differences in this pathway. This will help in understanding the immunobiology of the malarial parasite infection.

Aims and Objectives

1. To study the interaction of antigen presenting cells (MO/MQ & Dendritic Cells (THP-1cells) with different erythrocytes stages of *Plasmodium falciparum* and its components, and the effect of these on the expression of different cell surface molecules, DC maturation and cytokine secretion.

2. To study the alteration in the intracellular signaling in MO/MQ & Dendritic Cells due to phagocytosis of different stages of the parasites i.e ring, trophozoites & schizoints free hemozoin & synthetic beta-heamozoin molecules.

Work Achieved

Different parasite stages (ring stage, trophozoites, schizonts), hemozoin (parasite isolated pigment) & synthetic beta-hematin modulates/influence

macrophage co-stimulatory and other molecules expression differentially. The Human derived THP-1 monocytes & macrophages (after PMA treatment), were incubated with different erythrocytic stages of P.falciparum (ring, trophozoites, schizonts; parasitemia >50%), isolated malaria pigment-hemozoin & synthetic beta-hematin). One set of MO were stimulated with LPS. After 48 h incubation, the expression of various cell surface molecules was analyzed by FACS. Protein & RNA were isolated from the cells for studying expression of molecules involved in cell signaling. The culture supernatants were collected for cytokine profiling.

The parasite pigment has been implicated in immunosuppression and is also used as an immuno-adjuvant to enhance immune responses suggesting an important role for the pigment in modulating the immune response to the malarial parasite infection. In this context, we used the malaria pigment (hemozoin) and its synthetic analog (beta-hematin) to study the signaling response in MO/MQE). THP-1 monocytes engulfed with hemozoin containing schizonts reduces the HLA-DR expression by 35.95% and malaria pigment- isolated from parasites drastically reduces to 99.74% compare to controls. The ring and trophozoites enhances the molecule expression more than 30% percent. The synthetic beta-hematin also enhances the HLA-DR molecule expression significantly. Hemozoin is also reduces the expression of TLR9 by 31.89%, whereas the synthetic beta hematin enhances the mol. expression by 62.89% as compared to control. The ring stage parasite increases the expression of the moiety. CD40, CD80 expression is enhanced enormously by ring stage parasites whereas, Schizoint stage parasites increases expression of CD86. The Ring stage parasite, Trophozoites or Schizoints has no effect on CD45RB & CD11c expression. The expression of CD54 is not effected by Ring, Trophozoite, Schizoint, Pf-hemozoin & b-hematin treatments. However, isolated P.falciparum -hemozoin drastically down regulate expression of HLA-DR, CD80, CD86, CD11b,& TLR9 in MQ stimulated with LPS. The Synthetic B-hematin affects CD11b & HLA DR expression. However, beta-hematin has no influence on CD40, CD45RB and CD11c expression.

Future Work

Detailed study of the alterations in the intracellular signaling in APC's caused by exposure to different stages of malarial parasite i.e ring, trophozoites & schizoints, free parasite hemozoin & synthetic beta-hematin and its effect on expression of various molecules including cytokines.

Studies on immunomodulatory role of IL-3 in development of regulatory T cells

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Background

Regulatory T (T reg) cells play a crucial role in controlling autoimmunity, and prevent the development of chronic inflammatory and autoimmune diseases by suppressing autoreactive T cells. The forkhead transcription factor (Foxp3) is the master regulator for the development and function of T reg cells. The in vitro conversion of naïve $CD4^+CD25^-Foxp3^-$ T cells to $CD4^+CD25^+Foxp3^+$ T reg cells depends on the presence of TGF-β1 and IL-2 which is essential for TGFβ mediated induction of Foxp3. Both TGF-β1 and IL-2 play an important role in the induction, maintenance of Foxp3 expression and function of T reg cells under in vitro and in vivo conditions. T reg cells in rheumatoid arthritis (RA) patients have a defect in their ability to suppress proinflammatory cytokine production by activated T cells and monocytes. Also, deficiency of Foxp3 results in the paucity of $CD4^+CD25^+$ T reg cells and leads to severe multiorgan autoimmune diseases in both mice and humans. Thus, the expansion of functional T reg cells holds promise for the treatment of RA and other autoimmune diseases. IL-3, a cytokine secreted by Th cells, stimulates the proliferation, differentiation and survival of pluripotent hematopoietic stem cells. IL-3 is a potent inhibitor of osteoclastogenesis and bone resorption and has an anti-inflammatory activity. T reg cells are potent inhibitor of osteoclast differentiation and bone resorption, and prevent development of CIA. Therefore, we hypothesized that the anti-inflammatory activity of IL-3 may be through regulation of T reg cell development. In this study, we investigated the role of IL-3 in regulation of T reg cell differentiation.

Aims and Objectives

1. To investigate the role of IL-3 in development of regulatory T cells.
2. To investigate the in vivo role of IL-3 on natural and peripheral regulatory T cells

Work Achieved

IL-3Rα is Expressed by T reg cells.

To evaluate the role of IL-3 in T reg cell development we first determined the expression of IL-3Rα on both natural and induced T reg cells. We isolated $CD4^+CD25^+Foxp3^+$ natural T reg cells from splenocytes of mice, and induced T reg cells were generated from splenic $CD4^+CD25^-$ T cells by

stimulating with plate bound anti-CD3ε and soluble anti-CD28 mAbs in the presence of TGF-β1 and IL-2. After 4 days expression of IL-3Rα (CD123) was checked first at mRNA level by RT-PCR. We observed that both natural and induced T reg cells show strong expression of IL-3Rα (Fig.1A). Expression of IL-3Rα was also analyzed by FACS on both natural and induced T reg cells by labeling and gating on $CD4^+$ cells for expression of Foxp3 and IL-3Rα. Both natural (17.3%) and induced (43.2%) T reg cells expressed IL-3Rα (Fig. 1B). These results demonstrate that both natural and induced T reg cells express IL-3Rα at both gene and protein level.

IL-3 Promotes In Vitro Expansion of Both Induced and Natural T Reg Cells, and Potently Suppresses the Proliferation of Effector T Cells.

We next examined whether IL-3 promotes conversion of $CD4^+CD25^-$ T cells into Foxp3 expressing T reg cells. Splenic $CD4^+CD25^-$ T cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated with TGF-β1 with or without IL-2 and in the absence or presence of different concentrations of IL-3. After 4 days cells were analyzed for Foxp3 expression by FACS. We found that IL-3 in a dose-dependent manner increases the TGF-β1-induced conversion of $CD4^+CD25^-Foxp3^-$ T cells into $CD4^+CD25^+Foxp3^+$ T cells (Fig. 2A). To further check whether IL-3 enhances the number of natural T reg cells, splenic $CD4^+CD25^+$ T cells were incubated with TGF-β1

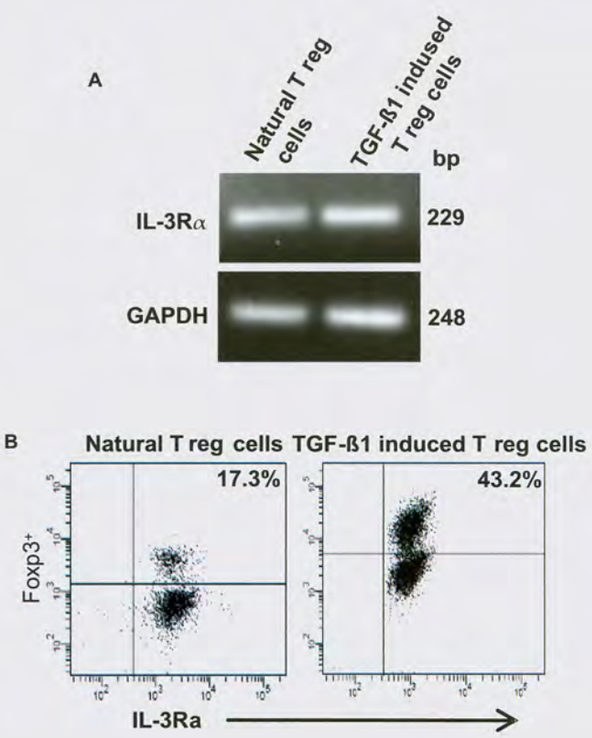
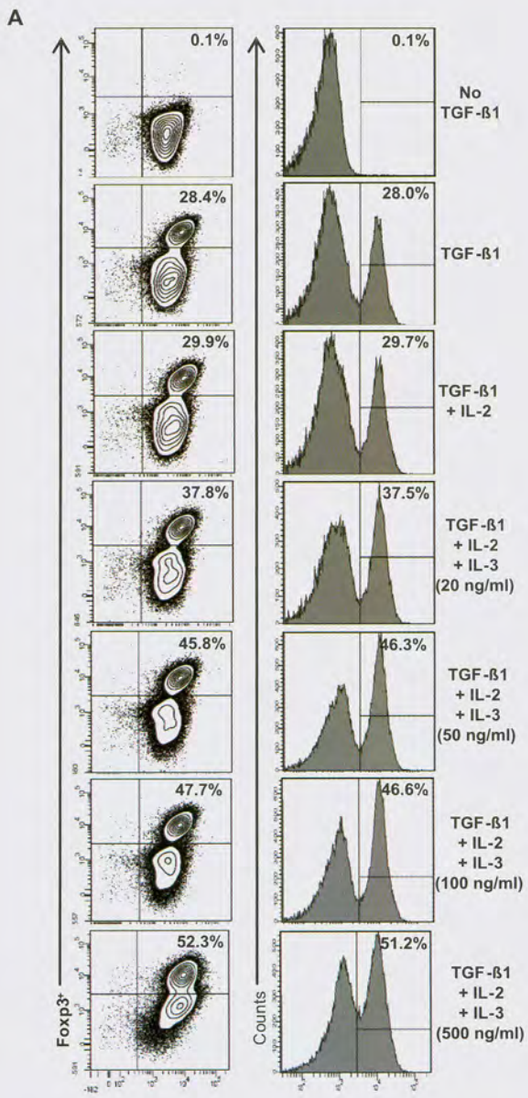
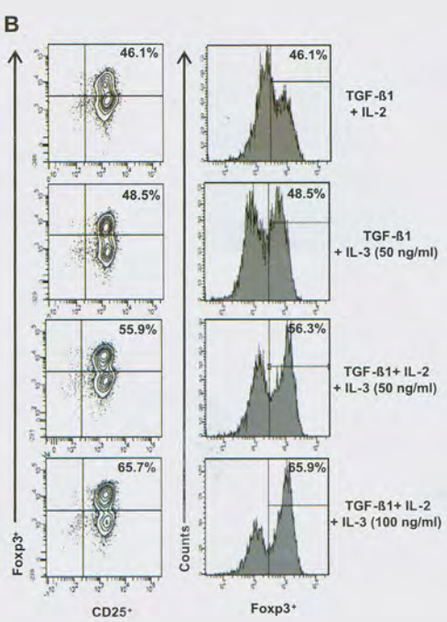


Fig. 1 IL-3Rα is expressed by both natural and induced T reg cells **(A)** $CD4^+CD25^+Foxp3^+$ natural T reg cells were isolated from splenocytes of 6-8 wk old BALB/c mice. Induced T reg cells were generated from splenic $CD4^+CD25^-$ T cells stimulated with plate bound anti-CD3ε (10 μg/ml) and soluble anti-CD28 (2 μg/ml) mAbs and incubated for 4 days in the presence of TGF-β1 (5 ng/ml) and IL-2 (20 ng/ml). Both cell populations were analyzed for mRNA expression of IL-3Rα (CD123) by RT-PCR. **(B)** Expression of IL-3Rα was also analyzed on both natural and induced T reg cells by FACS by labeling and gating on $CD4^+$ cells for Foxp3 and IL-3Rα expression. Results in (A) and (B) are representative of three independent experiments.





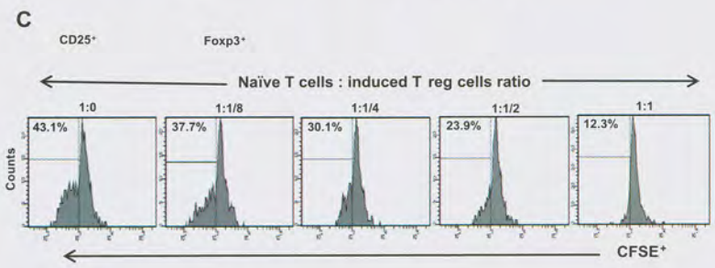


Fig. 2 IL-3 promotes in vitro expansion of both induced and natural T reg cells and suppresses the proliferation of effector T cells. **(A)** Splenic $CD4^+CD25^-$ T cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated with TGF-β1 (5 ng/ml) with or without IL-2 (20 ng/ml) in the absence or presence of different conc. of IL-3. After 4 days cells were analyzed for Foxp3 expression by FACS. Results are representative of three independent experiments. **(B)** $CD4^+CD25^+$ T cells isolated from splenocytes were incubated with TGF-β1 (5 ng/ml) with or without IL-2 (20 ng/ml) in the absence or presence of IL-3 (50 and 100 ng/ml). After 4 days, cells were analyzed for Foxp3 expression by FACS. Results are representative of two independent experiments. **(C)** Freshly isolated $CD4^+CD25^-$ T cells (2.5×10^5/well) labeled with CFSE were seeded in 96-well plates and co-cultured for 72 h with IL-3 expanded induced T reg cells at different cell ratios, with irradiated (800 Rad) syngenic T cell depleted splenocytes (5×10^4/well) and anti-CD3ε (1 μg/ml). Proliferation of effector T cells was analyzed by FACS for CFSE dilution. Data is representative of two independent experiments.

with or without IL-2 and in the absence or presence of IL-3. After 4 days, cells were analyzed for Foxp3 expression by FACS. We observed that IL-3 also increases the number of $Foxp3^+$ natural T reg cells (Fig. 2B). These results suggest that IL-3 enhances the expression of Foxp3 in both natural and induced T reg cells.

To check whether IL-3 expanded T reg cells are functional in suppressing the proliferation of responder/effector T cells, we performed CFSE based suppression assay. Freshly isolated, CFSE-labeled conventional $CD4^+CD25^-$ T cells were co-cultured with IL-3 expanded T reg cells at different cell ratios as indicated (Fig. 2C). We found that IL-3 expanded T reg cells inhibited proliferation of responder T cells in a cell density-dependent manner (Fig. 2C). Our results demonstrate that IL-3 enhances the number of functional T reg cells in vitro.

IL-3 enhances expression of Foxp3 in T reg cells via IL-2.

IL-2 is essential for TGF-β1 induced conversion of naive $CD4^+CD25^-Foxp3^-$ T cells into $CD4^+CD25^+Foxp3^+$ T reg cells. To check whether IL-3 enhances TGF-β1-induced T reg cell differentiation in absence of IL-2, we stimulated $CD4^+CD25^-$ T cells with anti-CD3ε and anti-CD28 mAbs for 4 days in presence of TGF-β1 with or without different conc. of IL-3. We found that IL-3 increased TGF-β1 induced T reg cell differentiation even in absence of IL-2 (Fig. 3A). Next, to determine whether the stimulatory effect of IL-3 on induced T reg cells is IL-2-dependent, $CD4^+CD25^-$ T cells were stimulated with anti-CD3ε and anti-CD28 mAbs for 4 days in TGF-β1 with or without IL-3 and in the absence or presence of anti-IL-2 mAb. Anti-IL-2 mAb completely neutralized the stimulatory effect of IL-3 on TGF-β1 induced T reg cells (Fig. 3B). Also, IL-2 secretion induced by IL-3 was significantly inhibited by anti-IL-2 mAb (Fig. 3C). These results suggest that IL-3 induced expansion of T reg cells is IL-2 dependent. This was further confirmed by dose-dependent effect of IL-3 on secretion of IL-2 in T reg cell cultures by cytometric bead array (CBA). We observed that IL-3 induces secretion of IL-2 in a dose-dependent manner. There was a drastic increase in IL-2 secretion at 100 and 300 ng/ml concentratins of IL-3 (Fig. 3D). Also, the enhanced secretion of IL-2 was directly co-related with simultaneous increase in expression of IL-2Rα (CD25) on T reg cells (Fig. 3E). Collectively, these results suggest that IL-3 induces expansion of T reg cells through secretion of IL-2.

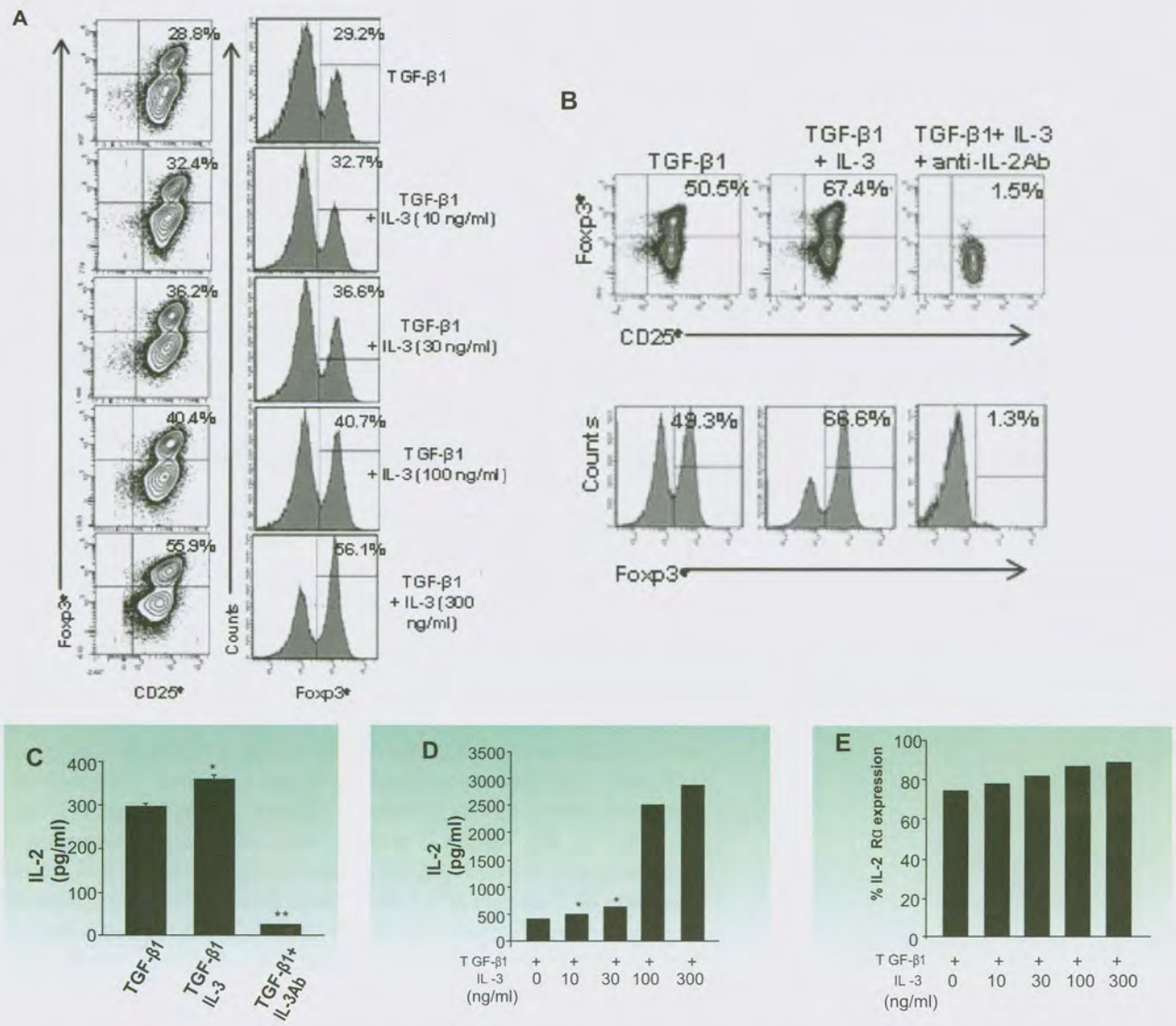


Fig. 3 IL-3 enhances Foxp3 expression in T reg cells through secretion of IL-2. **(A)** Splenic $CD4^+CD25^-$ T cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated with TGF-β1 (5 ng/ml) in the absence or presence of different conc. of IL-3. After 4 days cells were analyzed for Foxp3 expression by FACS. **(B)** Splenic $CD4^+CD25^-$ T cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated with TGF-β1 with or without IL-3 (100 ng/ml) or TGF-β1, IL-3 and anti-IL-2 (10 μg/ml) mAb. After 4 d cells were analyzed for Foxp3 expression by FACS. **(C)** Culture supernatants collected after 4 d from experiments in (B) were analyzed for IL-2 secretion by CBA as per manufacturer's instructions. *$p < 0.05$ vs TGF-β and **$p < 0.001$ vs TGF-β1 and IL-3. **(D)** Dose-dependent effect of IL-3 on secretion of IL-2 in T reg cells culture supernatants was analyzed by CBA. *$p < 0.001$ vs TGF-β1. **(E)** Dose-dependent effect of IL-3 on expression of IL-2Rα (CD25) in induced T reg cells. *$p < 0.05$ vs TGF-β1. Data in all above experiments are representative of three independent experiments.

Future Work

To investigate the role of IL-3 in prevention of collagen-induced arthritis in mice.

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

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Background

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

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. Later we have initiated a comprehensive study of all the HSP protein family members during HIV infection. Our results indicate that HSPs are differentially regulated during infection. As Heat shock factor-1 (HSF-1) is the major transcription factor

regulating HSP gene expression, we have also initiated studies on the role of HSF-1 in viral replication and pathogenesis. Recent results from these studies indicate that HSF-1 is involved in regulation of viral gene expression and 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 mitochondrial energy generating system in apoptosis exist, its exact role remains to be clearly understood. The OXPHOS system comprises of five enzyme complexes (Complex I, II, III, IV, V), subunits of which have been implicated in various functions in addition to their primary role in energy generating process. We have previously shown specific down regulation of the complex I subunit NDUFA6 with simultaneous impairment of mitochondrial complex I activity in HIV infection. Using differential gene expression analysis, we have now shown that Cytochrome Oxidase-II

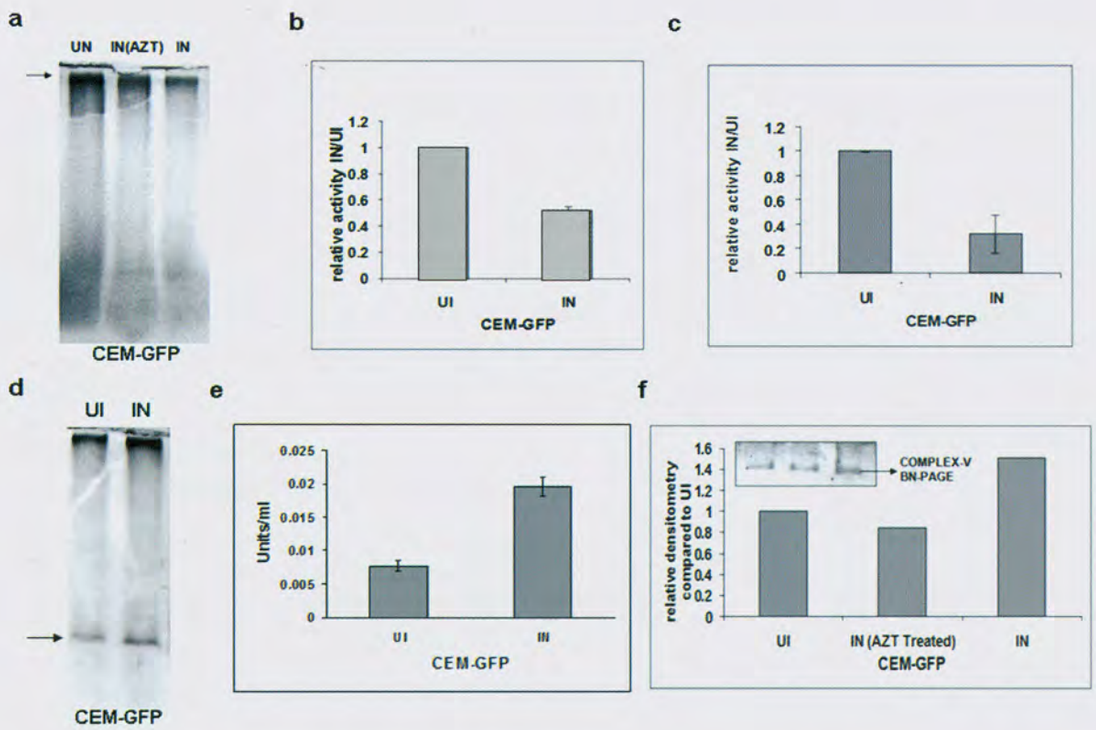

Fig. 1. Activities of different enzyme complexes of mitochondrial oxidative phosphorylation system during HIV-1 induced T cell apoptosis: **A.** BlueNative-PAGE in-gel activity for Complex-I. **B.** Spectrophotometric determination of Complex-IIactivity by spectrophotometry. **C.** Spectrophotometric determination of Complex-III activity. **D.** Blue Native-PAGE in-gel activity staining for complex IV activity. **e.** spectrophotometric determination of Complex-IV activity. **f.** Blue Native-PAGE in-gel activity staining for Complex-V (shown as inset) and bar graph showing the densitometry of the same. UN, uninfected CEM-GFP cells, IN (AZT), azidothymidine treated infected cells, IN, HIV-1 infected CEM-GFP cells (day-7).

(COX-II), a subunit of Complex-IV is induced in HIV infected apoptotic T-cells. Further analysis indicates increase in expression of majority of complex-IV subunits with concomitant increase in Complex-IV activity in HIV infected T cells. Silencing of COX-II expression leads to reduced apoptosis in infected T-cells, indicating its importance in apoptosis. Furthermore, our results also show that the activities of enzyme complexes I, II and III are decreased while those of Complex IV and V are increased at the time of acute infection and apoptosis (Fig-1). This differential regulation in activities of OXPHOS system complexes indicate a complex modulation of host cell energy generating system during HIV infection that ultimately leads to T cell apoptosis. Recently, we have also reported that the cell death regulator GRIM-19 (gene associated with retinoid-interferon-induced-mortality-19) is up-regulated in HIV-1 infected apoptotic T-cells. GRIM-19, a subunit of mitochondrial complex-I was previously implicated in Interferon-β and retinoic acid induced apoptosis in tumor cells. GRIM-19 mediated inhibition of STAT-3 is proposed to down regulate Bcl2 and Bcl-xL. Our results show a time dependent modulation of GRIM-19 expression in HIV-1 infected T-cells. Furthermore, siRNA mediated silencing of GRIM-19 led to significant reduction in apoptosis of HIV-1 infected T-cells by rescuing the expression of anti-apoptotic genes Bcl2 and Bcl-xL.

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. 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 APCs whereas 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 (IFNγ) secretion that controls T cell-mediated activation of APCs, and regulates differentiation of $CD8^+$ T cells into effector cells. Defective activation of APC by T cells that do not express CD40L could thus represent a primary event in the establishment of immunosuppression. 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 lacks 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 C (PLC-), mitogen-activated protein kinase (MAPKs), etc. We have used human monocytic cell lines THP-1 and U937 for analyzing cell signaling during HIV infection and

the role of CD40-CD40L interaction. We have observed modulation of ERK1/2 phosphorylation in HIV-1 NL4.3 infected monocytic cells based on the virus concentration used for infection. Our results with CD40 ligation also show modulation in ERK1/2 phosphorylation correlating with synthesis of IL-12 and IL-10. 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 have been 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. Our studies have resulted in identification of a number of novel derivatives of quinoline 2, 4-diol, β-carboline, dimeric phloroglucinol and caffeoyl anilide molecules showing potent anti-HIV activity. Some of these molecules have shown high safety or therapeutic index in cell based assays, which are now being further studied for potential development of a microbicide formulation. Mechanism of action studies for some of these molecules are also in progress.

Future Work

Our results till date indicate that heat shock proteins play an important role during HIV-1 infection. We are now trying to elucidate the role of different heat shock proteins in HIV replication and pathogenesis, which will provide us a comprehensive knowledge about the role of HSPs during HIV infection. In addition, we are trying to elucidate the role of HSF-1 in virus replication and pathogenesis, which seems to regulate virus gene expression and replication. We are also continuing characterization of several Nef interacting host cell proteins identified previously by yeast two hybrid system for their functional relevance in HIV lifecycle. Furthermore, we are studying the recruitment of Tat protein on the chromatin during HIV infection that might lead to the elucidation of the mechanism of Tat mediated regulation of both viral and cellular gene expression. Identification of differentially expressed genes and their relevance to HIV induced cell death is being continued, with a focus on mitochondrial oxidative phosphorylation system in infected cells. Our studies on the role of CD40 and CD40L in HIV infection will be taken further by initiating studies on human peripheral blood mononuclear cells and monocyte derived macrophages. Finally, studies to identify novel anti-HIV molecules will be continued with the objective to identify novel lead molecules with potential for use as anti-HIV microbicides.

Host-pathogen Interactions

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Background

Leishmania has evolved mechanisms to subvert the anti-parasitic functions to survive in the phagolysosomes containing reactive oxygen and acidic environment. We have previously shown that Selenophosphate synthetase of parasite is one of the key enzymes that is responsible for protection of parasite. We are interested in characterizing novel genes and their function in Leishmania and other infectious agents that are involved in host parasite infection.

Aims and Objectives

Leishmania has putative gene sequence for bax inhibitor-1 that is responsible for inhibition of apoptosis in higher organisms. The bax inhibitor-1 in plants is known to play role as anti-stress proteins. Leishmanial putative bax inhibitor-1 shares more homology with plants and therefore we want to see the role of bax inhibitor-1 in survival of parasite in macrophages.

Work Achieved

1. We have cloned the putative bax inhibitor-1 gene from Leishmania major and to confirm its antiapoptotic role we have expressed in heterologous yeast system that is sensitive to apoptosis. When Leishmanial bax inhibitor-1 was expressed using specific promoter in this yeast strain, survival and growth of yeast was seen confirming that the putative bax inhibitor-1 from Leishmania indeed functions as antiapoptotic protein.
2. Similar experiment was done in mammalian cell line to confirm the role of parasite gene.
3. In another infectious agent namely Candida albicans, invasion in host cells by fungal cells recruit zonula occludens-1 (ZO-1), a protein in the tight junctions of polarized epithelial cells to actin rings. This incorporation was mediated by the proline-rich region of the ZO-1 molecule during fungal invasion.
4. In animal model system, we observed that the sensitive mice to Leishmanial infection became more sensitive to parasite after malnourishment while the resistant mice remain resistance even after prolong protein malnourishment.

CD40 signaling in the regulation of immune response

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Background

Immune responses to auto-antigens, allo-antigens, pathogens and tumor antigens are regulated by activation of T-cells. It has been shown that the interaction between T-cell-expressed CD40-ligand (CD40-L) and antigen presenting cell-expressed CD40 plays a crucial role in T-cell activation. The CD40-CD40-L interaction results in signaling that is controlled reciprocally between two pathways. We have examined the role of protein kinase C (PKC), Ras and dual specific phosphatases (DUSP) in CD40 signaling with special reference to Leishmania infection.

Work Achieved

Ras isoforms differentially modulate CD40 signaling in macrophages

CD154-CD40 interactions in macrophages induce counteractive cytokines depending on the strength of signaling; however signaling intermediates remain unknown. Weak CD40 signals induce extracellular stress related kinase-1/2 (ERK-1/2)-dependent interleukin-10 (IL-10) production, stronger signals induce p38 mitogen-activated protein kinase (p38MAPK)-dependent IL-12 production, and intermediate CD40 signals induce both ERK1/2 dependent IL-10 and p38MAPK dependent IL-12 production. As Ras GTPases have been considered to be an important switch in various signaling events leading to regulation of diverse cellular functions, we investigated its role in CD40 signaling in macrophages. We observed the involvement of Ras GTPases in CD40 signaling in macrophages.

Ras GTPases have three isoforms namely H-Ras, K-Ras and N-Ras. Despite their great structural similarity, evidences suggest their functional disparity. Therefore, we studied their role in mediating CD40 signaling. And we observed isoform specific regulation of anti-CD40 stimulated p38MAPK or ERK1/2 activation and IL-10 or IL-12 production. H-Ras and K-Ras helped in mediating p38MAPK activation leading to IL-12 production, whereas N-Ras helped in mediating ERK1/2 activation leading to IL-10 production in CD40 signaling. Also, our observation revealed the mechanism of anti-CD40 induced Ras isoforms activation. We observed Syk dependent activation of N-Ras and Lyn dependent activation of H-Ras/K-Ras activation. Syk dependent N-Ras activation is mediated by Sos, a guanine nucleotide exchange factor (GEF), whereas Lyn dependent H-Ras/K-Ras activation is through Vav/Ras-GRP (GEFs). Thus, differential recruitment of

Ras GEFs by tyrosine kinases regulates the distinct functions of Ras isoforms in CD40 signaling in macrophages.

Leishmania major, a protozoan parasite skews CD40 signaling in macrophages towards ERK1/2 dependent IL-10 production and inhibits anti–parasitic IL-12 production. As a result, we checked the activation of Ras isoforms in CD40 signaling in Leishmania infected macrophages. And we observed enhance activation of CD40 induced N-Ras in Leishmania infected macrophages compared to uninfected macrophages. Thus, Leishmania targets N-Ras in order to manipulate CD40 signaling towards ERK1/2 dependent IL-10 production. Since N-Ras silencing activates more p38MAPK in CD40 stimulated macrophages, We checked whether N-Ras silencing would enhance the CD40 induced anti-leishmanial effect in L. major–infected BALB/c. Administration of anti-CD40 together with N-Ras shRNA resulted in reduced footpad thickness and parasite load in L. major–infected BALB/c mice, suggesting that N-Ras silencing can skew CD40 signaling through p38MAPK and help in enhancing CD40-induced anti-leishmanial effects in vivo. Together, our observations demonstrate that Ras isoforms differentially regulate CD40 signaling in macrophages.

Role of PKC isoforms in CD40 signaling

PKC is a family of serine threonine kinases. Members of PKC family are involved in various receptor signaling events and are also implicated in regulation of macrophage functions involved in host susceptibility and host defense against various infections. We studied the effect of CD40 stimulation on activation of various PKC family members and how these CD40 activated isoforms are regulating ERK1/2 and p38 MAP kinases, which are involved in CD40 signaling. We find maximum PKC activity after 10 minutes of anti-CD40 stimulation in mouse peritoneal macrophages. Anti-CD40 stimulation results in phosphorylation of various PKC isoforms in a time and dose dependent manner in Peritoneal Macrophages. We find change in expression of certain PKC isoforms (α, βI and βII isoforms are down regulated whereas δ and ζ are up regulated) after 72 hrs of Leishmania infection of macrophages. Then we studied phosphorylation status of PKC isoforms in a CD40 dose dependent manner in both Leshmania major infected (12hrs) and uninfected macrophages after 10 min of anti-CD40 stimulation. We find dose dependent response for PKC α, βI, βII and ε in uninfected macrophages, maximum phosphorylation was observed at 6mg/ml of CD40 concentration. which is impaired in case of L.major infection of macrophages. In case of PKCd and ζ in comparison to uninfected macrophages phosphorylation is enhanced in case of L.major infection and maximum phosphorylation was observed at 1mg/ml of anti-CD40 conc. We observed same kind of trend in membrane translocation of PKC isoforms. Further we studied effect of various PKC isoform specific inhibitors and SiRNAs on CD40 dependent phosphorylation of p38 and ERK1/2. We found that various PKC isoforms reciprocally regulated CD40 induced p38 and ERK1/2 phosphorylation. Inhibition of PKC α, β and ε resulted in decreased CD40 dependent p38 phosphorylation whereas

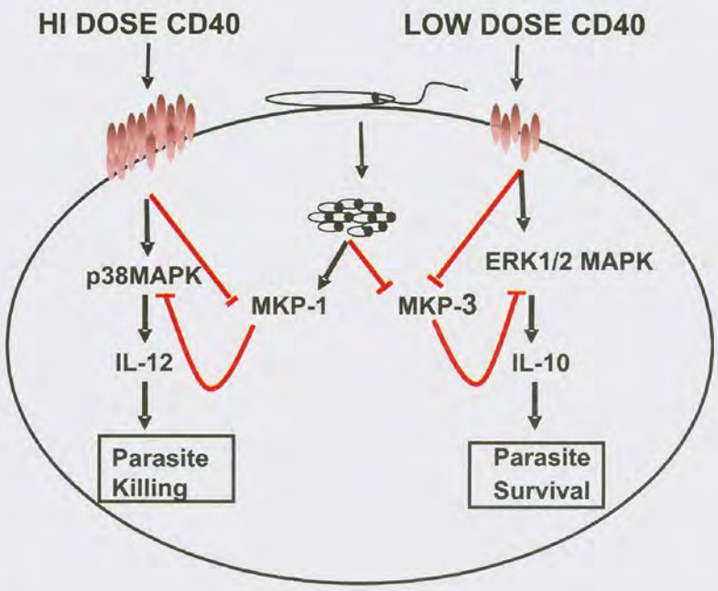


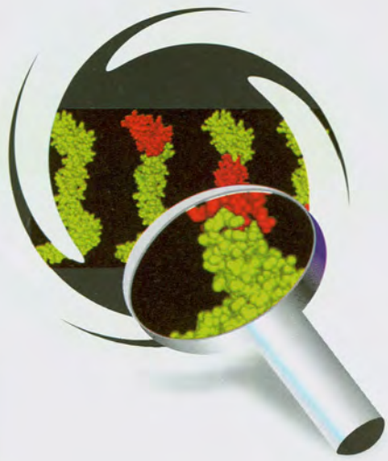
Fig.1 CD40 signaling in Leishmania-infected macrophages is MKP regulated.

phosphorylation of ERK1/2 was enhanced. Inhibition of PKC δ and ζ resulted in decreased CD40 dependent phosphorylation of ERK1/2 and enhancement of p38 phosphorylation. We also studied effect of PKC β, δ and ζ inhibitors on CD40 dependent IL12, IL10 and iNOS production. Inhibition of PKC β resulted in decreased IL12 and iNOS production whereas IL10 production was enhanced, indicating anti-parasitic role of PKC β. Inhibition of PKC δ and ζ resulted in decreased production of IL10 whereas IL12 and iNOS production was enhanced indicating pro-parasitic role of PKC δ and ζ. Further we found cholesterol to be responsible for this kind of differential regulation of PKC isoforms in CD40 signaling.

Regulation of CD40 signaling in Leishmania major infection by MKP-1 and MKP-3

Cellular outcome of any extracellular response is regulated by equal and balanced action of kinases and phosphatases. Mammalian MKPs are key regulators of MAP Kinase signaling, although they do regulate CD40 signaling of B cells but their involvement in CD40 signaling of macrophages and in Leishmania infection is not yet known. Since CD40 signaling in macrophages involves two reciprocally regulated MAP Kinases p38 and ERK1/2 and Leishmania is known to manipulate CD40 induced MAPK activation, expression of all the MKPs in Leishmania major infection was checked. Two phosphatases MKP-1 and MKP-3 were significantly altered in Leishmania major infection, expression and activation of p38 MAPK specific phosphatase, MKP-1 increases but the expression and activation of ERK1/2 specific phosphatase, MKP-3 decreases in infection. Differential expression of MKP-1 and MKP-3 is the function of the virulence of parasite since the avirulent parasite fails to do so. Leishmania

manipulates CD40 signaling reciprocally to augment ERK-1/2 phosphorylation and inhibit p38MAPK activation which results in increased IL-10 and decreased IL-12 production, respectively. Similarly expression and activation of MKP-1 and MKP-3 are reciprocally altered at different doses of anti CD40 stimulation. At higher dose of anti CD40 stimulation expression of MKP-1 decreases however expression of MKP-3 remains unchanged, whereas at lower dose of anti CD40 stimulation expression of MKP-1 gets upregulated but expression of MKP-3 gets downregulated.MKP-1 and MKP-3 reciprocally regulate CD40 induced MAPK signaling. MKP-3 inhibition enhances CD40 induced ERK1/2 phosphorylation, IL-10 expression and decreases p38 phosphorylation, iNOS2 and IL-12 expression but the inhibition of MKP-1 has reverse effect on CD40 induced effector functions .Further the mechanism of MKP-1 induction is mediated through IL-10 as its neutralization reduces its expression to basal level. To investigate the proparasitic functions of MKP-1, Triptolide a pharmacological inhibitor of MKP-1 or MKP-1 shRNA LV was used together with anti IL-10 neutralizing antibody or anti CD40 as a therapy against Leishmania major infection in susceptible BALB/c mice. A significant reduction in disease progression and parasite load was observed in susceptible BALB/c mice treated with MKP-1 inhibitor or MKP-1 shRNA LV. The observations described above suggest the involvement of two reciprocally regulated phosphatases MKP-1 & MKP-3 used by Leishmania major to skew the CD40 signaling as a part of their immune evasion strategy.



Role of viral complement control proteins in immune evasion

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Background

The innate immune mechanisms are diverse, evolutionarily ancient and possess the ability to trigger the adaptive immunity. The complement system suffices all these criteria. Since its discovery more than a century ago as a heat labile plasma factor that "complemented" the killing of a microbe by antibody, the understanding about the complement system as a composite arm of the host immune system, has come a long way. We now know that this ancient system present in invertebrates in rudimentary form has greatly evolved in mammals, has plasticity to recognize existing as well as newly emerging structures, and plays an instructive role in the adaptive immune response.

Unlike other innate immune mechanisms, the complement system has the ability to recognize the non-self targets, including viruses, with as well as without the help of pattern recognition molecules. Once recognized, it labels them as non-self by covalently tethering C3b, the activated form of complement protein C3, on their surface. This then leads to further activation of the complement pathways and destruction of the targets by various mechanisms. Because viruses have co-evolved with the host immune system, it is logical to presume that they must have adapted mechanisms to subvert the complement attack. Consistent with this, viruses have developed principles to elude the host complement system, one of which is encoding structural and functional homologs of the human complement regulators. Our laboratory primarily focuses on characterization of these viral homologs with respect to their function and role in viral pathogenesis.

Aims and Objectives

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

Work Achieved

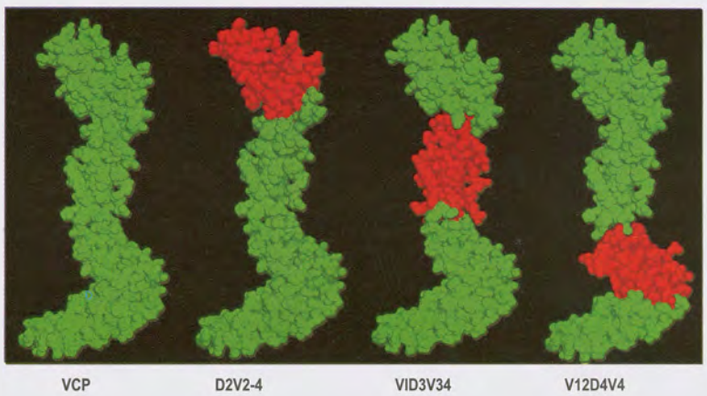
Vaccinia virus complement control protein (VCP), a four CCP module containing secretory protein, has been characterized earlier by us and others with respect to its mechanism of complement inhibition. The studies

revealed that it inhibits complement by targeting C3-convertases, the central enzyme in the complement system. The protein has been shown to inhibit the C3-convertases via two distinct mechanisms: i) by binding to C3b and C4b, the subunits of C3-convertases, and recruiting serine protease factor I for their proteolytic degradation (referred as cofactor activity), and ii) by binding and irreversibly dissociating the classical and to a limited extent the alternative pathway C3-convertase (referred as decay activity). We have also characterized the functional domains in VCP by deletion mutagenesis, wherein we identified the minimum domains important for binding to C3b and C4b and the functional activities. However, what principally remained unanswered was, what is the relative contribution of its individual domains in decay activity and cofactor activity? And which domains are important for interaction with factor I during cofactor activity, and for dissociation of C3-convertases during decay activity? To answer these questions, we swapped VCP domains with homologous domains of the human decay-accelerating factor (DAF; CD55) and membrane cofactor protein (MCP; CD46). We reasoned that since DAF possesses only decay activity and is devoid of cofactor activity, while MCP possesses only cofactor activity and is devoid of decay activity, swapping of VCP domains with homologous DAF or MCP domains would allow the identification of VCP domains(s) critical for factor I interaction and decay of protease subunits from the convertases, respectively

Identification of VCP domains critical for recruiting serine protease factor I for inactivation of C3b and C4b

Human DAF is devoid of cofactor activity and thus replacing VCP domains with homologous DAF domains is expected to selectively abrogate the recruitment of factor I, and as a result, loose the cofactor activity. The CCP modules 1-3 of VCP are homologous to CCP modules 2-4 of DAF, thus we swapped the respective modules of these proteins, generating mutants D2V2-4, V1D3V34 and V12D4V4 ('V' denotes VCP domain and 'D' denotes DAF domain; Fig. 1). Analysis of cofactor activity of these mutants revealed that of the three mutants, V1D3V34 and V12D4V4 were completely inactive, indicating that CCP modules 2 and 3 of VCP are critical for the cofactor activities of this molecule. The cofactor activity entails interaction of the complement regulator with the target protein (C3b or C4b) as well as factor I. We therefore measured binding of these mutants to C3b and C4b using Biacore. The abrogation in C3b and C4b cofactor activities of module 2 and 3 swap mutants did not correlate with binding to C3b and C4b. We therefore attribute the loss in the cofactor activities of these mutants to loss in their binding to factor I.

Because DAF possesses decay activity, it is expected that VCP-DAF chimeras would selectively loose cofactor activity and retain decay activity. In addition, given that DAF possesses significantly higher decay activity compared to VCP, it is expected that chimeras containing DAF modules vital for decay activity would show gain-in-function. As expected, all the chimeras retained the classical pathway (CP) decay activity, but intriguingly



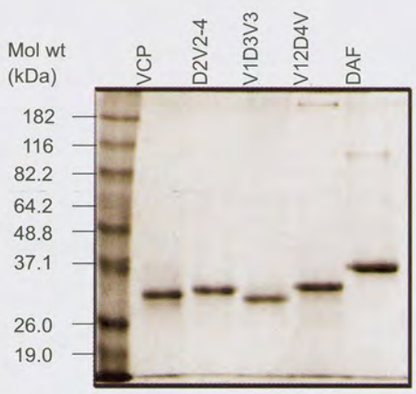


Fig. 1 Space-filling models and SDS-PAGE analysis of VCP and VCP-DAF domain swap mutants.

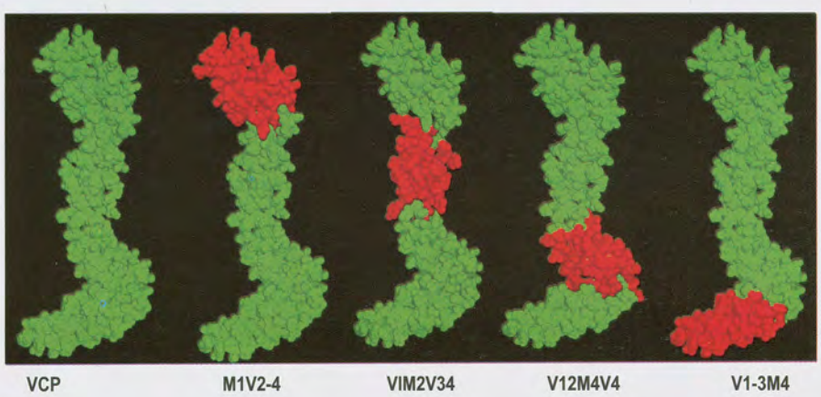
the mutant D2V2-4 demonstrated 55-fold increase in CP decay compared to VCP. Similarly, all the VCP-DAF chimeras retained alternative pathway (AP) decay activity, but there was a 670- and 106-fold increase in AP decay of V1D3V34 and V12D4V4, respectively compared to VCP.

Recently, the structure of the N-terminal four CCP modules of human complement regulator factor H in complex with C3b has been solved. The structure showed that all the four modules of factor H interact with the C3b molecule in a discontinuous manner. On the basis of the structure and previous studies, it was proposed that factor I inactivates C3b by interacting with the C3b-factor H complex at sites formed by the modules 1-3 of factor H and specific domains of C3b. The VCP domains 1-4 are structurally and functionally similar to factor H modules 1-4. Further, earlier during characterization of VCP deletion mutants, we noted that all the four domains are required for its optimal binding to C3b and C4b. Thus, we propose that like factor H, the four domains in VCP interact with the C3b molecule and modules 2-3 provide a docking surface for factor I. Because

domain requirements for ligand binding and cofactor activities in viral and human complement regulators are conserved, we suggest that the recognition sites for C3b/C4b and factor I are spatially conserved in both human and viral complement regulators, and that they employ a common mechanism to inactivate C3b and C4b.

Identification of VCP domains critical for decay of the protease subunit from the C3-convertases

Human MCP possesses cofactor activity, but is devoid of decay activity. It is therefore expected that swapping of VCP domains critical for its DAA with those of MCP would result in reduction/loss in decay activity. Because CCP modules 1-4 of VCP are similar to CCP modules 1-4 of MCP we swapped the homologous modules of VCP and MCP generating the mutants M1V2-4, V1M2V34, V12M3V4 and V1-3M4 (('V' denotes VCP domain and 'M' denotes MCP domain; Fig. 2). The measurement of CP decay activity of the four VCP-MCP domain swap mutants revealed that the mutant M1V2-4



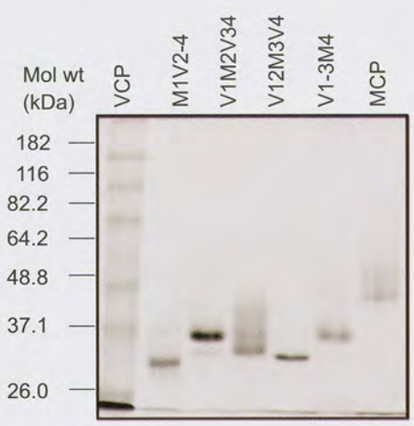


Fig. 2 Space-filling models and SDS-PAGE analysis of VCP and VCP-MCP domain swap mutants.

lacked this activity, while V1M2V34, V12M3V4 and V1-3M4 had no noticeable difference in their CP DAA compared to VCP. Clearly these data point towards the utmost importance of CCP1 in begetting the CP DAA in VCP. The AP decay data of these chimeras on the other hand revealed that the first three CCP modules of VCP are important for this activity as mutants M1V2-4, V1M2V34 and V12M3V4 demonstrated abrogation in their ability to decay the AP C3-convertase. As expected, all the MCP chimeras retained the cofactor activity against C3b and C4b.

Based on our functional analysis of these mutants and binding data obtained using Biacore we propose that while all the domains in VCP contribute to its optimal binding to C4b and C3b, domain 1 also contributes to destabilization of the convertase by competing for C2a and Bb interaction sites on C4b and C3b, respectively. And, in addition, domain 3 destabilizes the AP convertase by binding and presumably inducing a conformational change in C3b.

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.
4. Role of complement during influenza infection

Research Reports

Chromatin Architecture & Gene Regulation

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Tumor suppressor SMAR1: Crosstalks and gene regulation

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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. Proteins that specifically bind to these MAR elements regulate genomic DNA organization and nuclear 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.

Our group has demonstrated that this multi-faceted tumor suppressor is involved in modulation of NF-kB and TGFb signaling pathways. Moreover, previous studies have also shown that SMAR1 is involved in regulation of cell cycle and apoptosis, by its interaction with key transcription factors such as p53 and NF-kB and co-repressor complexes like mSin3-HDAC1, thereby maintaining cellular homeostasis. We continue to understand the role of SMAR1 in global gene regulation and how it governs cellular fate by interacting with other cellular proteins.

Aims and Objectives

1. Identification and characterization of SMAR1 interactome
2. Modulation of HIV-1 transcription by regulating TAP-TAT complex.
3. Regulation of NF-kB dependent chemokines gene expression in cancer

Work Achieved

Identification and characterization of SMAR1 interactome:

SMAR1 is a nuclear matrix attachment region binding protein and it is known to regulate various cellular processes. In order to understand its role in various cellular pathways, we sought to identify SMAR1 interacting partners using yeast two-hybrid screen. Repeated screening by colony lift β-

gal assay and CPRG mediated liquid β-gal assays followed by sequence alignment of the respective library clones led to identification of SMAR1 interacting partners. By yeast two hybrid experiment we came up with few candidate proteins such as AKR1a4, FGF4, FBXO40 and SRm160 that exhibited possible interaction with SMAR1.

AKR1a4 is a NADPH dependent aldo-keto reductase involved in various metabolic pathways like glucose metabolism, polyaromatic hydrocarbon activation, alcohol dehyrogenase activity and cytochrome p450. Although SMAR1 is predominantly localized to nucleus, in some cell lines like Hela and fibroblast lines such as NIH3T3, SMAR1 is also observed in the cytoplasm. Overexpression of SMAR1 leads to enhanced stability of AKR1a4 enzyme and reduced activity through direct interaction. The AKR1a4 enzyme activity is elevated upon knockdown of SMAR1 indicating that such interaction blocks the enzyme function under normal conditions. Earlier reports suggest that free radical scavenging machinery is defective in cancer cells and elevated free radical levels and ROS intermediates lead to oncogenic activation and cellular transformation. Higher metabolic rate in cancer cells leads to hyper-production of free radicals which in turn may cause apoptosis. Thus cancer cells have evolved with number of counteracting mechanisms such as hyper-activation of enzymes that scavenge free radicals by deregulating their inhibitory complexes. Many proteins are known to regulate activity of various enzymes. Here we report that MAR binding protein SMAR1 interacts and inhibits AKR1a4 enzyme activity in the cytoplasm. AKR1a4 enzyme activity is elevated due to loss of SMAR1 expression in case of higher grades of breast cancer, which may protect the cancer cells from anticancer drugs and free radical stress as well as helps the energy needs of cancer cell through increased metabolism. Thus the present study delineates yet another facet of tumor suppressor activity of SMAR1 in the cytoplasm. DNA damage and oxidative stress conditions lead to rapid dissociation of this complex in a time dependent manner. ATM kinase leads to dissociation of this complex through nuclear translocation of SMAR1 upon stress. This in turn facilitates activation of AKR1a4 enzyme to protect the cells by scavenging of free radicals which may further cause DNA damage. Thus, we propose a novel mechanism of ROS regulation by ATM through modulation of SMAR1-AKR1a4 complex (Fig. 1). We show that a small peptide of SMAR1 induces free radical stress by inhibiting AKR1a4 enzyme activity, which can be a potential anticancer therapeutic agent.

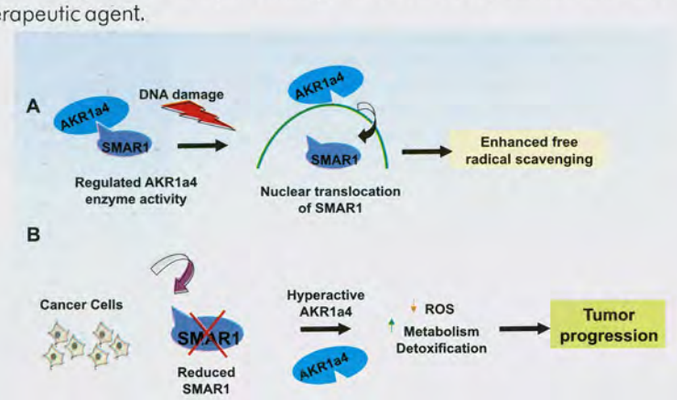


Fig.1 (A) Under endogenous conditions SMAR1 forms inhibitory complex with AKR1a4. DNA damage leads to activation of ATM kinase which facilitates nuclear translocation of SMAR1. This in turn activates AKR1a4 in the cytoplasm which scavenges excessive ROS. **(B)** In higher grades of breast cancer, expression of tumor suppressor protein SMAR1 is lost which lead to hyper-activation of AKR1a4 enzyme. This enzyme further may aid the cancer cells by efficient ROS scavenging, higher metabolic rate as well as it renders protection against anticancer drugs like doxorubicin leading to tumor progression.

Modulation of HIV-1 transcription by regulating TAP-TAT complex

We have previously reported that HIV-1 integration sites are flanked by MARs that stimulate transcriptional processivity from the HIV-1 LTR promoter independent of Tat. We also found that greater than 98% of HIV promoters contain a consensus MAR element in their 5' LTRs. SMAR1 binds to and tethers LTR-MAR to nuclear matrix and recruits the HDAC1/Sin3A corepressor complex thereby repressing LTR-mediated transcription. 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 and enhanced recruitment of RNAP II. Interestingly, overexpression of SMAR1 reduces virion production, while its knock-down induces basal HIV gene expression.

We further show that SMAR1 interacts with both p32/TAP and Tat. Tat is one of the most important viral proteins essential for HIV transcription and replication. TAP is known to be a coactivator of Tat and it enhances transactivation activity of Tat. Since SMAR1 interacts with both TAP and Tat, we wanted to know the functional effect of this interaction. SMAR1 and Tat bind to the same region of TAP. Luciferase assays show enhanced LTR-mediated transcription in presence Tat and TAP. Overexpression of SMAR1 leads to decrease in transcription. Therefore, SMAR1 prevents the binding of TAP to Tat and thus inhibits its coactivator activity. Further, we show by in-situ nuclear matrix staining that SMAR1-TAP, SMAR1-Tat and Tat-TAP interaction takes place in the nucleus. Using 3D immuno-FISH, we show that SMAR1, TAP and Tat associate on TAR in vivo (Fig. 2). Thus, on one hand SMAR1 directly binds to LTR and represses transcription, on the other hand, SMAR1 associates with TAR along with and inhibits coactivator activity of p32/TAP.

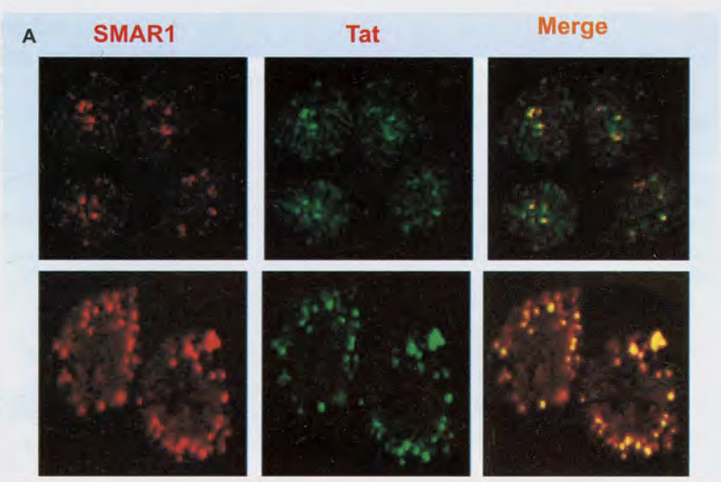
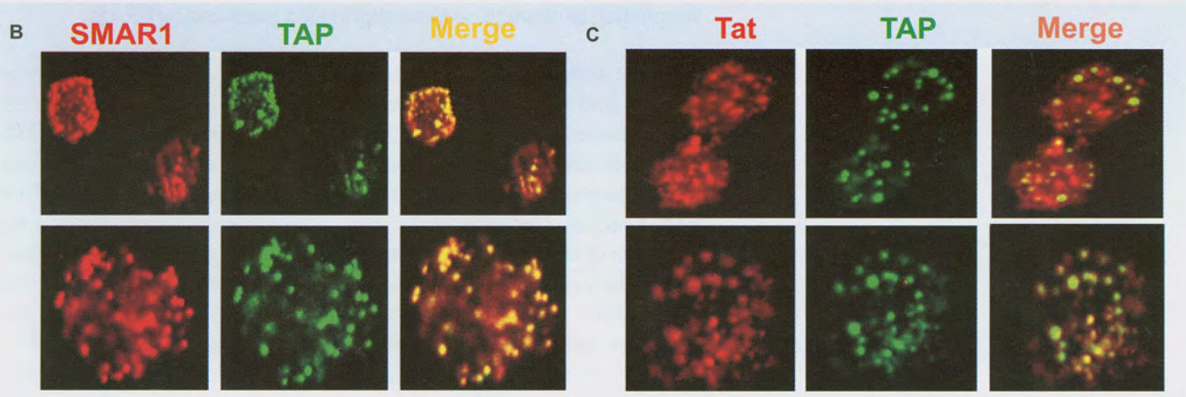


Fig. 2. SMAR1 interacts with p32/TAP and Tat in nuclear matrix. **(A)** In-situ nuclear matrix staining showing interaction between SMAR and Tat **(B)** SMAR1 and p32/TAP, and **(C)** Tat and p32/TAP in nuclear matrix. **(D)** Immuno-fluorescent in-situ hybridization showing colocalization of SMAR1, p32/TAP and Tat at TAR. **(E)** RNA-ChIP showing association of SMAR1, p32/TAP and Tat at TAR.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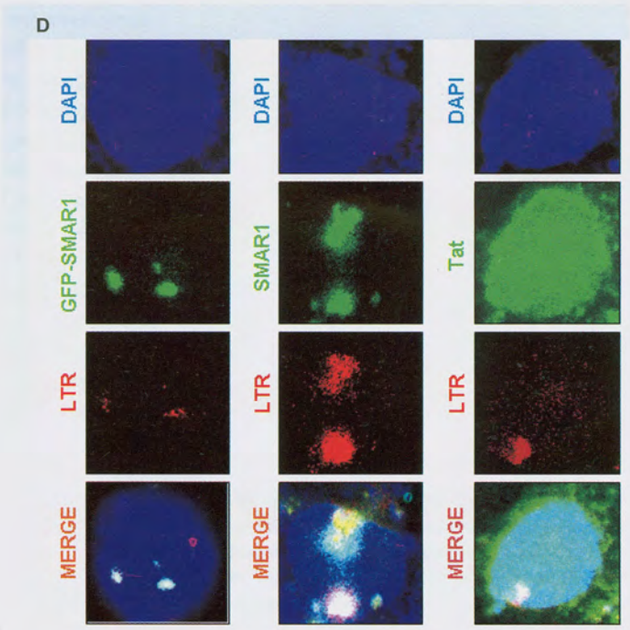
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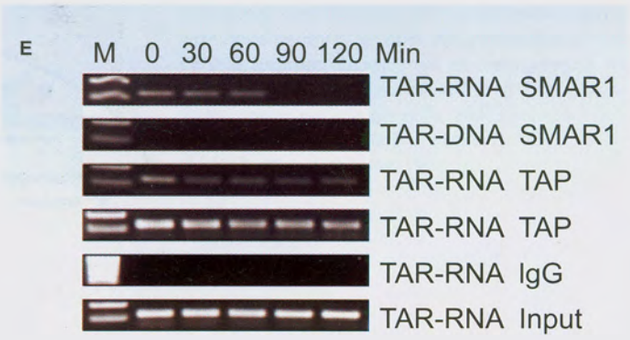
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Regulation of chemokine gene expression mediated by NF-κB:

Aberrant sustained activation of NF-kB has been reported in numerous tumors and implicated in various stages of tumorigenesis. NF-kB transcription factors can both induce and repress gene expression by binding to distinct DNA sequences, known as kB elements, in promoters and enhancers. The tumor suppressor SMAR1 regulates NF-kB activity by both direct and indirect mechanisms. Our previous studies showed that induction of tumor suppressor protein SMAR1 by doxorubicin or its ectopic expression in cancer cells leads to the downregualtion of IκBα. Molecular mechanisms revealed that SMAR1 binds to the MAR present in IkBα promoter, recruits HDAC1 dependent complex, together with p65/p50 at the locus and thus inhibits its transcription (Fig. 3). As a result of which there is increased nuclear accumulation of p65/p50 subunits, but these NF-kB complexes are transactivation deficient and fail to activate TNFα induced NF-kB signalling. The impact of such NF-kB regulation is that subset of kB target genes which belong to the category of cytokines, chemokines and angiogenenic molecules that are involved in tumor progression metastasis and angiogenesis are downregulated by SMAR1. IL8 is one such pleiotropic chemokine implicated in progression of several types of cancers. The higher grades of cancers show elevated level of this chemokine, which leads to metastasis and angiogenesis. The studies from our lab reveal that SMAR1 can downregulate IL8 expression by regulating its transcriptional activity. Taking breast cancer as model system we show that SMAR1 is reduced in metastatic breast cell lines like MDA MB 231 and MDA MB 468 which correlates with higher levels of IL8. Molecular mechanism behind the regulation of IL8 repression reveals that SMAR1 forms a corepressor complex on IL8 promoter which leads to its

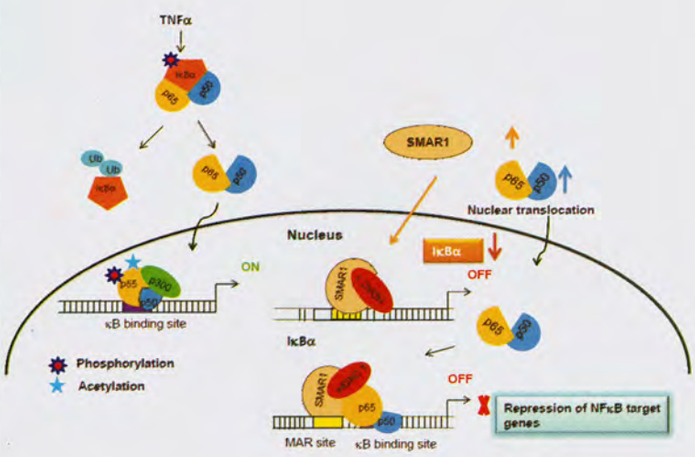


Fig. 3 Model demonstrating the regulation of NF-kB dependent transcription by SMAR1. Activation of NF-kB mediated transcription by TNFa in comparison to repression of NF-kB mediated transcription through MARs upon SMAR1 expression or induction by doxorubicin (atypical pathway). In the classical pathway, upon activation by TNFa, IkBa gets phosphorylated and ubiquitinated resulting in its proteosome mediated degradation. This allows nuclear translocation of p65/p50 and association with coactivators p300 leading to transcription from kB target genes. In the atypical pathway, SMAR1 induction by doxorubicin transcriptionally downregulates IkBa allowing nuclear translocation of p65/p50 complex. Additionally, upon nuclear translocation, p65/p50 associates with SMAR1-HDAC1 forming a repressor complex. This complex is then recruited to the MAR sites of kB target genes from where it dictates kB mediated transrepression.

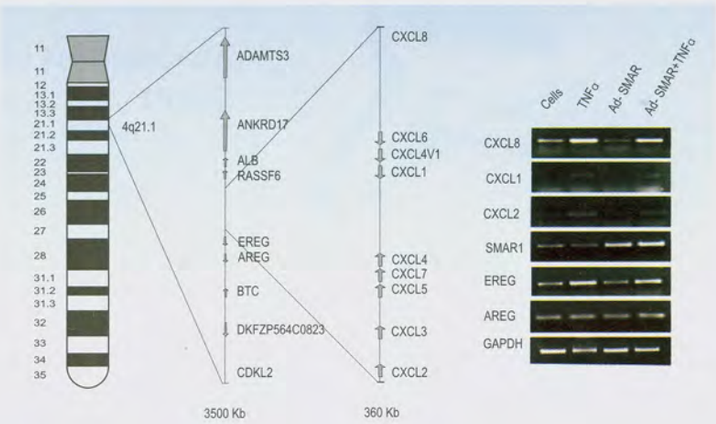
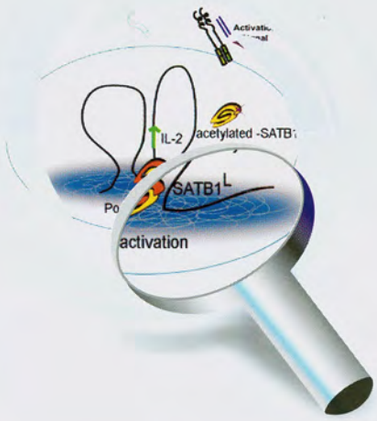


Fig. 4 CXC chemokines cluster located on human chromosome 4, and expression of candidate chemokines

transcriptional inhibition. In addition to this we also find that SMAR1 can inhibit the post translation modifications of p65 required for transactivation of IL8. SMAR1 induction by chemotherapeutics agents like Doxorubicin or ectopic expression in metastatic breast cancer cell lines, leads to the reduction in acetylation and phosphorylation of p65. Overall these results suggest that restoring SMAR1 function in metastatic breast cancer cells may inhibit cancer cell metastasis and angiogenesis by inhibiting the expression of angiogenic molecules like IL8. Apart from this other chemokines like CXCL1, CXCL2, CXCL3, CXCL7 are also regulated by SMAR1. Most of these CXC chemokines are clustered in human chromosome 4 within a locus of 365 kb (Fig. 4) We hypothesis SMAR1 might control the entire locus of these chemokines by binding to different MAR regions of this locus



Mechanisms of global gene regulation by SATB1

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Internal

Debashis Mitra

Background

Special AT-rich binding protein 1 (SATB1), a global gene regulator participates in organizing chromatin into topologically independent loop domains by tethering matrix attachment regions onto the nuclear matrix. The ability of SATB1 to act as a global regulator is conferred by various functional domains of SATB1. SATB1 binds to DNA in dimerization-dependent manner, aided by the PDZ-like domain in the N-terminal half. The N-terminal PDZ domain which aids in dimerization is also responsible for interaction with various cellular and viral proteins. The consequence of such protein-protein interaction(s) leads to the activation or repression of SATB1 activity. SATB1 recruits various chromatin remodelers such as HDAC1, NURD complex and PCAF depending on its post-translational modifications and thereby regulates gene expression. Furthermore, recent studies have revealed that acetylation- dependent interaction of SATB1 with the CtBP1 co-repressor determines the transcriptional status of SATB1 target genes via Wnt signaling during T cell development. Further, the functional interaction with PML via the PDZ-like domain of SATB1 dictates the chromatin loop architecture and transcription at the MHC-class I locus.

Studies using SATB1 knockout mice revealed that SATB1 orchestrates spatial and temporal expression of a large number of T-cell-specific and other genes. In SATB1 null mice, thymocytes are arrested at double positive (DP) stage thus compromising the number of functional CD4 and CD8 single positives (SPs). Within the thymus, maturation of thymocytes involves a series of discrete phenotypic stages that correspond to developmental checkpoints and are subsequently referred as CD4-CD8- (DN), CD4+CD8+ (DP), and CD4+CD3+ or CD8+CD3+ (SP). During T cell development cells undergo selection based on TCR re-arrangements and the TCR signals the thymocytes are exposed to via the stromal cells and subsequently the T cells mature into CD4+ or CD8+ cells (SPs). SATB1 mediated regulation of multiple genes important for T cell function such as CD8α, IL-2Rα, IL-2 and IL-7R suggested its role in T cell specific gene regulation.

To delineate the role of SATB1 in development of SPs from DP thymocytes we investigated the status of SATB1 in TCR activated 20 day old thymocytes (80% DP). Upon transcript profiling for Satb1 gene we detected presence of a novel transcript SATB1^{L} upon activation of T cells in addition to the natural transcript. Upon reverse transcription, cloning and sequencing it was observed that SATB1^{L} shared 98% homology with SATB1 and was localized at the same chromosomal location. This implicated that SATB1^{L} is an isoform of SATB1. Sequence analysis predicted SATB1^{L} as an alternatively

spliced isoforms containing an additional exon of 96 bp. Gene expression profiling of naïve and activated cells confirmed alternative splicing of Satb1 upon T cell activation. Surprisingly, induction of SATB1^{L} was found to be invoked due to PKC-θ activation mediated by TCR signaling. Coupled with the differential binding affinities of the two isoforms we further observed differential modulation of transcriptional activity by them in vivo with SATB1^{L} acting as an activator. Collectively, different lines of evidence suggested that induction of the alternatively spliced isoform SATB1^{L} helps in coordinated regulation of multiple SATB1 target genes as an effect of TCR activation by stringent modulation of their transcriptional status.

Aims and Objectives

1. Functional characterization of alternatively spliced form SATB1^{L}.
2. To delineate the differential regulation imparted by SATB1 and SATB1^{L}.
3. To study the role of SATB1^{L} in T cell development.

Work Achieved

SATB1^{L} is an isoform of SATB1

With a view to search for developmentally regulated T cell specific genes we identified 3 novel cDNA clones of unknown function. One of those expressed exclusively in lymphoid cells during activation and exhibited homology with the human SATB1 cDNA sequence. Sequencing of this novel clone differed from its natural variant by 32 amino acids (aa) and revealed that the novel variant spanned 795 aa. We further wanted to address whether the novel transcript SATB1^{L} is encoded by a single or multiple exons. A total of 13 independent genomic clones were isolated, one of which contained the additional exon. Using primers the sequence and the location of inclusion of the exon was identified to the distal of the DNA binding domain and immediately proximal to a continuous stretch of 15 glutamines (Fig. 1). The DNA sequence also revealed that the GT/AG rule for consensus donor and acceptor site was observed at intron-exon junctions of the extra exon of the novel variant, SATB1^{L}. Thus we conclude that SATB1^{L} is a spliced isoform of SATB1.

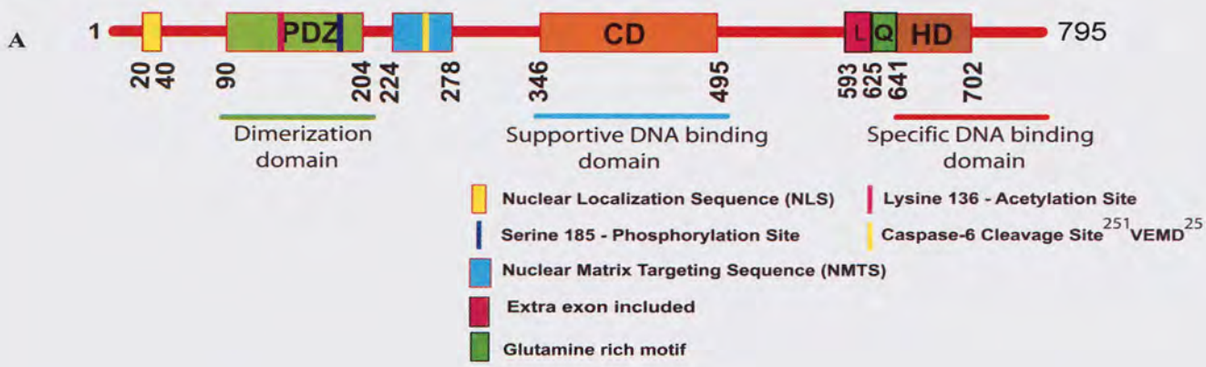

Fig.1 SATB1^{L} is an alternatively spliced isoform of SATB1. Schematic representation of SATB1^{L} is depicted to highlight its different domains and their positions.

2. SATB1 and SATB1^{L} have contrasting effects on gene expression at a global level

SATB1 has been demonstrated to act as a global gene regulator. Expression of SATB1^{L} results in alteration of transcriptional activity of SATB1. To ascertain the fact that SATB1^{L} results in alteration of function of SATB1 as a global regulator, we overexpressed SATB1 and SATB1^{L} in HeLa cervical epithelial cells that do not express SATB1 to test the effect of alternatively spliced isoforms on its ability to function as a global regulator at its target gene(s). Stably expressing clone with maximum overexpression was selected and used for gene expression profiling. The mRNA expression profiling was performed using 19K cDNA microarray chips. To our surprise, more than 25% of genes showed considerable difference upon overexpression of individual isoforms. Fold regulation of representative genes showed two distinct profiles. Almost 12% of the 50% dysregulated genes showed a reversal of expression upon SATB1 or SATB1^{L} overexpression. Cluster analysis of gene profiling data revealed that target genes of SATB1 and SATB1^{L} clustered separately indicating distinct mode of regulation imparted by the two isoforms (Fig. 2A). We then randomly selected 15 different genes and analyzed their transcript levels in HeLa cells in presence of SATB1 and SATB1^{L}. These results were further validated by quantifying the transcripts using real-time RT-PCR (Fig. 2B and 2C). Such analyses revealed that both the isoforms impart contrasting regulation on these randomly selected genes. When SATB1 acted as a repressor, SATB1^{L} acted as an activator and vice versa. However, few genes also exhibited similar repressive effect or activation by both the isoforms. We next wished to validate if a similar effect is exerted due to expression of SATB1^{L} upon its target gene(s) in the T cell model. Jurkat cells were activated using anti-CD3ε+C28 and cells were harvested at regular time intervals. The transcript analysis was performed using RNA extracted from these cells for a subset of target genes from the microarray analysis such as PPM1A, DUSP10, CHUK and GADD45A. The transcript profiling analysis revealed derepression of these genes upon activation of Jurkat T cells which is marked by expression of SATB1^{L} (Fig. 2D). The expression profiling of target genes corroborated the fold changes in gene expression obtained from the microarray data. Collectively, this analysis suggested that SATB1^{L} could act as a natural antagonist of SATB1.

3. Role of SATB1^{L} in T cell development

IL-2 is a key protein required for T cell development and proliferation. SATB1 represses IL-2 and IL-2R in naïve cells and are up-regulated upon TCR activation. Phosphorylated SATB1 recruits HDAC1 at the IL-2 promoter and represses it. However our in vivo reporter data clearly highlighted increase in IL-2 promoter reporter activity suggesting upregulation of the transcript. Upon Ionomycin activation SATB1 is acetylated resulting in de-repression of IL-2 accompanied with loss of occupancy of SATB1 from IL-2 promoter. However as an effect of TCR activation a huge surge in IL-2

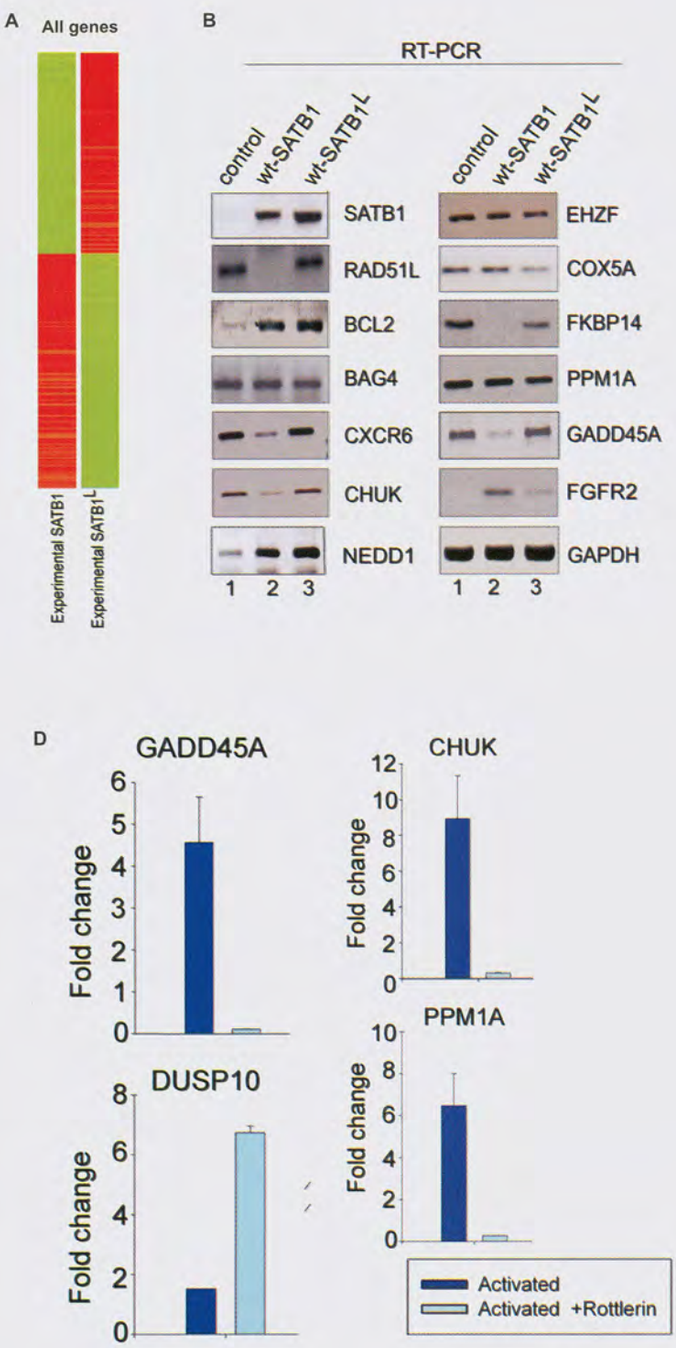

Fig. 2 Effect of SATB1^{L} upon transcription of gene(s) regulated by SATB1 **A.** Schematic representation of genes that were dysregulated in gene expression profiling of SATB1 and SATB1^{L}. **B.** Revalidation of gene expression was done of the various target gene(s) regulated by SATB1^{L} and SATB1 as obtained from the microarray analysis by performing RT-PCR analysis of 14 different genes. The transcript levels were compared between mock transfected cells and cells over-expressing wt-SATB1 and wt-SATB1^{L}. **C.** Real-time RT-PCR analysis was performed on the same 14 genes (on X-axis) to quantitate fold changes in gene expression (on y axis). In the graphical representation the grey bar represents the effect of over-expression of wt-SATB1 while black bar represents that of wt-SATB1^{L}. **D.** Revalidation of expression of target genes regulated by SATB1^{L} as obtained from the gene profiling data was performed for a few representative genes in Jurkat cells. Transcript analysis was performed in naïve as well as activated (with CD3-ε and CD28 at a conc. of 1.0 μg /ml) cells treated in absence and presence of Rottlerin at 1 μg /ml for 6 h. Real-time RT-PCR was performed on the same set of genes and fold change in expression level was plotted.

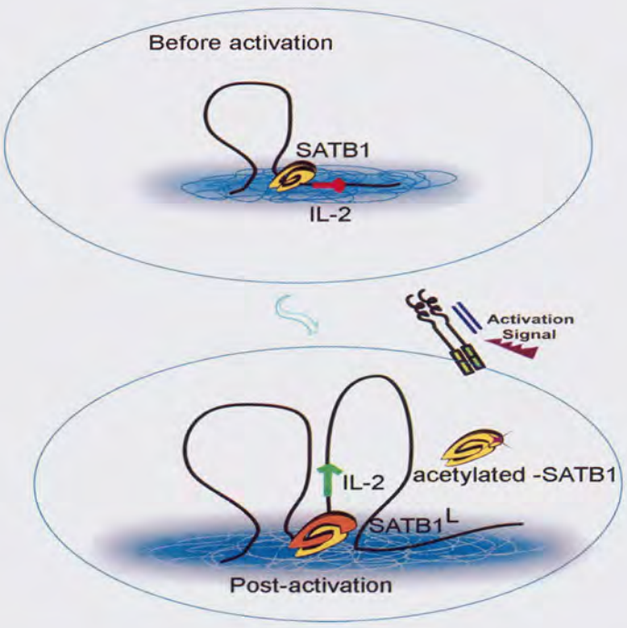

Fig. 3 Model depicting SATB1^{L} mediated TCR-dependent regulation of IL-2. In absence of TCR mediated signaling SATB1 occupies IL-2 promoter and represses the gene. However upon activation Satb1 is alternatively spliced generating SATB1^{L}. Activation leads to acetylation of SATB1 which loses its occupancy, whereas SATB1^{L} gains occupancy at the IL-2 promoter resulting in upregulation of gene expression.

expression that was accompanied with decrease in SATB1 occupancy and simultaneous increase in SATB1^{L} occupancy was observed. This ensured TCR activation mediated SATB1^{L} induction resulting in IL-2 expression.

In summary, we show that SATB1 undergoes alternative splicing to generate SATB1^{L} upon activation of T cells. Inducible expression of proteins as a result of splicing ensures diversified expression of protein repertoire. PKC-θ mediated splicing ensures role of SATB1^{L} in T cell development and proliferation. Activation-dependent changes in acetylation status of SATB1 decide the fate of its interaction with CtBP1 and with beta-catenin thus governing the regulation of SATB1 target genes in the Wnt pathway. Similarly, activation-dependent expression of SATB1^{L} may regulate the expression of various genes involved in TCR signaling and that are target of TCR activation. TCR activation which results in change in sub-cellular localization of SATB1^{L}could induce different functions by tethering same or different MARs and thus bring about coordinated gene regulation. This TCR mediated activation imposes differential task for SATB1^{L} in form of regulation of a small pool of SATB1 target genes with higher stringency. Its role as T cell specific gene regulator emphasizes its role during early or late thymopoeisis. We speculate that SATB1, through its alternatively spliced form SATB1^{L}, could exert a differential regulation on its target genes involved in T cell development. We further hypothesize that SATB1^{L} could also be responsible for inducing a dynamic status to the T cell chromatin organization especially during the activation phenomenon.

Support Units

Experimental Animal Facility

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Participants

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 a core service department of the Institute with an objective to breed, maintain and supply quality laboratory animals for research and development. The section also extends complete research technical support to facilitate animal experimentation in the Institute. 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/6
DBA/2J
DBA/1
129/SvJ
FVB/NJ
NOD/LtJ
SWISS#
BALB/c*
NMRI nu/nu**
NZB
AKR#

Genetically engineered mutant mice (knock-out, transgenic and mutant mice -32 lines)

RATS:

WISTAR

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 different inbred lines is structured in a three-tier/two tier format, i.e. the Foundation colonies (FC), Pedigreed expansion colonies (PEC) and the Production colonies (PC).

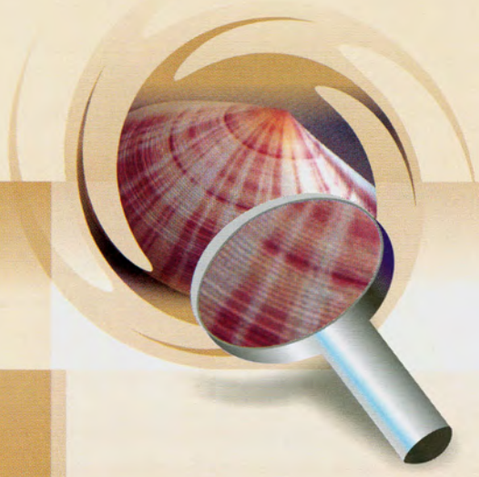
Strict full-sib pairing only propagates the animals in the FC and PEC. These colonies serve as nuclear colonies for the long-term propagation of the various strains.

The total number of mice strains, inbred, mutant and hybrids, being maintained at the Experimental Animal Facility stands at 45. The foundation/nuclear colonies of mice are housed in Individually Ventilated Caging systems. An aseptic/sterile routine standardized in-house is in practice for the housing, breeding and handling of these mice.

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 for various animal experiments. Over 9000 laboratory animals have been supplied on demand with nil or minimal waiting period during the reporting period.

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



Proteomics Facility

Participants

Dr. Vijay Sathe
Miss. Snigdha

The facility has three instruments:

1. 4800 MALDI TOF/TOF system is a tandem time-of-flight MS/MS system that is used for high-throughput proteomics research. The system can identify proteins by determining accurate masses of peptides formed by enzymatic digestion. Additionally, the system can more definitely identify and characterize proteins by isolating and fragmenting a molecular ion of interest and measuring the fragment ion masses. The number of samples analyzed is approximately 766 samples including 7 external samples from 01/01/2009 to 15/07/2010.

2. 4000 Q-Trap MS/MS system is hybrid triple quadrupole/linear ion trap mass spectrometer. The system is ideal for proteomic applications including post-translationally modified proteins discovery, protein identification, and biomarker validation. The number of samples (small molecules) using infusion syringe pump analyzed is approximately 61 samples including 8 external samples from 01/01/2009 to 15/07/2010.

3. Tempo Nano MDLC system is high performance, reliable, nano-scale liquid chromatography for proteomic applications. This Nano-LC system delivers precise and reproducible micro-scale gradients at constant flow-rates, creating ideal conditions for optimized mass analysis and a stable ionization spray. Currently, we are optimizing this system; we will start this system for routine analysis very soon.

Library

The NCCS Library caters to the information needs of its scientists, students and other staff members. It has collection in the frontier areas of biotechnology having relevance to NCCS research activities. The library holds approximately nine thousand seven hundred bound journals, two thousands three hundred fifty books, ninety NCCS Ph.D. thesis and subscribe to sixty nine scientific journals and twenty eight other periodicals in print form and six scientific online 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. The library is equipped with Linux based SLIM21 software with RFID interface for the library house keeping operation, and Web-OPAC for online searching of the library documents. It also has a barcode technology for circulation (Issue & Return) of library documents. The library maintains library information (In Hindi & English) on its webpage which includes free Online Medical database links, NCCS research publications, library forms, NCCS in News, Ph.D. thesis collection and list of NCCS Alumni. During the period under review, the library has created a Digital Archive for NCCS Ph.D. thesis.

Additional documentation facilities include local area network for library activities and access of PubMed database and CD-ROMs for books, journals & Ph.D. thesis. The NCCS 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.

The Library is also a member of DBT Online Journal Consortia which provides access to 916 Online Journals and "SCOPUS" citation database.

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 completed and the servers are functional thereby providing an additional storage space for the Mails, Domain based User Interface.

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

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

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

  The Centre has completed Installing Wireless Connections in all the labs. It is 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 one from Reliance (2 Mbps) and the other from TATA (2 Mbps). The process of upgradation of these leased lines to 2 Mbps is in progress thereby enhancing the internet bandwidth remarkably.

The Computer Section at NCCS is involved in the following 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, Softwares and Drivers and also provide support for installation and maintenance of hardware components.

b) Management of the invaluable information in the Institute.

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

d) Providing Support to the students and scientists for the research activities. Computer centre helps in the DTP work, CD writing, scanning of images and transperancy printing on color laserjet Printer.

e) Regular update of the NCCS website including individual scientist's web pages 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/support staff.

NCCS Facilities

DNA Sequencer

A total of 19, 000 samples were run on the machine during this period.

FACS Facility

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

Names of technicians in the facility:

1. Hemangini Shikhare
2. Pratibha Khot

The usage of the four equipments for the period under consideration is summarized in the tables below.

IMMUNOPHENOTYPING & CELL CYCLE analysis:

Equipment	Surface /Intracellular staining	DNA Cell cycle	CBA flex	CBA	Total Samples Acquired
FACS Calibur	4316	1294	-------	94	5704
FACS Canto II	5656	-----	152	---------	5808
FACS Vantage	295 (till Oct09)	27 (till Oct09)	-----	------	322

SORTING:

EQUIPMENT	SORTING		TOTAL
	UV laser	BLUE,RED laser	
FACS ARIA	82	225	307

Others

We have acquired following samples for outsiders like IRSHA Institute:

Surface/ Intracellular staining 101 and DNA cell cycle 58 on FACS Calibur.

FACS Aria II was purchased in March 2009 and has been installed in the new building in March 2010.

Confocal Microscopy

The Confocal 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 can be done making upto four parameter studies possible.

2. Advanced Spectral Confocal Microscope, Zeiss LSM510 META, with programmable CO2 incubator and temperature- humidity control. This system comprising 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 numbers of samples imaged during this year were approximately 2700 in-house and 330 came from various other institutes. Number of samples for live-imaging was 12.

Publications

Publications

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36. Joglekar MV, Taylor C and Hardikar AA. Isolation and molecular characterization of endocrine progenitors from human pancreas. Method. Mol. Biol. (in press)

37. Joglekar MV, Wei CJ and Hardikar AA. Quantitative estimation of multiple microRNAs and mRNAs from a single cell. Cold Spring Harb. Protoc. (in press).

38. Ansarullah, Jayaraman S, Hardikar AA and Ramachandran AV. Influence of Oreocnide integrifolia (Gaud.) Miq on IRS-1, Akt and Glut-4 in Fat-Fed C57BL/6J Type 2 Diabetes Mouse Model. Evid-Based Complement. Alternat. Med. (In press).

39. Wu Y, Li J, Saleem S, Yee S, Hardikar AA and Wang R. C-Kit and stem cell factor regulate PANC-1 cell differentiation to insulin- and glucagon-producing cells. Lab Investigation (in press)

40. Sasikala M, Swapna G, Pavan K, Rao GV, Hardikar AA and Reddy DN. Reduced expression of pdx-1 correlates with decreased beta cell function in chronic pancreatitis. Pancreas (in press)

41. Lee J, Jo J, Hardikar AA, Periwal V and Rane SG (2010). Cdk4 regulates recruitment of quiescent β-cells and ductal epithelial progenitors to reconstitute β-cell mass PLoS ONE 13;5(1): e8653

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43. Ranjan AK, Kumar U, Hardikar AA, Poddar P, Nair PD, Hardikar AA (2009). Human blood vessel-derived endothelial progenitors for endothelialization of small diameter vascular prosthesis. PLoS ONE 4(11): e7718.

44. Joglekar MV, Patil D, Joglekar VM, Rao GV, Reddy DN, Sasikala M, Shouche Y and Hardikar AA (2009). The miR-30 family microRNAs confer epithelial phenotype to human pancreatic cells. Islets 1(2): 137-47.

45. Parekh VS, Joglekar MV and Hardikar AA (2009). Differentiation of human umbilical cord blood derived mononuclear cells to endocrine pancreatic lineage. Differentiation 78(4): 232-40.

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48. Mulla SI, Hoskeri RS, Shouche YS and Ninnekar HZ. Biodegradation of 2-Nitrotoluene by Micrococcus sp. strain SMN-1. Biodegradation (in press).

49. Antony CP, Kumaresan D, Ferrando L, Boden R, Moussard H, Scavino AF, Shouche YS and Murrell JC. Active methylotrophs in the sediments of Lonar Lake, a saline and alkaline ecosystem formed by meteor impact. ISME J. (in press).

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51. Khandwekar AP, Patil DP, Shouche YS and Doble M. Surface Engineering of Polycaprolactone by Bio-macromolecules and Their Blood Compatibility. J. Biomater. Appl. (in press).

52. Salunke BK, Salunkhe RC, Dhotre DP, Khandagale AB, Walujkar SA, Kirwale GS, Ghate HV, Patole MS and Shouche YS (2010). Diversity of Wolbachia in Odontotermes spp. (Termitidae) and Coptotermes heimi (Rhinotermitidae) using the multigene approach. FEMS Microbiol. Lett. 307(1): 55-64.

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63. Tripathy MK and Mitra D (2010). 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 15: 28-40.

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69. International Stem Cell Banking Initiative (2009). Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. Stem Cell Rev. 5: 301-14.

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80. Vempati RK, Jayani RS, Notani D, Sengupta A, Galande S and Haldar D (2010). p300 mediated acetylation of histone H3 lysine 56 functions in DNA damage response in mammals. J Biol Chem. 285(37): 28553-64.

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84. Srikanth R, Wilson J and Vachet RW (2009). Correct identification of oxidized histidine residues using electron-transfer dissociation. J. Mass Spectrom. 44: 755-762.

85. Ryppa C, Niedzwiedzki D, Morozowich NL, Srikanth R, Zeller M, Frank HA and Bruckner C (2009). Stepwise conversion of two pyrrole moieties of octaethylporphyrin to pyridine-3-ones: Synthesis, mass spectral, and photophysical properties of mono and bis(oxypyri)porphyrins. Chem. Eur. J. 15: 5749.

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87. Sinha S, Malonia SK, Mittal SPK, Singh K, Kadreppa S, Kamat R., Mukhopadhyaya R, Pal JK and Chattopadhyay S (2010). Coordinated regulation of p53 apoptotic targets BAX and PUMA by SMAR1 through an identical MAR element. EMBO J. 29: 830-842.

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89. Sreenath K, Pavithra L, Singh S, Sinha S, Raut S, Dash PK, Siddappa NB., Mangaiarkarasi A, Ranga UK, Mitra D and Chattopadhyay S (2010). Nuclear matrix protein SMAR1 represses HIV-1 LTR mediated transcription through chromatin remodeling. Virology 400(1): 76-85.

90. Pavithra L, Sreenath K, Singh S and Chattopadhyay S (2010). Heat shock protein 70 binds to a novel sequence in 5' UTR of tumor suppressor SMAR1 and regulates its mRNA stability upon PGA2 treatment. FEBS Lett. 584(6): 1187-92.

91. Singh K, Sinha S, Malonia SK and Chattopadhyay S (2010). Tumor Necrosis Factor alpha (TNFalpha) regulates CD40 expression through SMAR1 phosphorylation. Biochem. Biophys. Res. Comm. 391(2): 1255-61.

92. Pavithra L, Chavali S and Chattopadhyay S (2010). Nutritional epigenetics- impact on metabolic syndrome. Review in book chapter on Molecular Mechanisms of Epigenetics (in press).

93. Kopikar S, Choudhari AS, Kumari A, Chattopadhyay S and Kaul-Ghanekar R (2010). Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. BMC Cancer 10: 210.

94. Shrihari P. Sanap, Sougata Ghosh, Amit M. Jabgunde, Rahul V. Pinjari, Shridhar P. Gejji, Shailza Singh, Balu A. Chopade and Dilip D. Dhavale (2010). Synthesis, computational study and glycosidase inhibitory activity of polyhydroxylated conidine alkaloids-a bicyclic iminosugar. Org. Biomol. Chem. 8: 3307.

95. Koparde P and Singh S (2010). Avian influenza and microRNA: Role of bioinformatics. J. Bioinform. Seq. Analysis (in press).

96. Ahmad M, Raut S, Pyaram K, Kamble A, Mullick J and Sahu A (2010). Domain swapping reveals complement control protein modules critical for imparting cofactor and decay-accelerating activities in vaccinia virus complement control protein. J. Immunol. (in press)

97. Khandwekar AP, Patil DP, Hardikar AA, Shouche YS, Doble M. (2010) In vivo modulation of foreign body response on polyurethane by surface entrapment technique. J. Biomed. Mater. Res. 95(2): 413-23

98. Joglekar MV and Hardikar AA. Epithelial-to-mesenchymal transition in pancreatic islet beta cells. Cell Cycle (in press)

99. Paila YD, Saha B and Chattopadhyay A (2010). Amphotericin B inhibits entry of Leishmania donovani into primary macrophages. Biochem. Biophys. Res. Commun. 399(3): 429-33.

Other Publications

1. Roychowdhary S, Patil D and Chintamani A. Building competitive advantages through supply chain management: A case for biotech sector. Published in Proceedings of International conference on Global meltdown: opportunities and challenges.

2. Roychowdhary S. Stores management: Challenges in the biotech arena. Published by Indian Institute of Materials Management.

Patents filed/sealed

1. Peptides that inhibit factor B, C2 and complement activation, and their uses. Sahu A and Kadam AP (Ref # 09100)

Memberships/ Awards/ Fellowships

Dr. Mrs. Vaijayanti P. Kale

- Member of International Society for Stem Cell Research
- Member of the International Society for Hematology and Stem Cells (ISEH)
- Associate Editor, International journal "Stem Cells and Development"
- Associate Editor, International journal "Annals of Neurosciences"
- Senior Editor, International journal "Insciences journal"

Dr. Nibedita Lenka

- Life Member, Indian Academy of Neuroscience.
- Active Member, International Society for Stem Cell Research (2005 – continuing).
- Life Member, Stem Cell Research Forum of India (SCRFI).
- American Society of Microbiology (ASM) (2010).
- Member Editorial Advisory Board, Annals of Neurosciences (2009-continuing).

Dr. Lalita S.Limaye

- Member International Society of experimental Haematology

Life member of

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

Dr. Vasudevan Seshadri

- Life member - Indian Society Of Cell Biology
- Life member - Society Of Biological Chemists, India

Dr. Mohan Wani

Award

- "National Bioscience Award for Career Development: 2009" by Department of Biotechnology, Govt. of India, New Delhi.

Memberships

- Chancellor nominee as a Executive Council (Governing Body) member, Maharashtra Animal and Fisheries Sciences University, Nagpur (2009-2012).
- Member of CPCSEA for NIV, Raj Biotech, NTC and Pune University.
- 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.

Dr. Jomon Joseph

- Member - Indian Society of Cell Biology

Dr. Sharmila Bapat

- 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 (IACR)
- Member, Indian Women Scientists Association

Dr. Anandwardhan A. Hardikar

- Vice-President, Islet Society, Stockholm, Sweden
- Barbara Walker Fellowship award, St. Vincents' Hospital, Melbourne

Dr. Debashis Mitra

- Member, Infectious Disease Biology Task force, Department of Biotechnology, Govt. of India
- Member, Expert Group on Microbicides, Indian Council of Medical research, Govt. of India

Dr. Arvind Sahu

- Fellow, National Academy of Sciences, India (2009)
- Member of the International Complement Society (since 1993)
- Member of the Molecular Immunology Forum (since 2005)
- Member of the American Society for Microbiology (since 2005)

Dr. Shailza Singh

Awards

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

Life Member of

- Biotechnology Society of India (BSI)
- Society of Biological Chemists, India
- Association of Microbiologists of India (AMI)
- Association for DNA Fingerprinting and Diagnostics

Dr Sanjeev Galande

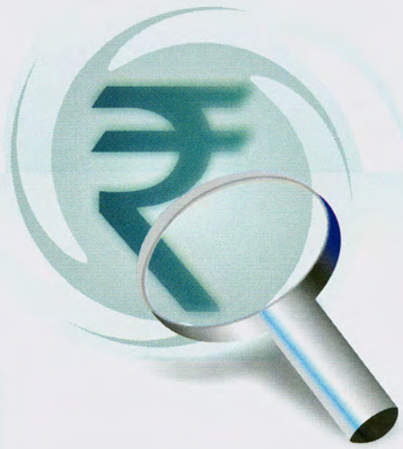
- Fellow, Indian Academy of Sciences

Dr. R. Srikanth

- Life Member of Indian Society for Mass Spectrometry
- Member of American Society for Mass Spectrometry
- Member of American Chemical Society

Dr. Samit Chattopadhyay

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



Extramural Funding

Dr. Mrs. Vaijayanti P. Kale

1. Stromal Cell Biology: Creation of an in vitro model of the BM niche and studies on its impact on the hematopoietic stem cells. DBT. 2009-2012.
2. Detection of minimal Residual Disease in acute leukemia by molecular methods. DBT. 2010-2013.
3. Identification of the molecular mechanisms involved in the induction of proliferative responses in primitive hematopoietic cells. DBT. 2008-2011.

Dr. Lalita S.Limaye

1. Ex-vivo and In-vivo studies on Megakaryocytes and Platelets generated in cultures from hematopoietic stem cells with special reference to role of nutraceuticals as supplement in the culture media. DBT.

Dr. Vasudevan Seshadri

1. Functional assessment of adult human pancreatic islets following autologous transplantation. DBT. 2010-2011.

Dr. Mohan Wani

1. Studies on understanding the role of IL-3 in regulation of human osteoclast and osteoblast differentiation. DBT. 2010-2012.

Dr. Jomon Joseph

1. Molecular characterization of the interaction between the tumour suppressor Adenomatous Polyposis Coli (APC) and the nucleoporin Nup358. DBT. 2008-2011.
2. Regulation of b-catenin function by the nucleoporin Nup358 in Wnt signaling. DBT. 2008-2011.

Dr. Sharmila Bapat

1. Two extramural project funded by Department of Biotechnology, India.

2. One Indo-Finish Project funded by Department of Biotechnology, India and Finish Academy of Science, Finland.

Dr. Padma Shastry

1. Exploring the potential of TF antigen binding property of lectin from Sclerotium rolfsii for Tumour suppressive activity. 2009-2012.

Dr. Anjali Shiras

1. 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.

Dr. Gopal C. Kundu

1. Role of Small Molecule Inhibitor(s) as Targeted Therapy in Pancreatic and Prostate Cancers using in vitro and in vivo Models. DBT. 2010-2013.
2. STAT3 a Key Regulator and Novel Therapeutic Target in Osteopontin-induced Tumor Growth and Angiogenesis in Breast Cancer. DST. 2010-2013.
3. Silencing Osteopontin and its Downstream Oncogenic Molecules Suppress the Tumor Growth and Angiogenesis in Breast Cancer. DBT. 2008-2011.
4. Role of Splice Variants of Osteopontin in Regulation of PI 3 Kinase Dependent/Independent ILK-mediated VEGF Expression Leading to Breast Tumor Growth and Angiogenesis. CSIR. 2010-2013.

Dr. Anandwardhan A. Hardikar

1. Differentiation of human fetal pancreatic progenitor cells for potential use in cell replacement therapy for type 1diabetes. DBT. 207-2010.
2. Biliary Duct Stem Cells for Cell Replacement Therapy in Diabetes. UKIERI. 2007-2010.

Dr. Yogesh S Shouche

1. DNA barcoding of butterflies from Western Ghat. DBT. 2007-2010.
2. DNA Barcoding of Amphibians from Western Ghat 2007-2010. DBT. 2007-2010.
3. Screening for Bio-molecules from microbial diversity collected from different ecological niches. DBT. 2007-2010.
4. Characterization of Hox complex and regulatory elements from Anopheles stephensi and Aedes aegypti. 2008-2011.

5. Establishment of Microbial Culture Collection and Biological Research Centre. DBT. 2009-2011.
6. Methanotrophic communities in a meteor impact crater lake in India,funded by UK India Research and Educational Initiative. UKIERI. 2008-2011.
7. Microbial Diversity & Development of Antibiotic resistance associated with industrial waste water treatment. Swedish Research Council. 2009-2011.
8. Mid gut bacteria in Aedes aegypti and vector competence, Forgarty International Research Collaboration Award

Dr. Debashis Mitra

1. 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
2. Dissecting the CD40L-CD40 mediated signaling pathway in HIV infection. DBT. 2006-2009. Co-Investigator: Bhaskar Saha (NCCS) and Sekhar Chakrabarti, IICB, Kolkata
3. 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.

Dr. Arvind Sahu

1. Role of vaccinia virus complement control protein in the vira pathogenesis. DBT. 2007-2010. Co-Investigator: Dr. D. Mitra

Dr. Sanjeev Galande

1. Centre of Excellence in Epigenetics. DBT. 2010-2015.
2. Frontiers of Science Award (Indo-US collaborative project). Indo-US Science and Technology Forum. 2010-2012.
3. Indo-Finland collaborative project. DBT. 2010-2013.

Dr. Samit Chattopadhyay

1. Molecular switch in Th1-Th2 response by SMAR1: Its implications in Mycobacterium Tuberculosis infection. DBT. 2007-2010.
2. Regulation of HIV-1 LTR mediated transcription by MAR binding protein SMAR1. DBT. 2007-2010.
3. Coordinated role of SMAR1 and p53 in tumorigenesis and apoptosis. DBT project. DBT. 2009-2012.

Seminars

Seminars delivered by Visiting Scientists

1. Prof. Srini V Kaveri, Equipe 16 - INSERM - U 872, Centre de Recherche desCordeliers PARIS, FRANCE, Immune homeostasis: what have natural antibodies got to do? 17th December, 2009.
2. Prof. Geeta Narlikar, University of California, San Francisco, USA, Mechanisms of regulation of chromatin structure and function, 23rd December, 2009.
3. Prof. Vivek Rangnekar, Department of Radiation Medicine University of Kentucky, Lexington KY,USA: Cancer Selective Apoptosis in Mouse Model, 15th February, 2010.
4. Dr. Luca Pellegrini, Department of Biochemistry University of Cambridge, UK Structural insights into the initiation of DNA synthesis in eukaryotic replication,16th February, 2010.
5. Dr. Marcin Andor, Europe, Applications of CMOS & EMCCD Camera, 22nd February, 2010.
6. Dr. Sourav Banerjee, Neuroscience Research Institute, University of California, Santa Barbara,, CA-93106, USA, MicroRNAs in Synaptic Plasticity, Tiny RNAs with Big Potential, 3rd March, 2010.
7. Dr. Girdhari Lal, Department of Gene and Cell Medicine, Mount Sinai School of Medicine New York, USA, Epigenetic Regulation of Foxp3 Expression in Regulatory CD4+ T Cells, 8th March, 2010.
8. Sach Jayasinghe, FlowJo Application Scientist, Australia, FlowJo, Flow cytometry Data Analysis software, 28th April, 2010.
9. Dr. Nagesh Narayan Panday, Beckman-Coulter, A new era in Flow Cytometric analysis: KALUZA, 2nd July 2010.
10. Dr. Dipankar Malakar, Application Scientist, Lab-India, QTRAP and MALDI OF/TOF, Application for proteomics, 21st July, 2010.

Seminars delivered by NCCS Scientists

Dr. Mohan Wani

- "Surgical techniques in laboratory animals" National Institute of Virology, September, 4, 2010.
- "Role of interleukins in bone remodeling" on the occasion of National Science Day, B J Medical College, Pune, March 4, 2010.

Dr. Sharmila Bapat

- Bapat SA. Isolation, Characterization and Applications of Cancer Stem Cells. International meeting on Research in Vision and Ophthalmology organized by the L.V. Prasad Eye Institute, on behalf of Asia-Arvo, at Hyderabad, India from 15th – 18th January, 2009. Chaired the session "New Insights from Cancer Stem Cell Biology"
- Bapat SA. Ovarian Cancer Stem Cell Biology. First International Undergraduate Symposium on "Molecular and Cellular Medicine" Organized jointly by Krishna Institute of Medical Sciences Deemed University, Karad, Moving Academy of Medicine and Biomedicine, Pune and Association of Indian Pathologist of North America on February 1st, 2009, at Karad, Maharashtra
- Bapat SA. Stem Cells and Cancer. Symposium on Emerging Trends in Biotechnology organized by the Indira College of Pharmacy, Pune on 28th February, 2009
- Bapat SA. Ovarian Cancer Stem Cells. Symposium on Ovarian Cancer organized at the All India Institute of Medical Sciences, New Delhi on 27th – 28th March, 2009
- Bapat SA. Systems Network Analyses of a gene expression based signature of serous ovarian cancer. 78th Annual Meeting of the Society of Biological Chemists (India) organized by the NCCS at Pune from 30th October – 1st November, 2009
- Bapat SA. Cancer Stem Cells. Symposium on Emerging Trends in Biotechnology organized by the International Institute of Information Technology, on 13th November, 2009
- Bapat SA. Plenary Talk - Cancer stem cells as determinants of tumor dormancy. 3rd Translational Meeting organized by the Institute of Life Sciences, Bhubhaneshwar from 18th – 21st December, 2009
- Bapat SA. Gene Expression - Protein Interaction Systems Network Modeling identifies Transformation Associated Molecules and Pathways in Ovarian Cancer. 29th Annual Convention of IACR at

Amritha Institute of Medical Sciences, Kochi 20st – 23rd February, 2009; also chaired a Session during the meeting.

- Bapat SA. Ovarian Cancer Stem Cells. Symposium on Recent Trends in Biology organized by the Department of Zoology, Pune University on 26th -27th February, 2010
- Bapat SA. Cancer stem cells as determinants of tumor dormancy. Symposium on Recent Trends in Stem Cells organized by the Modern College, Pune University on 13th-15th March, 2010
- Bapat SA. Comprehensive approaches towards understanding ovarian cancer stem cell biology. Talk delivered at the National Brain Research Institute, Gurgaon on 5th May, 2010.
- Bapat SA. Integrative Approaches for the identification and assessment of Cancer Biomarkers. Invited speaker at the 3rd DBT-AIST meeting held at Tsukuba, Japan 24th – 29th October, 2009.

Dr. Manoj Kumar Bhat

Invited talk at ST. Philomena College (University of Mysore) at Mysore on 9-10th April 2010. Title of the presentation: Advances in Cancer Chemotherapy.

Dr. Lalita S.Limaye

- Modern College of Arts , Science and Commerce in National Conference on Stem Cell Technology on 15th March 2010 on "Hematopoietic stem cells"
- National Institute of Immuno Hematology in "Advanced clinical applications of flowcytometry' on 26th Feb 2010 On "Applications of Flow cytometry in hematopoietic stem cell research"

Dr. Anandwardhan A. 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 -cells" Invited presentation at Center for Regenerative Medicine, University of Bath, UK, March 2009

Dr. Debashis Mitra

- Novel molecules and strategies targeting Human Immunodeficiency Virus-1 replication, Invited talk at Indo-Brazil symposium on Infectious Diseases, Kolkata, Dec 10-11, 2009.
- CD4+ T cell help is required for CD8+ T cell proliferation during HIV infection. Md. Zulfazal Ahmed, Bhaskar Saha and Debashis Mitra, Work presented at Molecular Immunology Forum 2010, Indian Institute of Chemical Biology, Kolkata, January 15-17, 2010.
- Cellular and Viral Micro RNAs in HIV-1 pathogenesis. Invited talk at RNA 2010, A National Symposium of the RNA group of India, University of Pune, January 18-19, 2010.
- Role of cytokines in modulation of immune response to HIV-1 antigens gp120 and Tat. Invited talk at International Seminar on new trends in AIDS vaccine development and treatment, January 30, 2010. International Institute of Information Technology, Pune.
- Regulation of HIV-1 gene expression: Role of Heat Shock Factor-1. Pratima Rawat and Debashis Mitra. Work presented at 13th Transcription Assembly Meeting 2010, Jawaharlal Nehru University, New Delhi, February 26-28, 2010.

Dr. Anjali Shiras

- RNA Biology Meeting - A novel mechanism of oncogenesis by a non-coding RNA Ginir at University of Pune, 2009.
- Modern Trends in Biotechnology- Popular Lecture arranged by Modern College of Pharmacy, Pune, 2010.

Dr. Yogesh S Shouche

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

Dr. Shailza Singh

- Invited talk on 9th Feb. 2010 at Modern College of Arts, Science and Commerce (University of Pune). Title of Presentation "Gene to Drug In Silico: Bioinformatics in the post-genomic era."

Dr. Sanjeev Galande

- "Acetylation of chromatin organizer SATB1: A double-edged sword for regulation of gene expression upon Wnt signaling" at the Annual meeting of the Cell Biology Society, December 10-12 2009, University of Hyderabad, Hyderabad.
- "Evolution of organismal complexity and genome size: lessons from the genome sequencing projects" at the International Meeting on Evolution, November 14-16, 2009, Alexandria, Egypt.
- "Novel nuclear function of Fibronectin" at the Turku Biotechnology Centre, Turku, Finland, January 7, 2010.
- "Unfolding the chromatin 'loopscape': integrating higher-order chromatin architecture with gene regulation" at the University of Calcutta, February 12, 2010.
- "From Sequence to Consequence" at the Computational Research Laboratory, Pune, April 2010.

Dr. Samit Chattopadhyay

- Transcriptional regulation of apoptotic genes by p53. International conference on cancer biology–Molecular mechanisms and novel therapeutics. IIT – Chennai, February 18-20, 2010.
- Role of SMAR1 in T helper (Th) cell differentiation, Molecular Immunology Forum, Raichawk, January 15-17, 2010.
- Stem loop structure of 5'UTR is required for mRNA stability and tumor suppressor activity of SMAR1 RNA meeting at Department of Biotechnology, University of Pune, January15-16, 2010.
- Attended Guha Research Conference at Mangalore during December 19 to 22, 2009

Conferences / workshops attended by Scientists

Dr. Mrs. Vaijayanti P. Kale

- Monika Sharma, Lalita Limaye and Vaijayanti Kale (2010) Three-dimensional hydrogel-based cultures of mesenchymal stem cells closely mimic the in vivo hematopoietic stem cell regulatory-niche. Abstract accepted for an oral presentation in the annual meeting of International Society of Experimental Hematologists (ISEH) to be held at Melbourne, Australia from 15th to 18th September 2010.
- Vaijayanti Kale. Invited speaker in Biologic India" (Dec 1-4, 2009) organized at Hyatt Regency, Mumbai, by Terrapin, Singapore. Delivered the seminar on: Therapeutic Potential of artificial bone marrow-like environments generated in vitro.
- Vaijayanti Kale. Hands on training on BD FACS ARIA II and BD Pathway 855 at San Jose, California, USA (14th to 25th September 2009)
- Smita Paranjape, Monika Sharma and Vaijayanti P. Kale (2009) Soluble Jagged-1 presented in the context of marrow stromal cells promotes proliferation of hematopoietic stem cells. Poster presentation at 50th Annual Conference of Association of Microbiologist of India (AMI), held in NCL from 15th Dec to 18th Dec 2009.

Dr. Lalita S.Limaye

- Attended workshop on image analyzer and FACS Aria in BD ,San Jose , California, USA, in Sept 2009.

Dr. Nibedita Lenka

- DBT sponsored Brain Storming session on human ES cells, NIRRH, Mumbai (2010).

Dr. Anjali Shiras

- Oral Presentation in Annual Conference of the Indian Society of Neuro-oncology -2010 Meeting at Chennai, India : Establishment Of Model System Of Cancer Stem Cells For Deciphering Mechanisms Of Cellular Signalling

Dr. Anandwardhan A. Hardikar

- First meeting of the Islet Society, Stockholm, Sweden (July 2010)
- Australian Diabetes and islet transplantation meeting, St. Vincent's Research Institute, Melbourne, Australia (November 2009)
- Stem cells and Diabetes Annual meeting at the University of New South Wales, Sydney, Australia (December 2008)
- Pancreas Developmental Biology meeting at the Center for Regenerative Medicine, University of Bath, UK on March 2009
- EMBO workshop in Peebles, UK February 2009

Dr. Yogesh S Shouche

- Annual Meeting of Association of Microbiologists of India, Pune , 2009
- Annual meeting of Federation of European Microbiological Society, Gothenborg, 2009
- European Culture Collection Organization meeting, Gothenborg, 2009

Dr. Debashis Mitra

- Indo-Brazil symposium on Infectious Diseases, Indian Institute of Chemical Biology, Kolkata, Dec 10-11, 2009
- Molecular Immunology Forum 2010, Indian Institute of Chemical Biology, Kolkata, January 15-17, 2010.
- RNA2010, A National Symposium of the RNA group of India, University of Pune, January 18-19, 2010.
- International Seminar on new trends in AIDS vaccine development and treatment. International Institute of Information technology, Pune, January, 30, 2010.
- 13th Transcription Assembly Meeting 2010, Jawaharlal Nehru University, New Delhi, February 26-28, 2010

Dr. Arvind Sahu

- Molecular Immunology Forum (2010), Kolkata.

Dr. Sanjeev Galande

- Trendys, August 16-17, 2009, Institute of Life Sciences, Bhubaneshwar.

- Darwin Now, International Meeting on Evolution organized by the British Council, November 14-16, 2009, Alexandria, Egypt.
- Indo-US workshop on Epigenetics, December 16-18, Centre for Cellular and Molecular Biology, Hyderabad.
- Young Investigator Meeting, February 8-12 2010, Fort Raichuk, Kolkata
- Cold Spring Harbor Symposium on Quantitative Biology: Nuclear organization and function, June 2-7, 2010, Cold Spring Harbor, USA.

Dr. Shailza Sigh

- Worshop on "Structure based Drug Design", 8th December 2009, organised by Sinhgad College of Pharmacy and Schrodinger, USA.

Conferences / workshops attended by students

- Poster presented at XXXIII All India Cell Biology Conference, Hyderabad Dec10-13 2009, Shardul D. Kulkarni, Bhavana Muralidharan and Vasudevan Seshadri, Glucose induced translation regulation of insulin mediated by 5'UTR binding proteins.
- Poster presented at XXXIII All India Cell Biology Conference, Hyderabad Dec10-13 2009, Amaresh C. Panda and Vasudevan Seshadri, Novel splice variant of mouse insulin2 mRNA: implications for insulin expression
- Poster presented at 5th Graduate students meet, ACTREC, Navi Mumbai, Dec 18-19, 2009, Shardul D. Kulkarni, Bhavana Muralidharan and Vasudevan Seshadri, Glucose induced translation regulation of insulin mediated by 5'UTR binding proteins. (Best poster, second prize)
- Poster presented at 5th Graduate students meet, ACTREC, Navi Mumbai, Dec 18-19, 2009, Amaresh C. Panda and Vasudevan Seshadri, Novel splice variant of mouse insulin2 mRNA: implications for insulin expression
- Oral presentation at RNA 2010 meeting, University of Pune, Pune, January 18-19, 2010, Shardul Kulkarni, Glucose induced translation regulation of insulin mediated by 5'UTR binding proteins.

- Poster presented at 78th Annual Meeting of Society of Biological Chemists (India), National Centre for Cell Science, Pune, October 30- November 1, 2009. Baskar B., Sarmishtha K.,Galande S., Ramanamurthy B., Parab P.B., Kohale K. and Seshadri V.. Dense Cataract and Microphthalmia (dcm) in BALB/c Mice is caused by mutation in GJA8 gene
- Prayag Murawala, Mukesh Mani Tripathi, Pankhuri Vyas, Aparna Salunke and Jomon Joseph: Nup358 interacts with APC and plays a role in cell polarization. 33rd All India Cell Biology Conference at University of Hyderabad, Hyderabad held in 10th to 13th December 2009.
- Prayag Murawala, Mukesh Mani Tripathi, Pankhuri Vyas, Aparna Salunke and Jomon Joseph: Nup358 interacts with APC and plays a role in cell polarization. 5th All India graduate student meet at ACTREC, Mumbai held in 18th to 19th December 2009.
- Pankhuri Vyas attended 35th Mahabaleswar Seminar on the Evolution of Molecular function and principles of protein design at Mahabaleswar, India on 21st to 28th February 2010.
- Pabitra Kumar Sahoo attended 33rd All India Cell Biology Conference at University of Hyderabad, Hyderabad held in 10th to 13th December 2009.
- Raj Kumar S. Kalra, Avinash Mali, S.A. Bapat. mAb150, a monoclonal antibody mediates cytoreductive effects in vivo against Ovarian Cancer Epithelial Cells, Translation Cancer Research. Bhubaneswar, Orissa, December 18-21, 2009.
- Pandey, V., Vijaykumar, M.V. and Bhat, M.K.: Hyperglycemia enhances the cytotoxicity of DNA damaging drugs carboplatin and 5-Fluorouracil in MCF-7 cells: involvement of ROS. 3rd International Symposium on Translational Cancer Research, 18th – 21st December 2009, Bhubaneswar – 751023, Orissa, India.
- Ajay, A.K. and Bhat, M.K.: Purturbation of caeveolin-1 induces cell death in cells with functional cevaeolin-1. 3rd International Symposium on Translational Cancer Research, 18th – 21st December 2009, Bhubaneswar – 751023, Orissa, India.
- Sharma, A. and Bhat, M.K.: Chemotherapy and Hyperthermochemotherapy : Functional Significance of Heat Shock Proteins in Hepatocellular Carcinoma. 3rd International Symposium on Translational Cancer Research, 18th – 21st December 2009, Bhubaneswar – 751023, Orissa, India. (awarded best poster prize)

- Kumari, R., Sharma, A., Ajay, A.K. and Bhat, M.K.: Mitomycin C Induced Bystander Killing in Hepatoma Cells: Mechanism and Significance. 3rd International Symposium on Translational Cancer Research, 18th – 21st December 2009, Bhubaneswar – 751023, Orissa, India.
- Meena, A.S.,Sharma, A., Kumari, R. and Bhat, M.K.: Molecular mechanisms underlying chemotherapeutic drug resistance in Hepatocellular Carcinoma: Role of membrane and cellular signalling proteins. 3rd International Symposium on Translational Cancer Research, 18th – 21st December 2009, Bhubaneswar – 751023, Orissa, India.
- Oral presentation- 29th Annual Convention of Indian Association for Cancer Research, Feb 20-23, 2010, AIMS, Cochin. Loss of function of Rictor enhances MMP-9 activity and invasion, independent of PKC-□ in gliomas (Involvement of Raf 1/ MEK/ERK pathway) by Gowry Das and Padma Shastry. Awarded Sitaram Joglekar award for "The Best Oral Presentation by Young Scientist"
- Dr. Varsha Shepal - Presented Poster at Translational Research Meeting at Bhubaneswar India Nov 210. Awarded "Second Best Poster award" Studies of novel non-coding RNA with transformation potential.
- 78th Annual Meeting of Society of Biological Chemists (India), National Centre for Cell Science, Pune, October 30- November 1, 2009. Host Cellular factors in HIV-1 pathogenesis: interactions and interplay, Poster no 182, Sohrab Z. Khan, Pratima Rawat, Renu Singh, Neeru Dhamija, Manoj K. Tripathy, Sanchita Roy, Zulfazal Ahmed and Debashis Mitra. Best poster Award to Sohrab Z. Khan.
- Muzammil Ahmad attended EMBO Meeting 2009 at Amsterdam and presented a poster entitled "The complement control protein domain 2 (CCP2) of vaccinia virus complement control protein plays critical role in imparting factor I cofactor function", August 29 - September 1, 2009.
- Kalyani Pyaram and Viveka Nand Yadav attended the 78th Society of Biological Chemists (India) meeting hosted by NCCS at YASHADA, Pune, October 30 – November 1, 2009.
- Nidhi Chaudhary and Jinu Mary Mathai attended an International conference on cancer biology- molecular mechanisms and novel therapeutics. Held at IIT- Chennai, February 18-20, 2010.

Students awarded PhD

Ms Ashwini Hinge

Title of the Thesis: Studies on the effects of plant lectins on Hematopoietic stem cells.

Guide: Dr. Mrs. Vaijayanti P. Kale

Bhavana Muralidharan

Title of the Thesis: Role of Untranslated Regions of Insulin mRNA in its Translational Regulation by Glucose.

Guide: Vasudevan Seshadri

Mr. Syed Khund Sayeed

Title of the Thesis: Studies on Merozoite surface protein gene expression of Plasmodium falciparum

Guide: Dr. D. Prakash, Dr Vasudevan Seshadri and Dr Sanjeev Galande

Mr. Nawneet K. Kurrey

Title of the Thesis: Transcription Factors Snail and Slug in Ovarian Cancer Invasiveness, Metastasis and Cell Survival;

Guide: Dr. S. A. Bapat.

Mr. Amrendra Kumar Ajay

Title of the Thesis: Relevance of p53, E6, Caveolin and other cellular proteins in ensitivity of cancer cells to chemotherapeutic drugs

Guide: Dr. Manoj Kumar Bhat

Dr. E. Maheswara Reddy

Title of the Thesis: Cloning, Characterization and Gene expression studies with a newly identified transcriptional activator Dlxin-1; Study of its role in cell cycle progression and growth regulation.

Guide: Dr. Anjali Shiras

Dr. Santosh Kumar

Title of the Thesis: Investigation into cellular and molecular mechanisms underlying diabetic cardiomyopathy: Role of oxidative and Nitrative stress: Therapeutic potential of multiple antioxidants.

Guide: Dr. Sandhya L. Sitasawad

Dr. Sandeep Kumar

Title of theThesis: Hyperglycemia induced contractile dysfunction and stress signaling changes in diabetic heart: Role of oxidative and nitrosative stress. Protection afforded by insulin and antidiabetic drugs

Guide: Dr. Sandhya L. Sitasawad

Dimple Notani

Title of the Thesis: Signal-dependent gene regulation by SATB1: role of its PDZ- like domain and interaction partners

Guide: Dr. Sanjeev Galande

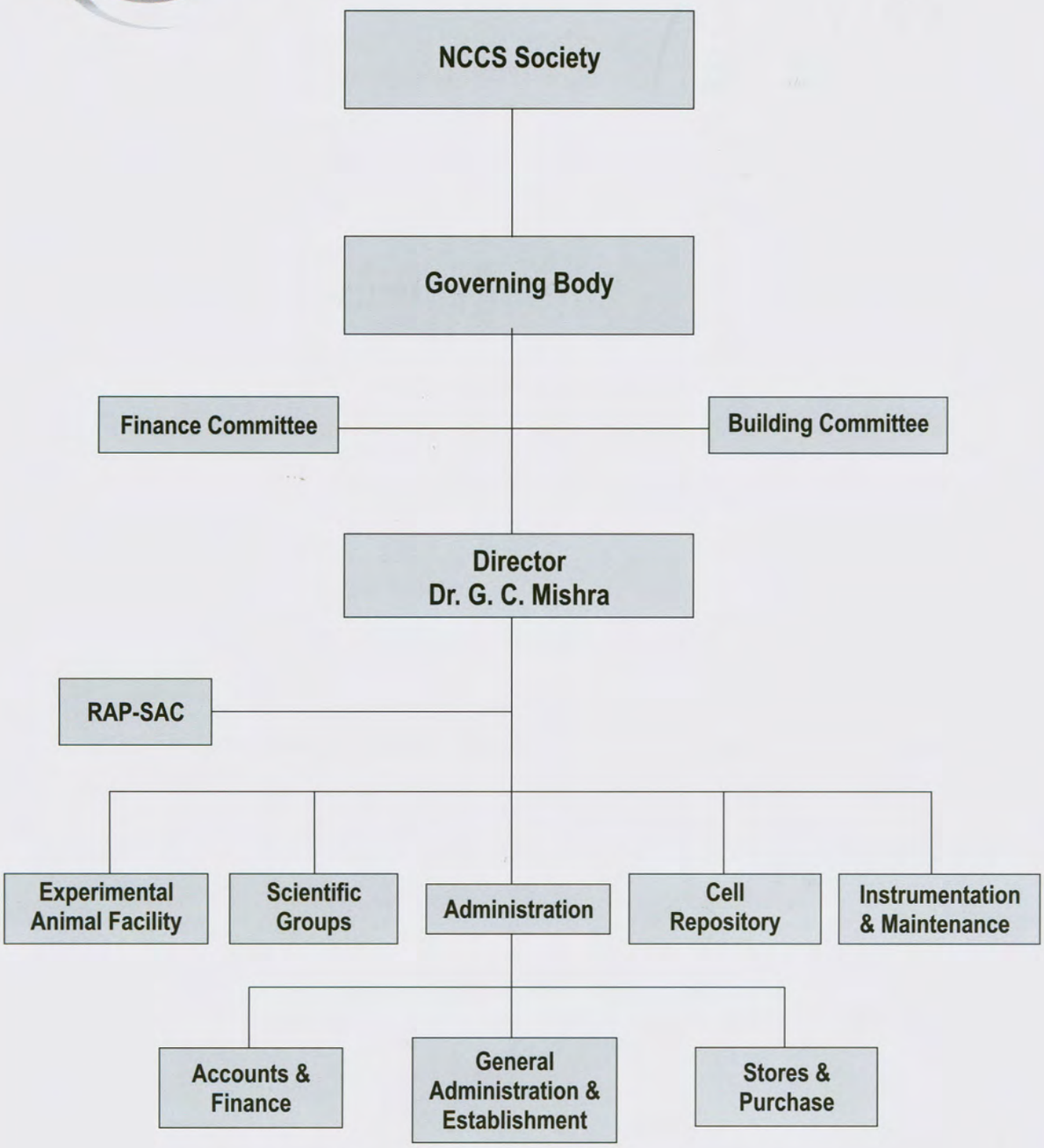
Amita Limaye

Title of the Thesis: Functional characterization of the novel alternative spliced form SATB1L during T-cell differentiation and development

Guide: Dr. Sanjeev Galande

Organizations

NCCS ORGANIZATION



NCCS COMMITTEES

Society

1.	Shri Prithiviraj Chavan, Honourable Minister of State (Independent Charge), Ministry of Science & Technology & Earth Sciences, Ministry of Personnel, Public grievances & Pensions and Minister of State in the Prime Minister's Office, Ministry of Parliamentary Affairs, Anusandhan Bhawan, 2, Rafi Marg, New Delhi. 110 001.	President
2.	Prof. M. K. Bhan, Secretary, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi. 110 003	Member
3.	Dr. Vishwa Mohan Katoch, Secretary, Department of Health Research and Director General, Indian Council of Medical Research, Ansari Nagar, Post Box 4911, New Delhi. 110 029.	Member
4.	Dr. S. Ayyappan, Director General, Indian Council of Agricultural Research And Secretary, Dept. of Agricultural Research & Education, Krishi Bhavan, New Delhi. 110 114.	Member
5.	Prof. R.K. Shevgaonkar, Vice Chancellor, University of Pune, Ganeshkhind, Pune. 411 007.	Member
6.	Prof. Umesh Varshney, Dept. of Microbiology & Cell Biology, Indian Institute of Science, Bangalore. 560 012	Member

7.	Ms. Sheila Sangwan Additional Secretary & Financial Adviser, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi. 110 003 8.	Member
8	Shri Sukdeb Sinha Adviser, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi. 110 003	Member
9.	Dr. (Mrs.) S.S. Ghaskadbi, Head, Department of Zoology, University of Pune, Ganeshkhind, Pune. 411 007.	Member
10.	Dr. Kanuri Rao, Sr. Scientist & Head, Immunology Group, International Centre for genetic Engineering And Biology Aruna Asaf Ali Marg, New Delhi. 110 067	Member
11.	Dr. Padma Shastry, Scientist 'G' National Centre for Cell Science, Ganeshkhind, Pune. 411 007.	Member
12.	Dr. G. C. Mishra Director National Centre for Cell Science, Ganeshkhind, Pune. 411 007.	Member Secretary

Governing Body

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

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

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

4. Prof. R.K. Shevgaonkar, Member
Vice Chancellor,University of Pune,
Ganeshkhind,
Pune. 411 007.

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

6. Ms. Sheila Sangwan Member
Additional Secretary & Financial Adviser,
Department of Biotechnology,
Ministry of Science & Technology,
Block No.2, 7th floor,
CGO Complex, Lodi Road,
New Delhi. 110 003

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

8. Dr. Kanuri Rao, Member
Sr. Scientist & Head,
Immunology Group,
International Centre for genetic
Engineering And Biology,
Aruna Asaf Ali Marg,
New Delhi. 110 067

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

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

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

Finance Committee

1.	Ms. Sheila Sangwan, Additional Secretary & Financial Adviser, Department of Biotechnology, Ministry of Science & Technology, Block No.2, 7th floor, CGO Complex, Lodi Road, New Delhi. 110 003	Chairperson
2.	Dr. Srikanth Tripathy, Scientist 'F', Clinical Sciences Dept., National Aids Research Institute, G-Block, P-73, Near Electronic Sadan, Bhosari, Pune 411 026	Member
3.	Dr. Satish Kumar Gupta, Chief- Reproductive Cell Biology Laboratory, and Scientist H National Institute of Immunology, Aruna Asaf Ali Road, New Delhi 110 067	Member
4.	Prof (Mrs.) S.S. Ghaskadbi, Head, Department of Zoology, University of Pune, Ganeshkhind, Pune 411 007	Member
5.	Shri Sukdeb Sinha, Adviser, Department of Biotechnology, Block No. 2, 7th - 8th Floor, CGO Complex, Lodi Road, New Delhi 110 003.	Special Invitee
6	Dr. G. C. Mishra Director National Centre for Cell Science, Ganeshkhind, Pune 411 007.	Member Secretary

Building Committee

1.	Dr. A.C. Mishra, Director, National Institute of Virology, Dr. Ambedkar Road, Pune - 411 001	Chairman
2.	Dr. B. Ravindran, Director, Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar – 751 023	Member
3.	Dr. V.S. Rao, Ex-Director, Agharkar Research Institute, Pune	Member
4.	Dr. J. Nagaraju, Staff Scientist, Centre for DNA Fingerprinting And Diagnostics, Bldg. 7, Gruhakalpa, 5-4-399/B, Nampally, Hyderabad – 500 001	Member
5.	Shri Sukdeb Sinha, Adviser, Department of Biotechnology, Block No. 2, 7th Floor, CGO Complex, Lodi Road, New Delhi – 110 003	Member
6.	Chief Engineer, Pune (PW) Region, New Central Building, Pune 411 001	Member
7.	Shri Sanjay Goel, Director (Finance), Department of biotechnology, Block No. 2, 7th Floor, CGO Complex, Lodi Road, New Delhi 110 003	Member
8.	Dr. G.C. Mishra, Director, National Centre for Cell Science, Ganeshkhind, Pune	Member
9.	Shri P.Y. Bhusnale, Tech. Officer 'C' (I&M) NCCS, Pune	Convener
10.	Shri B.G. Acharya, Officer 'D' (Sr. Officer-Admin) NCCS, Pune	Special Invitee
11.	Dr. M.S. Patole Scientist 'F' and In-charge -Accounts NCCS, Pune	Special Invitee

Scientific Advisory Committee

1. Dr. Kanuri Rao — Chairman
International Scientist
International Centre for Genetic
Engineering & Biotechnology
Behind JNU Campus, Aruna Asaf Ali Marg
New Delhi 110 067

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

3. Dr. N. K. Mehra, — Member
Head,
Dept of Transplant Immunology &
Immunogenetics,
All India Institute of Medical Sciences,
New Delhi – 110 029

4. Dr. Rajesh S. Gokhale, — Member
Director, Institute of
Genomics and Integrative
Biology Mall Road, Delhi-110 007

5. Dr. Soniya Nityanand, — Member
Professor and Head, Dept of Hematology,
Sanjay Gandhi Post Graduate Institute of
Medical Sciences, Immunology Division,
Raebareli Road Lucknow 226 014

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

7. Dr. Samit Adhya, — Member
Head, Molecular and Human
Genetics Division,
Indian Institute of Chemical Biology,
4, Raja S. C. Mullick Road,
Kolkata- 700 032, West Bengal

8. Dr. Amitabha Mukhopadhyay, — Member
Scientist,
National Institute of Immunology,
Aruna Asaf Ali Marg,
New Delhi – 110 067

9. Dr. V. Nagraja, — Member
Professor,
Microbiology & Cell Biology,
Indian Institute of Science ,
Bangalore 560 012

10. Dr. Kumarvel Somasundaram, — Member
Associate Professor
Microbiology & Cell Biology,
Indian Institute of Science ,
Bangalore 560 012

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

12. Dr. R. Varadarajan, — Member
Professor,
Molecular Biophysics Unit,
Indian Institute of Science ,
Bangalore – 560 012

13. Shri. S. Sinha, — Member
Advisor,
Department of Biotechnology,
Block 2, 7th Floor,
CGO Complex, Lodi Road,
New Delhi – 110 003

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	:	26
Administrative	:	41
Technical	:	61
Total	:	128

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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.

Hindi diwas chief guest Dr. Padmaja Ghorapade (left) Head, Hindi Department, S. P. College, Pune, being greeted by Dr. Padma Shastry (right), Scientist, NCCS.

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 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 'F', 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