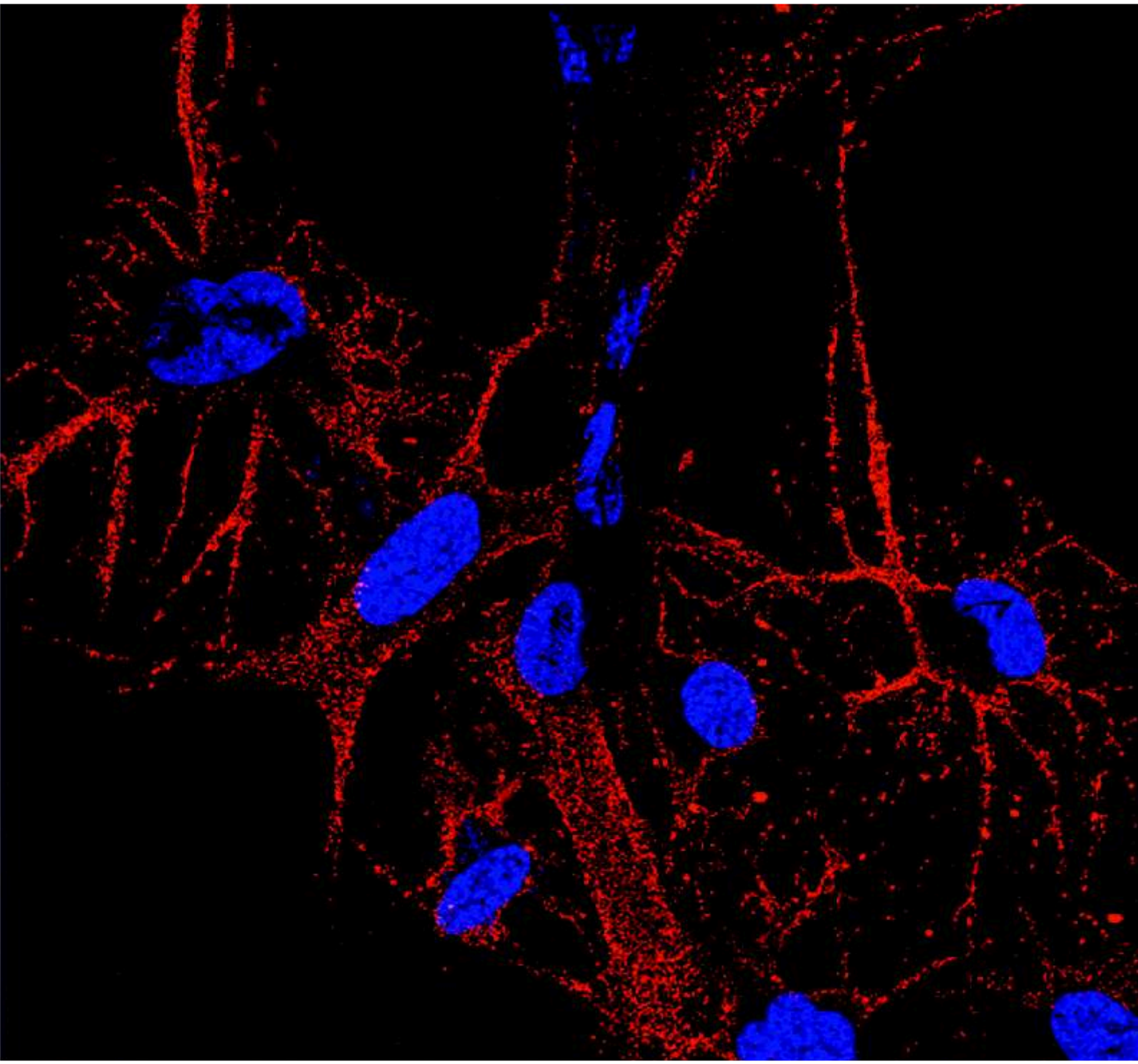
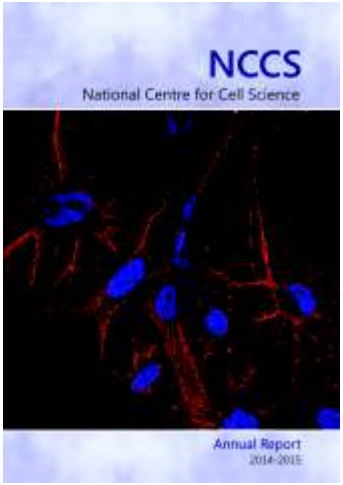
NCCS

National Centre for Cell Science



Annual Report

2014-2015



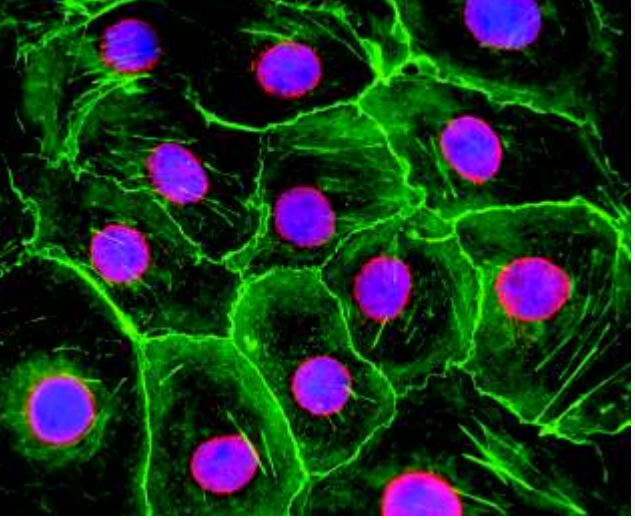
Cover page image

Mesenchymal stem cells from human placenta subjected to neural differentiation stained positive (red) for MAP2 (microtubule associated protein 2). Nuclei are stained with DAPI (blue).

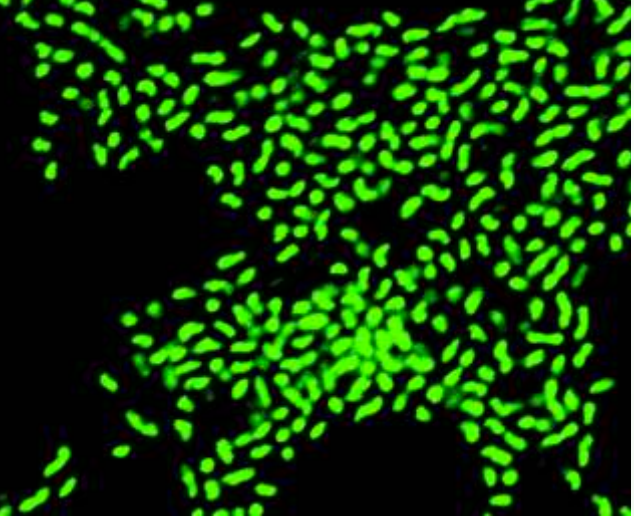
(Image courtesy of Dr. L. S. Limaye, Ms. Manasi Talwadekar & the NCCS confocal facility)

National Centre for Cell Science

Annual Report 2014 - 2015



Contents



Mandate of NCCS

- To receive, identify, maintain, store, grow and supply:
  - Animal and human cell cultures.
  - Newly developed and existing (typed) cell lines.
  - Hybrid cells including hybridomas.
  - Tissues, organs, eggs (including fertilized ones) and embryos.
  - Unicellular, obligate pathogens, parasites and vectors.
  - Plasmids, genes and genomic libraries.

- To develop, prepare quality control and supply culture media, other reagents and cell products independently and in collaboration with industry and other organizations.

- Research and development.

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

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

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

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

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

From the Director's Desk

I am privileged to present the Annual Report of the National Centre for Cell Science (NCCS), Pune, for the year 2014-15. NCCS continues to uphold its legacy of carrying out frontline research in major areas of modern Cell Biology and supplying cell lines to researchers across the country, while also providing training to develop high quality manpower in cell biology. During this year, NCCS provided four thousand five hundred and eight cell lines to four hundred organizations as a part of its commitment to supporting research in cell biology across the country. Towards nurturing young scientific talent, NCCS admitted twenty eight research scholars into its PhD programme, and fifteen research scholars of NCCS registered as Ph.D. students with the University, bringing the total number of research scholars who are registered for a Ph.D. to 133 during this year. NCCS also trained twenty one summer trainees and forty four project trainees.

The research at NCCS is dedicated to understanding the functioning of the cellular machinery in health and disease. Some key findings of a few of our research groups are summarized.

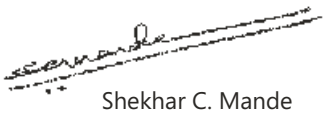
Cancer, aptly described by Siddhartha Mukherjee as "The Emperor of All Maladies", is a leading cause of death worldwide. At the molecular level, cancer arises mainly due to the inactivation of malignancy-suppressive genes and/or the activation of malignancy-promoting genes. The well-known tumor suppressor, p53, is considered as 'the guardian of the genome', owing to its important role in combating myriad cellular stresses. Under stress conditions, p53 can either induce senescence, arresting cell growth to enable the repair of any damage in the genome, or can direct damaged, potentially cancerous cells towards apoptosis, thereby preventing the development of cancer. Surprisingly, even though the activation of p53 is significantly compromised by the oncoprotein, MDM2 in the majority of cancers, substantial activation of p53 is often observed upon chemo- or radiotherapy used to treat cancer. The mechanism underlying this activation was hitherto not well understood. The research findings of Dr. Manas Santra and his group have recently shed more

light on this. They found that the tumor suppressor, FBXO31 gets stabilized in the cells upon exposure to known cancer therapy regimens, including chemo- and radio-therapy. The resultant increase in the levels of FBXO31 is then able to effectively degrade the oncoprotein MDM2 through a proteasome-mediated pathway, leading to the restoration of p53 activity. These findings, which reveal for the first time the missing link in the p53 activation pathway operative upon the administration of cancer therapy, were published in PNAS in July, 2015. The activation of p53 could enhance the response of cancerous cells to chemo- and radiotherapy, since both these work best if the tumor cells have functional p53. Therefore, resolving the underlying molecular mechanisms holds promise for the development of improved therapeutic approaches for treatment of cancer.

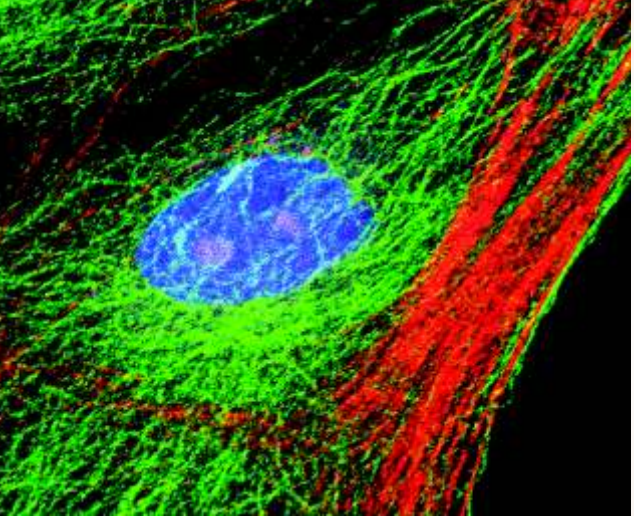
While cancer can develop in many different parts of the body, other diseases such as multiple sclerosis (MS) are localized to specific areas, like the central nervous system (CNS). MS is a T cell-mediated inflammatory autoimmune disease of the CNS, which is characterized by demyelination and axonal damage in the brain and spinal cord. Inflammatory CD4 T cells such as Th1 and Th17 play a very important role in the pathogenesis of MS. Transendothelial migration (TAM) of CD4 T cells at the blood-brain barrier (BBB) involves complex cellular and molecular responses, including various types of signalling, membrane trafficking and remodelling of cell-cell junctions. Under homeostatic conditions at the BBB, inter-endothelial junctions restrict the passive entry of soluble factors and blood cells into the CNS. Under diseased conditions, however, the BBB is compromised, enabling inflammatory cells to migrate into the CNS and damage the tissue, leading to the development of autoimmunity. Using an animal model of MS, Dr. Girdhari Lal's group investigated how inflammation that is induced by cytokines and chemokines produced in the peripheral tissues can affect the BBB endothelial cells. Their findings revealed that the Th1-specific cytokine, IFN-γ, increased the expression of cell adhesion molecules like ICAM-1, VCAM-1 & PECAM, and redistributed the tight-junction molecules, VE-Cadherin, zonula occludens-1 (ZO-1), occludins and claudin molecules, at the endothelial junction in a STAT-1 dependent manner. They found that blocking these molecules or downstream signals prevented the transmigration of CD4 T cells across the endothelial barrier. Further understanding the cellular and molecular mechanisms involved in BBB breakdown could help design novel therapeutic strategies to control neuronal inflammation and autoimmunity.

The role of free radicals in biology and medicine is being studied by Dr. Sandhya Sitaswad's research group. Their current research is focused on elucidating the mechanisms that underlie the pathogenic processes occurring in the heart, which lead to diabetic cardiomyopathy (DCM). Mitochondrial redox regulation provides valuable insights into the molecular mechanisms responsible for altered cardiac function in diabetes. This group is therefore studying the mitochondrial sources of reactive oxygen species (ROS) and the antioxidant mechanisms involved in DCM. Having earlier reported that the mitochondrial flavoenzyme, monoamine oxidase A (MAO A) is an important source of oxidative stress in the myocardium, this group sought to further determine whether MAO A plays a major role in modulating DCM. Their findings revealed that it promotes cardiac dysfunction, apoptosis, and fibrosis in DCM. These findings were published in Free Radical Biology & Medicine, in June, 2015. Discerning the operative molecular mechanisms could provide useful insights for improving therapy for DCM. This group also studies cancer biology. They had earlier isolated and patented a novel, plant-derived triterpenoid, AECHL-1, with anti-cancer properties. During recent studies on the anti-angiogenic properties of this compound, they found that it targets tumor neo-vasculature and impairs the endothelial cell cytoskeleton, making it a potential candidate for therapeutic drug development. These findings were reported in Angiogenesis in July, 2015.

We are happy to have welcomed new faculty members over the past three years, with expertise in cutting-edge research areas dealing with structural biology, stem cell biology, deciphering the role of RNA in biological control processes and the cellular and molecular basis of memory. We aspire to continue building the team with brilliant young minds who will support the vision of NCCS to expand into newer research areas, even as we further strengthen research in our core areas. We will also explore translating our promising scientific breakthroughs into tangible benefits for the people.



Shekhar C. Mande
Director



Human Resource Development

During the year 2014-15, twenty eight research scholars joined NCCS for the Ph.D. programme, under the guidance of different faculty members. Fifteen research scholars of NCCS registered as Ph.D. students with the University during this year, taking the total number of registered Ph.D. students to 133, as on 31st March, 2015. One student submitted a thesis to the University and ten students were awarded the Ph.D. degree during the said year.

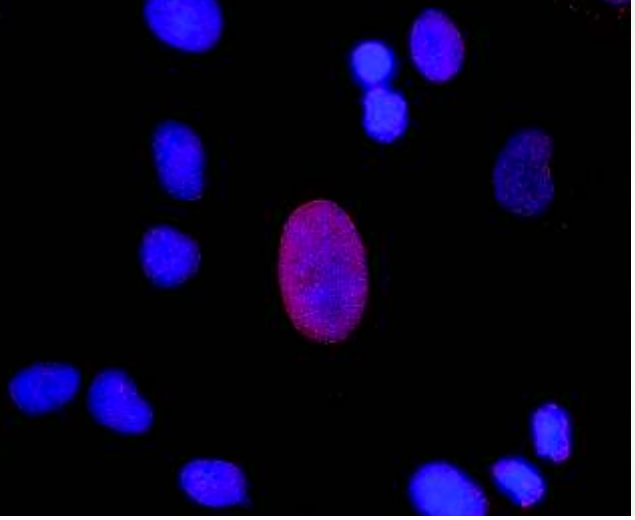
NCCS also conducts training programmes for students every year, as given below:

(a) 6-months' project training is imparted twice a year, i.e. during January-June and July-December.

(b) Summer training is conducted for 2 months during May-June. The summer trainees are selected from among the Indian Academy of Sciences Summer Research Fellows of the respective year.

The number of students who received training under these programmes during 2014-15 is as follows:

Project trainees : 44
Summer trainees : 21



Cell Repository

NCCS serves as a national cell bank for animal cell lines. The repository manages the expansion, cryopreservation and distribution of cell lines to research and academic institutions throughout the country. In the year 2014-15, four thousand five hundred and eight cell lines were supplied to four hundred research institutions.

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Ms. Anjali M. Patekar

Mr. Bhimashankar G. Utage

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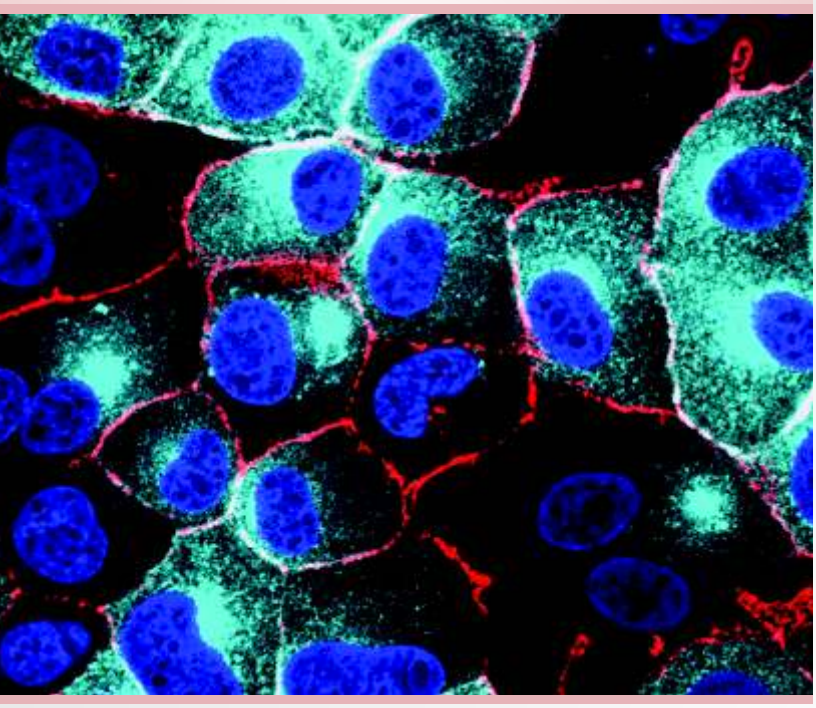
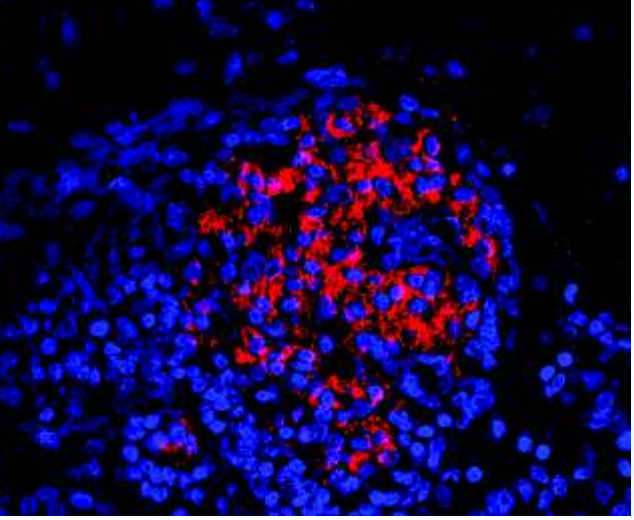


Research Reports



Index of Research Reports

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Tumor Heterogeneity and development of targeted combination cancer immuno- and chemo- therapies

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Background

Tumor heterogeneity and the presence of drug sensitive and refractory populations within the same tumor are almost never assessed in the drug discovery pipeline. Such incomplete assessment of drugs arising from spatial and temporal tumor cell heterogeneity reflects on their failure in the clinic and considerable wasted costs in the drug discovery pipeline. Following our established leads of resolution of CSCs in ovarian cancer, we mapped abberant stem cell hierarchies through label-chase of vital membrane labeling dyes (PKH) and achieved eneumeration of all cell populations with differential regenerative potentials, along with those arising from genetic instability and differential cell cyling within xenografts. Such resolution of intra-tumor heterogeneity defined the 'tumor cytotype' comprising of 18 discrete, well-defined cell fractions. With a view towards understanding cross-talks between various populations, we applied the tumor cytotype in drug screening, evaluation and prediction of responses to cancer drugs. Additional profiling of specific molecular drug targets in the tumor cytotype also revealed cell groups that could evade the most stringent individual therapies. More recently, we integrated all such information towards the development of a pipeline to derive optimal therapeutic combinations that could achieve total remission. Some of the salient findings of the project are presented.

Aims and Objectives

- Identification and functional relevance of cell-molecular drug target associations in each cell component of the tumor cytotype.
- Derivation of optimal combinations to achieve total remission.

Work Achieved

Profiling the cellular heterogeneity in residual xenografts in immunocompromised mice following drug exposure (paclitaxel, cisplatin, gemcitabine; epigenetic drugs such as 5Aza-dC, TSA, LSD1 inhibitors, curcumin; and a novel monoclonal antibody developed in our lab viz. mAb150) provide a proof-of-principle of the necessity to consider tumor heterogeneity in the assessment of drug responses. Despite significant tumor regression following each individual drug therapy, none of them achieved total remission. Essentially, three striking observations emerged regarding the response to cytotoxic drugs -

(i) rapid depletion of differentiated cells that constitute tumor bulk,
(ii) re-entry of quiescent CSCs into a state of active cell division and
(iii) emergence of parallel aneuploid hierarchies.

Each of the above contributes to disease relapse; the first could lead to incomplete drug evaluation, the second is in stark contrast to the dogma that CSCs evade drug action through quiescence to potentiate tumor regeneration on completion of treatment, while the third deems genetic variability essential for survival under further environmental stresses through shifting clonal dominance. Drug induced CSC 'enrichment' reported earlier by several groups including ourselves can be attributed to label kinetics that maintains cycling CSCs in the PKH^{hi} fraction for 3-4 doublings resulting in a logarithmic increase in frequencies of CSCs. Such drug-resistant cycling CSCs generate proliferative, maturation-blocked progenitors and aneuploid cells, while deeply quiescent CSCs may yet be retained to tackle further challenges. The latter along with slow-cycling progenitors and growth arrested aneuploid cells remain an important determinant of minimal residual disease. Further emergence of parallel hierarchies is determined by the selective pressures of the microenvironment that determine the 'fittest' clone for tumor survival.

The residual cytotype profiles were also extremely useful by identifying specific cellular targets of each drug and provided cues for derivation of improved drug combinations. While several such combinations were tested, the most efficacious one comprised of mAb150 (cytotoxic to CSCs and progenitors expressing ANXA2 which is the target antigen recognized by mAb150) along with gemcitabine (targets RRM2 expressing cycling cells) and the epigenetic drug 5Aza-dC (restricts emergence of aneuploidy in progenitor and differentiated fractions; its efficacy is determined as enhanced expression of its target biomarker PTGIS). Of the four epigenetic drugs screened, 5Aza-dC was

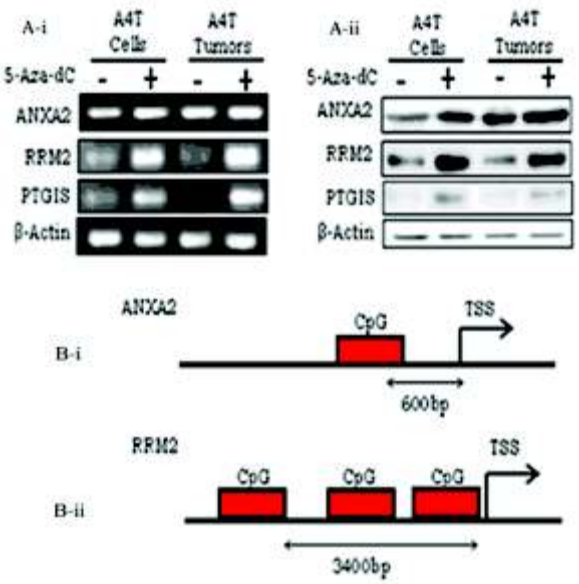

Fig. 1: **A.** Representative RT-PCR profiles (A-i) and Western blots (A-ii) of ANXA2, RRM2, PTGIS and β-actin (control) in A4 cells and A4 xenografts with and without 5Aza-dC treatment; **B.** Schematic representation of CpG islands in promoter region of AnxA2 **(B-i)**, RRM2 **(B-ii)**, identified using the EMBOSS CpG Finder tool

earlier reported to de-repress several genes including ANXA2. Indeed, following 5Aza-dC treatment upregulation of all ANXA2, RRM2 and PTGIS was detected (Fig.1). This was an important consideration in defining the components of the three-drug combination.

The first convincing indication of synergistic effect of the three-drug therapy was observed as almost complete tumor regression (trace residual granulation tissue at the tumor site) and extended survival from 54 days in untreated controls to at least 93 days in treated animals (Fig.2A). Effects following one course of three doses and recovery period (Day 28) included effective targeting of differentiated cells and enrichement of slow-cycling progenitors and CSCs. This *status quo* was maintained further even after the second course at Day 41; however activation of an euploid CSC fraction following two more courses (Days 54 & 67 respectively) generated a drug resistant tumor hierarchy towards replenishing tumor volumes (Figs.2A, right panel). However, this regeneration was short-lived and by the end of 6 cycles of chemotherapy (93 days), almost no tumor tissue was evident. Mice were however sacrificed for analysis and remnant tumor tissue harvested revealed a high number of host cells (local tissue) along with trace xenograft-derived differentiated cells and growth-arrested progenitors that were too few for functional validation.

Further profiling RRM2-ANXA2-PTGIS over the deconstruction platform in residual tumors at specified intervals during the three-drug combination treatment provided insights of mode of action. Enhanced expression of all three

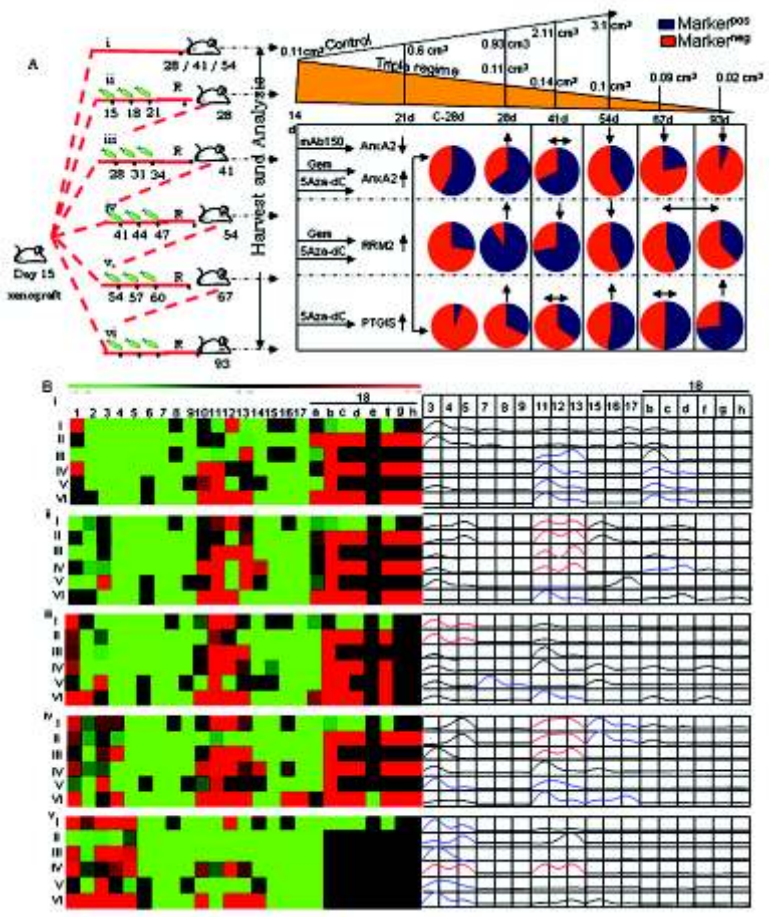

Fig. 2: 2A-left panel: 3-drug combinatorial drug regime design in **i.** Untreated, **ii.** 28 day, **iii.** 41 day, **iv.** 54 day, **v.** 67 day, **vi.** 93 day tumors (R–recovery); - right panel: Representative pie-charts indicating modulation of targets/biomarkers in residual tumors on Days 28, 41, 54, 67 and 93 within triple combinatorial treatment, upper panel represents increasing and decreasing tumor volumes (cm^3) of controls (only till 54 days after xenograft initiation) and residual tumors respectively; **B.** Left panels - Heat-map representation of fold-change in frequency of tumor fractions 1-18h based on fold-change with respect to controls in residual tumors where **i.** AnxA2^{neg}, **ii.** AnxA2^{pos}, **iii.** RRM2^{neg}, **iv.** RRM2^{pos}, **v.** PTGIS^{neg}, **vi.** PTGIS^{pos} following combinatorial drug treatment at - **i.** 28 days, **ii.** 41 days, **iii.** 54 days, **iv.** 67 days, **v.** 93 days, Right panel - Representative cell cycle profiles following combinatorial drug treatments of cycling fractions within tumors, where red blue and black line represent active cycling, cell cycle arrest and slow cycling tumor fractions respectively of tumors analyzed at the specified time points following combinatorial drug treatment. 1-host (red bar), Yellow bars indicate PKH^{neg} subsets (2-PKH^{neg}EuG0, 3-PKH^{neg}EuG1, 4-PKH^{neg}EuS, 5-PKH^{neg}EuG2M, 6-PKH^{neg}AneuG0, 7-PKH^{neg}AneuG1, 8-PKH^{neg}AneuS, 9-PKH^{neg}AneuG2M), Blue bars indicate PKH^{lo} subsets (10-PKH^{lo}EuG0, 11-PKH^{lo}EuG1, 12-PKH^{lo}EuS, 13-PKH^{lo}EuG2M, 14-PKH^{lo}AneuG0, 15-PKH^{lo}AneuG1, 16-PKH^{lo}AneuS,17-PKH^{lo}AneuG2M, 18- PKH^{hi} EuG0;18a-PKH^{hi}EuG0, 18b-PKH^{hi}EuG1, 18c-PKH^{hi}EuS, 18d-PKH^{hi}EuG2/M, 18e-PKH^{hi}AneuG0, 18f-PKH^{hi}AneuG1, 18g-PKH^{hi}AneuS, 18h-PKH^{hi}AneuG2/M.

markers was observed after one course of therapy (due to 5Aza-dC mediated promoter hypomethylation); continuing drug regimes increased PTGIS expression and gradually depleted ANXA2^{pos} and RRM2^{pos} cells (continual targeting by gemcitabine and mAb150; Fig.2B,left panel). Although marker^{pos} CSCs initially re-enter into the cell cycle, consequent growth arrest leads to their elimination by completion of treatment regime. Regenerative potential appeared to be associated with ANXA2^{pos} and RRM2^{pos} progenitors although some intermittently cycling marker^{neg} cells were also present. Significantly, treatment restricted emergence of regenerative aneuploid populations, yet did not drastically diminish differentiated fractions. On completion of treatment regime, residual tumor profile indicated abolishment of almost all regenerative fractions although trace, non-cycling RRM2^{pos} remained (Fig. 2B, right panel). In summary, the three-drug combination achieved highest efficacy through-

(i) inducing maximal expression of drug target genes,

(ii) inhibition of aneuploidy,

(iii) gradual decline and elimination of quiescent as well as cycling CSCs (PKH^{hi}) over the course of the treatment, and

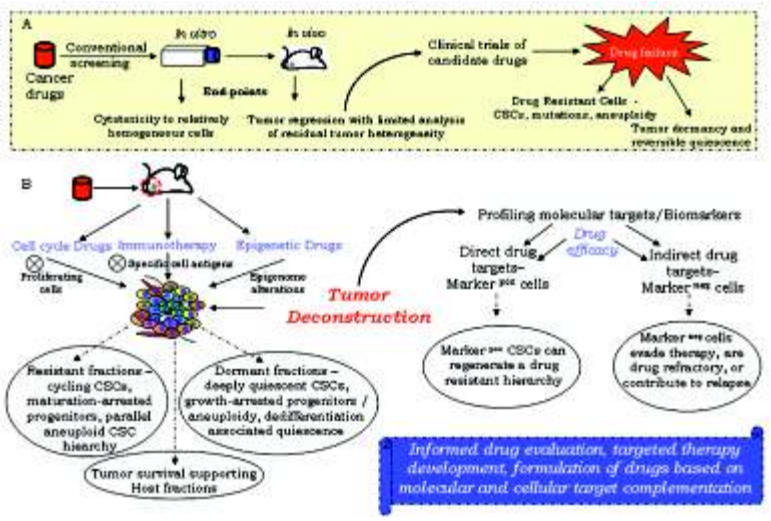

Fig.3. Overview of conventional vs. tumor deconstruction based drug screening and efficacy.

(iv) lack of development of drug resistance (most re-emerging populations on continued treatment appeared slow-cycling and/or growth arrested).

Functional analyses of residual populations have also been carried out in several instances and correlate well between predicted regenerative potential of each fraction. Conclusively, the deconstruction and derivation of the tumor cytotype lends a precision to the drug screening pipeline by overcoming the limitations of conventional approaches and may now be integrated as a convenient tool in drug discovery programs (Fig.3).

Future Research Plans

Elucidation of molecular and cellular cross-talks in the key processes of oxidative stress, metabolism and metastases during tumor progression on the background of tumor heterogeneity.

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Cancer, Chemotherapy, and Metabolic disorders

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Background

Obesity/overweight, resulting from excessive adiposity, is a serious public health problem worldwide with imminent clinical complications and economic burden. Epidemiological studies and meta analyses support a possible link between obesity and risk of breast, colon, pancreatic and cervical cancers as well as melanomas. Approximately 20% of all cancers are attributed to obesity and overweight causing late-stage disease, poor prognosis, cancer aggravation and impairment of chemotherapy by imposing chemoresistance. Adiposity deleteriously alters the production of proliferative, inflammatory, anti-inflammatory factors which influences the development and growth of cancer by releasing several factors/hormones collectively termed as adipokines. The precise mechanisms of tumor progression under obesity are still not clear, and studies deciphering precise effects of altered serum profile on cancer progression are scarce. In order to counteract the tumor promoting effect of obesity and adipokines, there is growing interest in exploring the possibility of whether weight loss therapies could reduce cancer-related deaths.

Adipose tissue plays an important role in tumorigenesis, invasion and metastasis. In obesity, secretory profile of adipokines from adipose tissue is altered leading to development of oxidative stress, pro-inflammatory and proliferative microenvironment. Adipokines exert their effects through receptors or membrane-associated molecules and activate various cellular signalling pathways. Cancer cells express receptors for most of the adipokines which activate multiple signalling pathways including PI3K/Akt, MAPK and JAK/STAT. Activated status of these pathways eventually supports cancer cell

growth and proliferation by modulating genes or proteins involved in tumor progression. Leptin and resistin are the major adipokines associated with obesity, and their role in growth and proliferation has been extensively explored in breast and prostate cancers. However, the involvement of these adipokines in melanoma is not well understood.

Melanoma is one of the most aggressive and obesity-promoted human malignancies. It is a fatal form of skin cancer which occurs in the proximity of subcutaneous adipose tissue. Being resistant to many anticancer drugs, it accounts for about 75% of skin cancer-related deaths worldwide. Therefore, consideration of life style factors or metabolic diseases becomes integral to the management of various aspects of tumorigenesis and tumor progression. In pharmacological front, orlistat, the FDA approved antiobesity drug, is a relatively tolerable and safe agent used to induce weight loss in obese individuals. It primarily acts by preventing absorption of dietary lipids through reversible inhibition of gastrointestinal lipases. At cellular level, orlistat has also been shown to irreversibly inhibit fatty acid synthase (FASN), a key enzyme in *de novo* synthesis of fatty acids. Orlistat, at higher dosage, has been reported to exhibit antitumor properties as cancer cells rely on availability of fatty acids and related molecules for their survival. However, the equivalent anticancer dose of orlistat in humans, due to its severe adverse side effects, could be clinically unfeasible.

Although a number of antiobesity drugs are available, diet-control interventions still remain to be the preferred line of therapy for effective management of obesity. Also, the role of dietary and nutritional factors towards cancer risk has been recently reported by many research groups. However, the comprehensive investigations on the impact of effective management of obesity on tumor progression are lacking. Therefore, we hypothesized that controlling obesity would be an appropriate approach in minimizing the risk of obesity-promoted cancer progression. In this study, we investigated the implications of therapeutic and dietary interventions for controlling obesity on the progression of melanoma. The underlying molecular events and role of specific adipokines were explored using appropriate *in vitro* and *in vivo* models. We demonstrate that controlling obesity is associated with normalization in levels of obesity-associated factors which parallels with reduction in melanoma progression and it may possibly be true for other cancer types too.

Aims and Objectives

- To study the impact of controlling obesity on melanoma progression and the molecular events involved.
- To evaluate the role of adipokines (leptin and resistin) on melanoma cell growth and proliferation.

Work Achieved

Controlling obesity using orlistat or dietary intervention hampers rapid progression of melanoma in HFD mice

To study whether controlling obesity has an impact on progression of melanoma, if any, we developed diet-induced obesity in C57BL/6J or NOD/SCID mice by feeding with high fat diet (HFD) for 6 months. In order to control obesity, we then started the treatment with orlistat (10 mg/kg, oral on every alternate day) and/or employing dietary intervention by shifting these mice to normal diet. Fifteen days later, we injected 2×10^5 B16F10 (murine origin) or 5×10^5 A375 (human origin) melanoma cells subcutaneously on the right flank of mice. Tumor progression and changes in body weight were followed up for 8 weeks with continuous treatment of orlistat and/or shifting to normal diet. At the end of the experiments, mice were sacrificed, and tumors and organs were preserved for further analysis. To check whether orlistat at antiobesity dose affects melanoma progression in non-obese phenotype, the similar experiments were also performed in normal diet (ND) fed C57BL/6J mice.

We observed that melanoma progression was significantly reduced in obese (HFD) mice treated with orlistat or in those shifted from high fat diet to normal diet (Figure 1Ab-c and 1Bb-c). The diminished tumor progression was associated with reduction in body weight/fat mass (Figure 1Aa and Ba), and normalization in obesity-associated parameters including serum lipids, insulin and adipokines. Interestingly, the normalization in obese parameters and reduction in tumor size was much more effective and pronounced when dietary intervention was combined with orlistat treatment (Figure 1Aa-c). However, we did not observe any significant changes in these parameters in non-obese (ND) mice treated with orlistat (Figure 1Ca-c).

Reduction in adipocyte size influences tumor cell proliferation and angiogenesis

As for cellular and molecular events those are associated with reduced tumor progression in HFD mice following orlistat treatment or diet shifting, we found

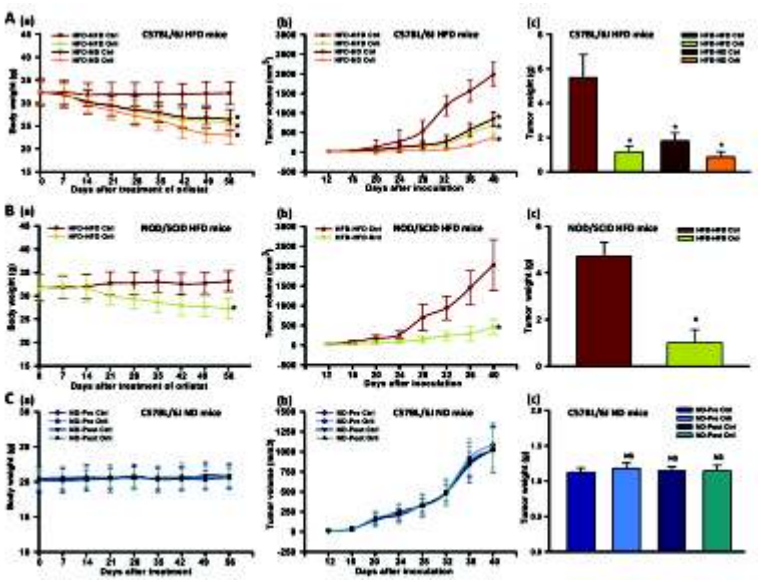

Fig. 1: Orlistat treatment and/or diet shifting reduces tumor progression and decreases tumor size in HFD but not in ND mice. Trends of changes in body weight (a), Tumor progression (b), and Tumor weight (c) in HFD C57BL/6J (A), HFD NOD/SCID and ND C57BL/6J mice.

increased necrotic areas and reduced cell density in tumors excised from mice administered with orlistat and/or shifted to normal diet as compared to their respective controls. Immunohistochemical analysis showed that the levels of CD31 (a marker for angiogenesis) were significantly reduced, which could lead to increase in necrosis in these tumors because of decreased angiogenesis.

At molecular level, drastic reduction in protein levels of FASN and Cav-1, which are elevated in melanoma under obesity, was detected in tumors from both HFD C57BL/6J and NOD/SCID experimental mice when compared to their respective controls (Figure 2A). These changes were associated with reduced levels of activated Akt. The level of apoptotic markers caspase-3 and caspase-7 were unchanged in all the tumors, while a significant decrease in the protein levels of proliferating cell nuclear antigen (PCNA) and cyclin D1 were detected (Figure 2A). However, the levels of these molecules remained unaltered in tumors of orlistat treated ND C57BL/6J mice (Figure 2A).

Role of adipocyte-secreted factors/adipokines in melanoma

Adipocyte-secreted factors favor growth, survival, proliferation and invasiveness of cancer cells. Leptin and resistin are major adipokines reported to promote proliferation of many cancer cells including breast and prostate cancers. However, the role of these adipokines in melanoma is not very clear. As we noted decreased serum levels of these adipokines in HFD mice treated with orlistat and/or shifted from high fat to normal diet, the involvement of these adipokines on melanoma growth was explored. A375 cells were treated with varying concentrations (0.1-100 ng/ml) of recombinant human leptin and

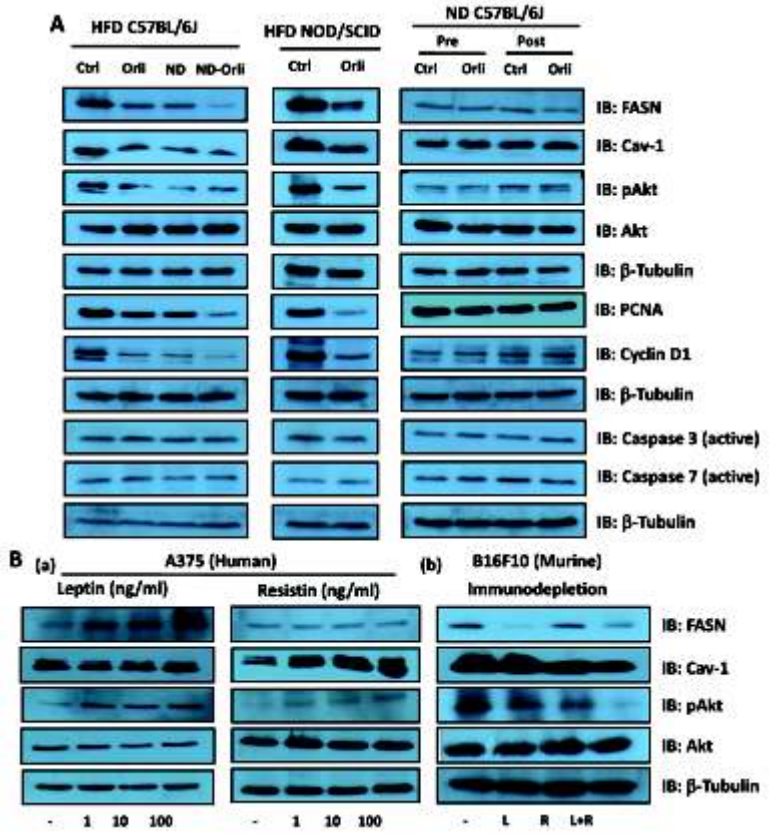

Fig. 2: Molecular events associated with melanoma progression in mice. (A) Levels of FASN, Cav-1, activated Akt, proliferative and apoptotic proteins in tumors of HFD C57BL/6J, HFD NOD/SCID, and ND C57BL/6J mice. (B) Role of leptin and resistin in modulation of FASN and Cav-1 proteins in melanoma cells.

resistin individually, and it was found that both these adipokines enhanced proliferation of cells. Akt pathway is reported to be hyper activated in melanoma tumors in obese mice. Interestingly, we found that leptin and resistin treatment not only caused activation of Akt but also enhanced the protein levels of FASN and Cav-1 respectively in A375 cells (Figure 2Ba). To confirm this finding in murine melanoma cells, we collected serum from HFD C57BL/6J mice and immunodepleted it of leptin and/or resistin. As expected, in B16F10 cells cultured in serum depleted of leptin and resistin, protein levels of FASN and Cav-1 were reduced respectively, with concomitant reduction in the levels of activated Akt. When both the adipokines were depleted simultaneously, protein levels of FASN as well as Cav-1 were reduced (Figure 2Bb). At transcription levels, however, no differences in both FASN and Cav-1 were detected which was also consistent with the unaltered protein levels and the nuclear localization of transcription factors which regulate FASN and Cav-1. The modulation of FASN and Cav-1 by these adipokines could be due to increased stability or through reduced degradation.

In summary, our study establishes a link between controlling obesity and melanoma progression through involvement of obesity-associated factors. The

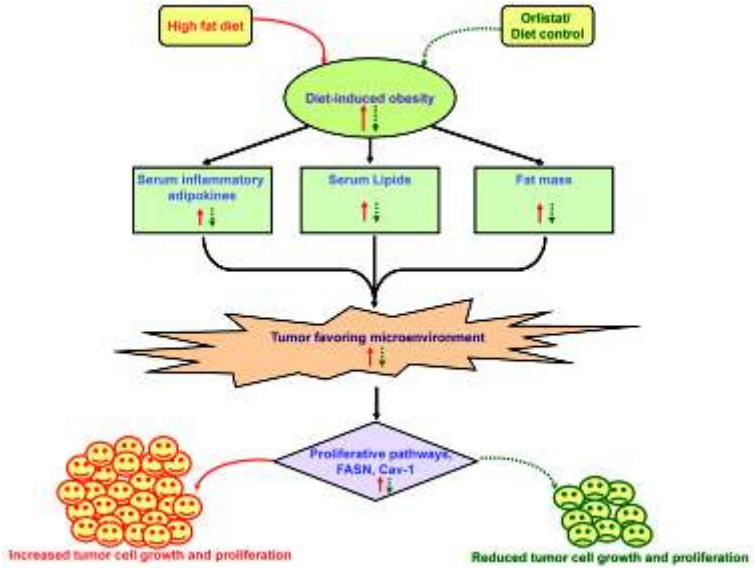

Fig. 3: Schematic representation of impact of targeting obesity on melanoma progression. Obesity-associated factors increase melanoma progression by inducing chronic low-grade inflammation and tumor-favoring microenvironment (solid arrow marks). On the other hand, controlling obesity causes reduction in fat mass, serum lipids and pro-inflammatory adipokines. This, in turn, reduces melanoma growth and progression through modulating molecules and pathways associated with increased tumor progression (dotted arrow marks).

study suggests that controlling obesity could be crucial to prevention of obesity-promoted cancer progression and also the outcome of cancer chemotherapy by virtue of normalizing serum levels of adipokines those affect tumor promoting molecules and signalling pathways (schematic overview shown in Figure 3). Therefore, strategic means of controlling obesity by reduced caloric diet or with antiobesity drugs treatment may render obesity-promoted tumor progression in check, improve chemotherapeutic response and prolong the survival of patients.

Future Research Plans

The World Health Organization (WHO) predicts that the obese and diabetic population will double from the year 2000 to 2030 and the epidemiological data clearly establish a link between metabolic disorders and cancer. Bulk of epidemiological studies available, support the interrelation-ship between the two, though only limited attempts have been made to explore in-detail mechanisms based functional correlation between these two diseases at cellular level or at molecular level. In this direction, by applying appropriate *in vitro* as well as in vivo models, the future objectives of our laboratory are:

To study the impact of obesity or associated factors on the outcome of cancer chemotherapy using appropriate in vivo and *in vitro* models, and elucidation of molecular signatures.

To study the hyperglycemia induced alterations at gene level as well as signalling cascade in solid tumors cells and role of glucose lowering drugs.

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An insight into the various functions performed by chromatin remodeling protein SMAR1

Background

The nuclear matrix provides structural framework to the nucleus tethering several proteins that are important for many processes like transcription, replication, splicing, DNA repair etc. The nuclear chromatin is organized in loops by the nuclear matrix thus modulating the chromatin architecture. The chromatin that remains bound to the nuclear matrix is known as the MAR (Matrix Attachment Region) sequences and proteins that help attachment of such MAR sequences to the nuclear matrix are called MARBPs (Matrix Attachment Region Binding Protein). SMAR1 is one such MARBP, which shares similarities with other MARBPs like Cux, SATB1, PARP, etc. SMAR1 houses a BEN domain which is crucial for DNA-protein and protein-protein interaction. SMAR1 acts as a docking site for various chromatin modifiers thus regulating the expression of certain genes like cyclin D1 (Ramapalli et.al., MCB, 2005) IKB (Singh et.al., 2009), Bax, PUMA (Sinha et.al., EMBO J, 2010). SMAR1 as a stress response protein was characterized as its levels were found to be elevated upon DNA damage (Singh et. al., 2007). Upon DNA damage, SMAR1 plays a dual role by interacting with Ku70 and repairing damaged DNA at one hand and on the other, prevents entry of Bax in mitochondria thus dictating cell survival upon DNA damage (Choudhary et. al., Cell Death and Disease, 2014) SMAR1 was first identified in mouse double positive thymocytes and SMAR1 transgenic mice show abnormal V(D)J recombination (Kaul-Ghanekar et. al., 2003). T cell polarization is controlled by SMAR1 as this protein allows the T cells to commit to Th2 lineage and suppresses the Th1 and Th17 lineage commitment. Absence of SMAR1 causes increased Th17 differentiation and thus

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inflammation (Chemmannur et. al., Mucosal Immunology, 2015), Fig. 1. FoxP3, a major factor in Treg cell differentiation is controlled by SMAR1 and this maintains the fine balance between Treg and Th17 phenotype. Our results suggest a protective role of a novel Scaffold/ Matrix Associated Region binding protein SMAR1 both in colitis and rheumatoid arthritis disease progression. Results show SMAR1 transgenic mice are resistant to DSS induced colitis as a function of repressed Th1 and Th17 response by SMAR1. (Mirlekar et. al., Mucosal Immunology, 2015).

Pre-mRNA splicing is a complex regulatory nexus modulated by various trans-factors and their post-translational modifications to create a dynamic transcriptome through alternative splicing. Signal-induced phosphorylation and dephosphorylation of *trans*-factors are known to regulate alternative splicing. However, the role of other post-translational modifications such as deacetylation/acetylation, methylation and ubiquitination that could modulate alternative splicing in either a signal-dependent or -independent manner remain enigmatic. We demonstrate that nuclear matrix-associated protein SMAR1 negatively regulates alternative splicing through HDAC6-mediated

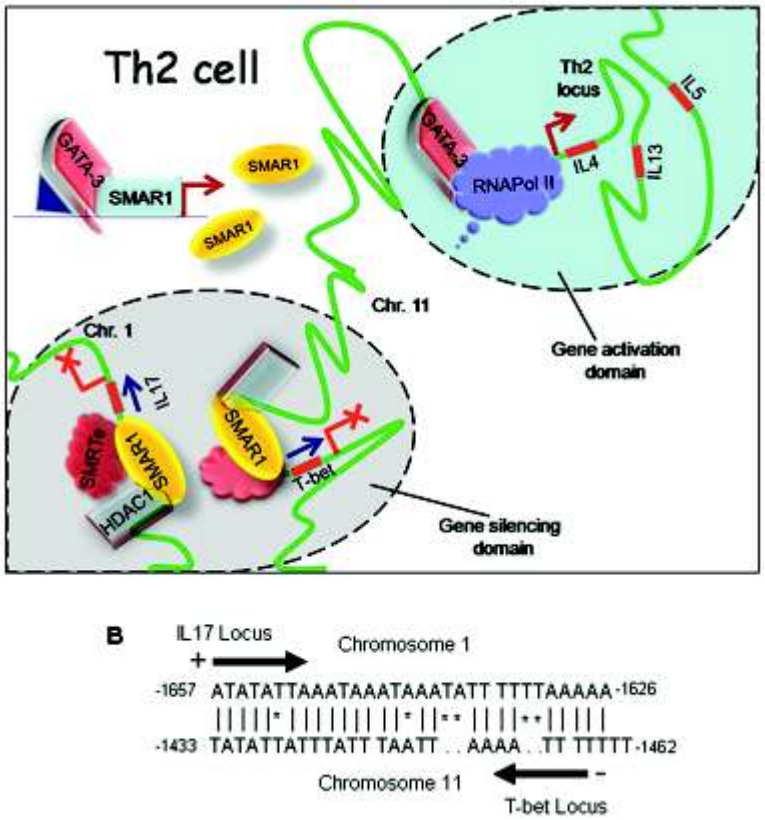
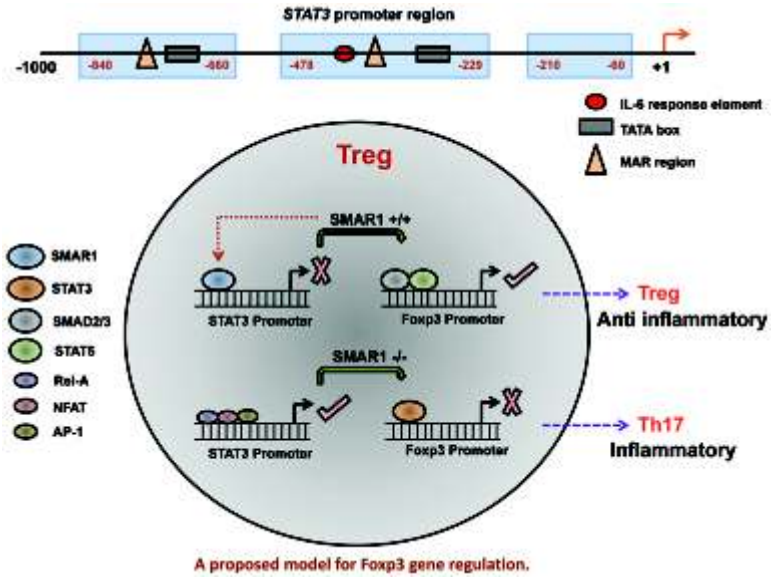


Fig. 1: Working model. In type 2 helper T (Th2) cells, GATA-3 induces SMAR1 expression. SMAR1 functions as a molecular switch in the maintenance of Th2 cells by inhibiting both Th1 and Th17 pathways by directly binding and suppressing the transcription of T-bet (T-box protein expressed in T cells) and IL-17 through specific matrix attachment region (MAR) sequences present on the promoters. Binding of SMAR1 recruits chromatin-modifying complexes that keep the gene silenced presumably by targeting these genes to specific silencing domains within the nucleus. This model also hypothesizes differential regulation of genes (T-bet and Th2 locus) present in the same chromosome (Chr). Chr. 11. In Th2 cells, T-bet is silenced and IL-4/IL-13/IL-5 genes are kept transcriptionally active possibly through spatial recruitment of genes to various subnuclear compartments. Thus, SMAR1 functions as a critical molecular switch in dictating the stability of Th2 cells by silencing Th1 and Th17 pathways.

Fig. 2: Schematic representation of in silico docking analysis for the binding of Sam68 (white) and HDAC6 (Orange) on SMAR1 (green) protein. Structure specific RNA is binding to the grove between SMAR1 and Sam68.



deacetylation of RNA-binding protein Sam68. SMAR1 is enriched in nuclear splicing speckles and associates with the snRNAs that are involved in splice site selection. ERK-MAPK pathway that regulates alternative splicing facilitates ERK-1/2 mediated phosphorylation of SMAR1 at threonines 345 and 360, localizes SMAR1 to the cytoplasm, abrogating its interaction with Sam68. Our studies showed that endogenously, SMAR1 through HDAC6 maintains Sam68 in a deacetylated state. However, knockdown or ERK-mediated phosphorylation of SMAR1 releases the inhibitory SMAR1-HDAC6-Sam68 complex, facilitating Sam68 acetylation and alternative splicing. Thus, our results reveal the complex molecular mechanism underlying SMAR1-mediated signal-dependent and -independent regulation of alternative splicing via Sam68 deacetylation (Nakka et al., PNAS, 2015), Fig. 2

Aims and Objectives

To study:

- The role of SMAR1 in global gene regulation through interaction with tumor suppressor p53.
- Metabolic regulation of epigenetic changes in tumor suppressor SMAR1 promoter.
- The role of SMAR1 mediated downstream genes in Wnt signaling pathway.
- Proteomic profiling of SMAR1 regulated genes and their implication in MHC processing.
- The maintenance of cellular homeostasis through transcriptional regulation of MAR binding protein. SMAR1 and its role in cellular homeostasis.

Work Achieved

Role of SMAR1 in global gene regulation through interaction with tumor suppressor p53

Down-regulation of SMAR1 in higher grades of cancer has its own implication which leads to increased expression of Cyclin D1 and Cytokeratin 8 leading to enhanced cell proliferation, survival and apoptosis, regulation of chemokine expression. Previous studies from our lab have shown that SMAR1 directly interacts with p53 and activates p53 through its serine-arginine rich motif and this leads to delayed tumor progression and cell cycle arrest. Since SMAR1 interacts with and modulates the function of p53, it acts as an important switch in regulating the transcription (Malonia et al, BBA Reviews in Cancer).

Along with many protein-coding gene targets, SMAR1 can potentially regulate the expression of certain microRNAs (miRNAs). miRNAs are 20-25 nucleotide long, non-coding RNAs which bind to the 3` UTR of mRNA bringing about post-transcriptional regulation. miRNAs have various implications in cancer and tumor progression, maintenance of stem cell pluripotency, and many more. Regulation of miRNAs by SMAR1 will thus elucidate a novel mechanism of gene regulation. Thus we speculated that SMAR1 could be a global gene regulator along with p53 and also regulate the miRNA cluster. With an aim to identify novel gene targets of SMAR1 in presence and absence of p53, a high throughput ChIP-sequencing approach was undertaken. The ChIP-seq analysis predicted a plethora of SMAR1 gene targets, to which SMAR1 can bind in the presence and absence of p53. A significant number of genes, however, favor the binding of SMAR1 irrespective of p53 status. Motif analysis of the SMAR1 target sequence reads identified a stretch of 20bp long T[CG] consensus as a putative SMAR1 binding motif. It was found that SMAR1 binds within the gene body with higher affinity than to any other part of the gene.

In addition to the gene targets, we wanted to elucidate the role of SMAR1 in the regulation of microRNAs, namely, miR-371-373 cluster. After confirmation of SMAR1 binding upstream of miR-371-373 by ChIP-PCR and luciferase assays, we went ahead to validate the binding by electrophoretic mobility shift assays. EMSA experiments confirmed and strengthened the binding of SMAR1 to the upstream sequence of miR-371 in a dose-dependent manner. As this microRNA cluster has been reported to have roles in tumorigenesis and metastasis, we investigated the role of SMAR1 in tumor formation via miR-371-373. *In vivo* data suggested that SMAR1 inhibited tumor formation and progression in Adeno-SMAR1 treated mice as compared to control mice. Thus,

these results conclude the role of SMAR1 in tumor repression by binding and negatively regulating the miR-371-373 cluster (Mathai et al. Mnuscript in preparation).

Metabolic regulation of epigenetic changes in tumor suppressor SMAR1

SMAR1 is a MAR binding protein which tethers chromatin to the nuclear matrix and modulates the architecture of chromatin by forming inactive loops. Perturbations of chromatin structure can cause inappropriate gene expression and genomic stability, resulting in cellular transformation and malignant outgrowth. Therefore, the proteins that control chromatin organization are the key players in cancer progression. Rapidly proliferating cells show significant increase in glycolysis known as the "Warburg effect". The role of SMAR1 as a stress response protein in repair of DNA damage is already reported from our lab. A rapidly proliferating cancer cell has to circumvent many stresses in order to survive and continue proliferation one of which is the metabolic stress. A cancer cell has high energy requirements because of their highly proliferative nature thus it was interesting to check the effect of glucose deprivation on levels of SMAR1.

Epigentic regulation of a gene is primarily obtained by methylation of the DNA stretch and also the methylation and acetylation of histones. Methylation of cytosine residue is mainly observed where a methyl group gets attached to cytosine. This is brought about by the DNMTs; mainly Dnmt1 which is a maintenance methyl transferase, Dnmt3a and Dnmt3b which are the de-novo DNMTs. Upon methylation several methyl binding proteins like MeCP2, Sin3a etc. localise and bind to the methylated cytosine moieties on the DNA. Methylation of the promoter region causes two major events; firstly it recruits HDACs which de-acetylates the histone. This causes the second change i.e, change in the chromatin conformation making the DNA inaccessible to the RNA polymerase machinery. In a cell, the only methyl donor that provides the –CH3 group to cytosine or to the histones is S-adenosyl methionine (SAM). SAM is generated primarily through the folate pathway in normal cells but in case of malignant cells, where glycolysis is increased multifold, SAM is also generated through the one-carbon metabolism pathway as an offshoot to the glycolysis pathway.

We showed that the levels of SMAR1 are altered by changing the glucose supply to the cell i.e, by changing the cellular metabolism which in turn causes the change in the methylation status of the *SMAR1* promoter. We found that

MeCP2 interacts with both HDAC1 and HDAC2 in untreated cells. We also observed that in untreated cells there is methylation of H3K9 and H3K27 and these methylation marks are lost upon glucose deprivation. Apart from these findings, we also observed an overall decrease in the levels of HDAC1 and HDAC2 upon glucose deprivation. We can speculate that since the overall levels of the HDACs go down and since there is a loss of methylation, together it causes the transcriptional activation of the tumor suppressor gene *SMAR1*. To validate this further we treated the cells with Trichostatin A, which is a general HDAC inhibitor and this treatment allowed transcription of SMAR1 gene (Das et al., unpublished data).

Role of SMAR1 in the Wnt Signaling pathway

In cancer cells there is aberrant activation of signaling pathways that help the cancer cells maintain their cancerous phenotype and their proliferation potential. One such signaling cascade is Wnt signaling. Few tumor suppressors that negatively regulate Wnt signaling have been reported so far in mammals. TCF4/LEF1 can act both as an activator or repressor depending on their binding to β-catenin. Studies have reported that when HDACs bind to LEF1 there is repression of the gene thus regulating the down-stream targets. SMAR1 has been reported as a tumor suppressor and was shown to recruit HDAC1/mSin3a to the promoter of Cyclin D1. This de-acetylation represses the expression of Cyclin D1 bringing about G1/S arrest. Our studies have revealed that SMAR1 is a negative regulator of β-catenin and may prevent the Wnt signaling activation.

We found that SMAR1 negatively regulate β-catenin and thus prevent activation of Wnt signaling. Our findings also show that a Wnt 3a activation result in the down-regulation of SMAR1 and over-expression of SMAR1 was found to revert the effect. CDC20 is reported to be stabilized upon Wnt 3a activation and is also responsible for SMAR1 degradation. Hence, prevention of SMAR1 degradation using small molecule compounds or peptides can serve as a potential therapeutics in cancer. Since SMAR1 suppresses β-catenin, we checked LEF1/TCF4 promoter binding of β-catenin. Over-expression of SMAR1 caused reduced activity of Super 8X Topflash luciferase promoter, whereas down-regulation caused enhanced activity. Thus, SMAR1 regulates β-catenin/Wnt signaling pathway by regulating β-catenin. We also found that SMAR1 occupies the β-catenin promoter thus bringing about regulation of β-catenin.

We are reporting for the first time that SMAR1, a tumor suppressor protein regulates β-catenin at the transcriptional level. Most of the reports have shown

to regulate β-catenin either by degradation or by preventing its binding to TCF4/LEF1. Inhibition of Wnt signaling pathway is an important event in controlling some of the cancers where Wnt/ β-catenin is very active (Taye et al., Unpublished data).

Proteomic profiling of SMAR1 regulated genes and their implication in tumorigenesis and antigen processing and presentation

SMAR1 triggers cell cycle arrest and apoptosis through transcriptional regulation of specific target genes. SMAR1-dependent regulation of the up-regulated protein calnexin was further studied. To delineate the mechanism of how SMAR1 regulates calnexin gene expression, a bioinformatics analysis of calnexin promoter was performed. Interestingly SMAR1 and GATA2 binding sites were observed proximal to each other in calnexin promoter. Chromatin immunoprecipitation confirms the binding of SMAR1 and GATA2 on calnexin promoter. SMAR1 forms triple complex with GATA2 and HDAC1. Recruitment of HDAC1 results in deacetylation of GATA2, under deacetylated condition GATA2 acts as repressor resulting in downregulation of calnexin gene. This study mechanistically highlights the co-ordinated regulation of calnexin gene by SMAR1 and GATA2. SMAR1 controls the expression of these proteins suggesting direct role of SMAR1 in ER homeostasis.

We also found SMAR1 as one of the ER responsive protein. Further we are checking the role of SMAR1 in MCF7 resistance against tunicamycin and antigen processing and presentation. Preliminary findings indicates mycobacterium antigen ESAT6 downregulates SMAR1 and at the mean time overexpression of Calnexin suggesting its role in antigen processing and presentation. Thus, this study reveals protein targets of SMAR1 and highlights the role of SMAR1 during various biological responses (Alam et al., Unpublished data).

Maintenance of cellular homeostasis through SMAR1 and its role in embryogenesis

All the activities of the cell including proliferation and multitude of genomic functions (replication, repair, transcription, splicing, etc.) are carefully regulated during development to maintain homeostasis. One such requirement is to maintain orderly arrangement of nuclear domains which is brought about by anchorage of specific sequences (MARs) to nuclear matrix. Essentially, SMAR1 is recognized as a mammalian protein but alignment studies using bioinformatics tools have shown the presence of a 66% identical sequence in zebrafish.

Comparison of SMAR1 amino acid sequence with those of other vertebrate species of interest using Clustal Omega multiple sequence alignment revealed a high conservation amongst vertebrates. ScanProsite predicted the region 223 to 319 as BEN domain. The BEN domain is predicted to function as an adaptor for the higher-order structuring of chromatin, and recruitment of chromatin modifying factors in transcriptional regulation. String v9.1 algorithm further predicted the functional partners of zebrafish SMAR1 which included Setmar, tp53, aspartate β-hydroxylase, etc.

Whole mount RNA in-situ hybridization revealed spatial distribution pattern of SMAR1 mRNA in the developing embryo. Probe showed localization around brain especially emarginating the brain ventricles and also around the skeletal muscle in the middle part of body, which is supposed to be highly hematopoietic region. This suggests that SMAR1 might be involved in some of the development pathway regulating the early development of zebrafish embryos.

To understand the role of SMAR1 in zebrafish development, a dose of 400-500 µM of SMAR1-MO was chosen to study the effect of SMAR1 knockdown in development. At this dose, we found embryonic or larvae malformations, developmental delay, smaller heads, pericardial edema and a linear heart tube phenotype in SMAR1-MO injected zebrafish. These results indicate that SMAR1 can influence the development of zebrafish, especially with regard to the heart. Taken together, these results indicate that MO mediated knockdown of SMAR1 caused abnormal heart tube development in zebrafish embryos (Patel et al., Unpublished data).

Future Research Plans

- Telomerase (hTERT) is activated in most of the cancer cells, rendering cancer cells immortal. Using colorectal cancer model we show an inverse correlation between SMAR1 and hTERT.
- To understand the function of SMAR1 in global regulation of alternative splicing through its interaction with hnRNPU.
- BEN domain has a novel DNA binding activity and is found in a conserved family of transcriptional repressors one of them being SMAR1. We are looking for BEN-domain interacting partners to gain further insights into the function of this domain.

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Structural and Functional Studies on components of the Nuclear Pore Complex

Background

The nuclear pore complexes (NPCs) embedded in nuclear membrane bilayer solely mediate transport of all kind of macromolecules between nucleus and cytoplasm, and regulate nearly most cellular processes such as gene expression, mitosis, cell differentiation etc. Additionally, alternations in NPC and its associated proteins have been linked to several human diseases, such as cancer, genetic disorders and viral diseases. The architecture of the NPC is evolutionarily conserved from yeast to human and is a highly modular structure. Each NPC is comprised of ~30 different proteins called nucleoporins (Nups) that are arranged in multiple copies to yield a size of 65 MDa (yeast) or 125 MDa (vertebrate). In order to understand the molecular mechanisms of NPC assembly formed by these ~30 nups and its versatile functions, the high-resolution structures are highly desired but complexity and the size of the NPCs pose tremendous challenges. A rational strategy therefore would be to disintegrate the components of NPC based on their structural and functional specificity and employ integrative approaches to learn about the roles of Nups in NPC assembly and cellular physiology.

Our laboratory routinely utilizes various structural biology tools such as X-ray crystallography, spectroscopic methods etc and we work in collaboration with cell biologists to understand the versatile functions of NPCs, such as how Nups participate in nucleocytoplasmic transport, gene regulation and cell differentiation

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Aims and Objectives

- Reconstitution of minimally interacting regions of Nup93 subcomplex to understand their roles in assembly of the NPC.
- X-ray crystallographic studies on reconstituted minimal complexes of Nups.
- Analysis of the Nups in regulating transport activity and various cellular functions

Work Achieved

The lab research focus is on one of the main sub-complex of the NPC, Nup93 sub-complex. It is comprised of mainly five Nups, Nup93, Nup205, Nup188, Nup155 and Nup35. Among them Nup93 is key to anchor central channel (Nup62•Nup54•Nup58 complex). Our lab is using two parallel approaches to understand the role of these Nups in NPC assembly and their subcomplexes:

1) Characterization of native sub-complexes of the human NPC, and structural analysis by cryo-EM methods.
2) Reconstitution of nucleoporin complexes and their structure determination by x-ray crystallography.

Characterization of native sub-complexes of the human NPC, and structural analysis by cryo-EM methods

Using this approach we are aiming to analyze the larger complexes of the human NPC such as Nup62 subcomplex (Nup62, Nup54 and Nup58) and

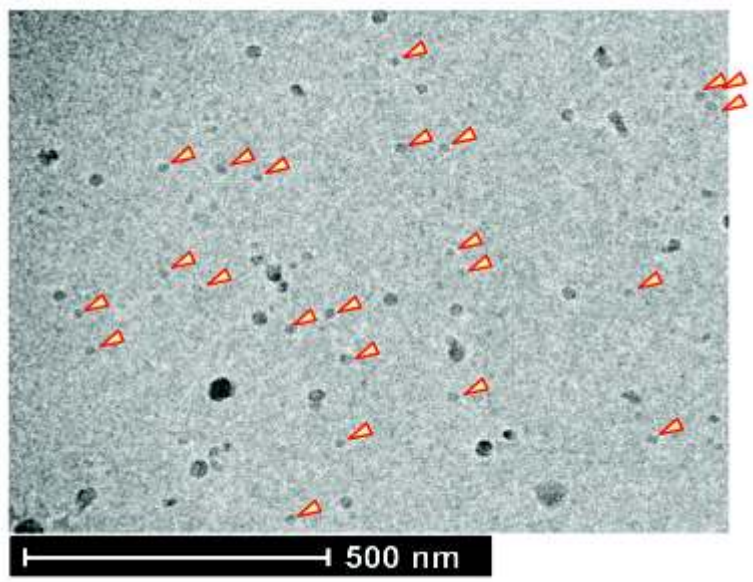

Fig. 1: Preliminary Cryo-electron microscopy (Cryo-EM) analysis of partially purified Nup62 subcomplex. Images were taken using the Tecni-G2 (FEI) TEM in vitrified ice conditions at IGIB New Delhi. Few selected Nup62 complexes are marked with the arrows.

Nup93 subcomplex (Nup93, Nup205, Nup188, Nup35, Nup155). So far we are able to isolate native Nup62 complex. Briefly, His6 tagged Nup62 encoding gene was integrated into the transcriptionally active region of the genome of the HEK293 FlpTrex (invitrogen) cells and its expression was induced by tetracycline. Large cultures of these cells were used to isolate Nup62•Nup54•Nup58 complex using affinity chromatography. A preliminary cryo-EM analysis revealed the homogenous spherical particle shape of the complex (Figure 1). Currently we are aiming to collect larger cryo-EM dataset for Nup62•Nup54•Nup58 complex for single particle reconstruction analysis.

Reconstitution of nucleoporin complexes and their structure determination by x-ray crystallography

We are currently reconstituting Nup93•Nup205 and Nup93•Nup62•Nup54•Nup58 complexes for structural studies. We have employed co-immunoprecipitation and yeast two hybrid approaches to identify minimal interacting domains of interacting Nups. This approach is very challenging but has potential to reveal the details of protein-protein interactions, which can enable us to dissect the roles of various Nups in NPC assembly and its diverse functions.

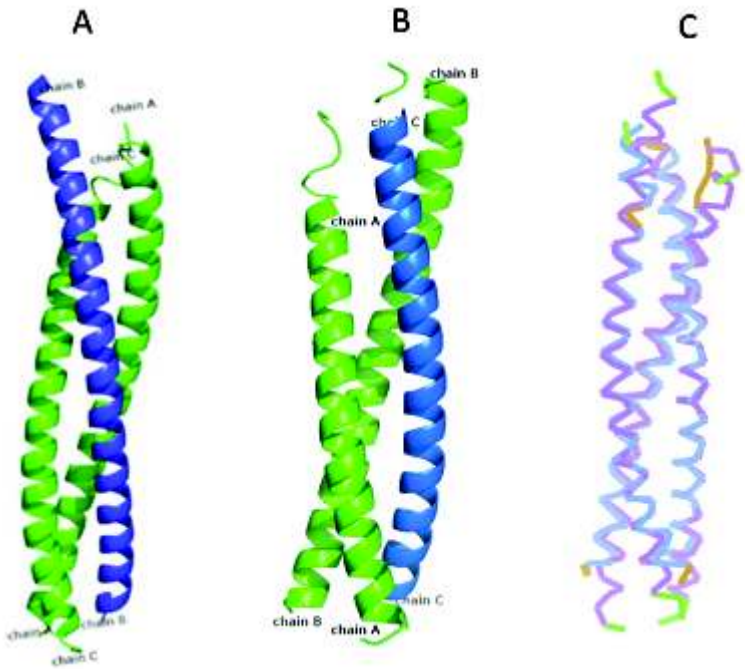

Fig. 2: Crystal structure of: **A.** Nup62(362-425)•Nup54(346-407) hetero-trimer; **B.** Nup62(362-425) homotrimer and **C.** Superimposition of heterotrimer and homotrimer structure with rmsd of 0.9 Å.

One of the channel Nups, Nup62 can interact with either Nup54 or Nup88. We have crystallized Nup62 (362-425) region and x-ray diffraction data is collected at ELETTRA synchrotron, Italy. Its 3D structure is determined by molecular replacement and revealed homotrimeric structure form, similar to Nup62•Nup54 heterotrimeric structure (Figure 2). The comparative study of Nup62(362-425) homo-trimeric structure to its hetero-oligomeric assemblies revealed that inspite of less than 5% identity between Nup62 and Nup54, Nup62(362-425) homotrimer acquired similar parallel helix packing as in Nup62•Nup54(346-407) complex. Also the Nup62 homotrimer structure analysis showed the lower stability of Nup62 homotrimer than Nup62(362-425)•Nup54(346-407) heterotrimer. It was earlier shown that Nup62(327-525) alpha helical region could interact with Exo70, a component of exocyst complex. Our studies have shown that Nup62(362-425) region interacts with Exo70(1-100) region. This region of Exo70 is primarily alpha helical with coiled-coil packing. Althogether our data indicate that Nup62(362-425) region can self oligomerize or interact with Nup54 and Nup88 within NPC and Exo70 outside the NPC thus playing an important role in both nucleocytoplasmic transport ans well as exocytosis. We aim to further characterize the significance of these interactions.

Future Research Plans

- X-ray diffraction structure determination of vertebrate Nup93 and Nup205 of the nuclear pore complex.
- Native isolation of the Nup93•Nup205 and Nup62 subcomplex from stably expressed HEK cell lines followed by low resolution and high resolution cryo-electron microscopy studies.

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Role of Nup358 in miRNA pathway

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Background

Regulation of gene expression at the translational level is recently shown to be involved in diverse cellular processes, and has emerged as an area of intense investigation. Small non-coding RNAs, particularly microRNAs (miRNAs), appear to contribute to this layer of regulation significantly. miRNAs are of ~22 nucleotides length, which suppress translation of mRNAs that possess partial or complete sequence complementarity, mostly at the 3'-untranslated region (UTR). Prediction based on sequence analysis has indicated that miRNAs could target over 50% of human protein-coding genes. miRNA genes are generally transcribed by RNA polymerase II to produce primary-miRNAs (pri-miRNAs), which are recognized and processed into preliminary miRNAs (pre-miRNAs) in the nucleus by the microprocessor complex containing Drosha and DGCR8. The pre-miRNA, in complex with Exportin-5 and RanGTP, is exported through nuclear pore complex (NPC) into the cytoplasm, where it is processed by Dicer into mature double stranded miRNA. One of the strands is stably associated with Argonaute (Ago) proteins, to generate a functional miRNA-induced silencing complex (miRISC). Humans have four Ago isoforms, namely, Ago1-Ago4. A glycine-tryptophan (GW) rich protein, GW182 (also called TNRC6), interacts directly with Ago proteins and is essential for the miRISC-mediated translational repression and/or degradation of target mRNAs through recruitment of deadenylation and decapping complexes, which is believed to occur in the cytoplasmic foci termed 'processing bodies' or 'P bodies'.

Although the subcellular location where the loading of miRNAs to Ago proteins (miRISC formation) and association of miRISC with the target mRNAs occur is

not well understood, recent studies have indicated a role for endoplasmic reticulum (ER) in these processes. It was shown that Arabidopsis Ago1 associates peripherally with ER, and miRISC could inhibit the translation of target mRNAs on the ER. Another study indicated that rough ER could be the site for miRNA and siRNA loading to Ago proteins and translational regulation of target mRNAs. A central question that is yet unresolved is how miRISC identifies the target mRNAs in vivo. Although a sorting mechanism could be envisaged that couples the RNAs exported from the nucleus with the miRISC, possibly at the ER, there is no available evidence for the existence of such machinery.

The nuclear envelope (NE) is made up of a double layered membrane, which is interspersed with NPCs that act as molecular gates for the transport of macromolecules between the nucleus and the cytoplasm. The protein components of NPCs are generally termed as nucleoporins (nups), and each mammalian NPC contains around 30 different nucleoporins in multiple copies. The spatial distribution of individual nucleoporins within the NPC structure could vary. For example, some nucleoporins are located at the nuclear basket (e.g. Nup153, Tpr, Nup50), some are localized to the central core region (e.g. POM121, Nup103, Nup133) and some are present on the cytoplasmic side (e.g. Nup358, Nup214, Nup88). Although the nucleoporins are fundamentally expected to be involved in the regulation of nucleo-cytoplasmic transport, several of them are shown to have multiple other functions.

Apart from the localization to NPCs on the NE, some nucleoporins also accumulate in the cytoplasm as part of annulate lamellae (AL), which are stacked ER membrane containing pore-like structures. These AL pore complexes show gross structural similarities to that of NPCs at electron microscopy level and share many nucleoporins. Although AL structures have been extensively analyzed in male and female gametes, other proliferating non-germ cells also possess varying quantities of AL. However, the functional role for these structures in any cellular processes, if any, is unclear.

Nup358 is a nucleoporin that localizes to the cytoplasmic side of the NPC and has been implicated in several functions. Depletion of Nup358 does not appear to grossly affect transport of macromolecules across the NE, although many studies suggest requirement of this nucleoporin for specific receptor- and cargo-dependent transport.

Based on the preliminary results (see below), we hypothesize that Nup358, as a component of cytoplasmic AL structures, would be involved in coupling miRISC to target mRNAs. Also, AL could act as cytoplasmic platforms for sorting and regulating the fate of exported mRNAs.

Aims and Objectives

- Understand the functional relevance of AL associated Nup358.
- Role of Nup358 in miRNA pathway
- Understand the molecular mechanism by which Nup358 functions in miRNA pathway

Work Achieved

Nup358 positive AL associate with stress granules (SGs) and P bodies

Staining for endogenous Nup358 using a specific antibody in HeLa cells showed distinct cytoplasmic accumulation, in addition to the NPC localization. Consistent with the previous reports, Nup358 along with a set of other nucleoporin localizes to AL. The origin of AL has been unclear and to monitor this, we expressed GFP-Nup358 and analyzed the dynamics of Nup358 labeled AL using live-cell imaging. We observed that AL were highly dynamic and were often undergoing homotypic fusion with neighboring AL structures. Interestingly, we observed that some AL structures were budding off from the NE and fusing with pre-existing cytoplasmic AL. These results suggest that

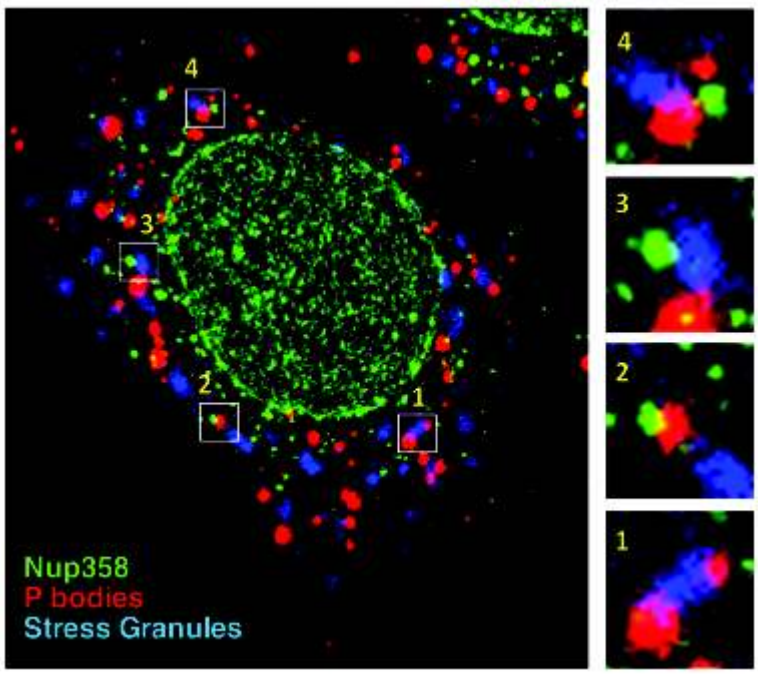

Fig. 1: Nup358 positive AL structures are juxtaposed to SGs and P bodies. HeLa cells were subjected to sodium arsenite treatment, fixed and stained for endogenous proteins. Nup358 was stained in green, Dcp1a (P body marker) in red and eIF3η (SG marker) in blue.

cytoplasmic AL could originate from NE and also that they are extensively dynamic entities.

Towards identifying a possible role for cytoplasmic AL structures in general, and Nup358 in particular, we investigated the distribution of AL in relation to other cytoplasmic structures. Interestingly, we found that two cytoplasmic messenger ribonucleoprotein (mRNP) granules, namely stress granules (SGs) and P bodies, were often associated with or present juxtaposed to AL (Figure 1). As SGs are induced upon mild stress, we subjected HeLa cells to oxidative stress through sodium arsenite treatment, and immunostained for endogenous Nup358, eIF3η (SG marker) and Dcp1a (P body marker). As reported earlier, P bodies and SGs were found often juxtaposed to each other in the cytoplasm. Interestingly, we observed that many individual Nup358 positive AL structures were present beside SG or P bodies, and in some cases, all three structures appeared to physically associate with each other (Figure 1). The physical association was much more striking when GFP-Nup358 was exogenously expressed with RFP-Dcp1a (P body marker) or RFP-G3BP1 (SG marker). Together, these studies show a physical and dynamic interaction between AL, SGs and P bodies in the cytoplasm.

Depletion of Nup358 disrupts P body formation

The physical association between Nup358 positive AL and SGs or P bodies prompted us to investigate if Nup358 is functionally connected with these mRNP granules. Initially, we wished to test if Nup358 depletion caused any effect on the assembly of these structures. HeLa cells were treated with control or Nup358 specific siRNA and were subjected to sodium arsenite treatment to induce SGs. Nup358 depletion did not have any gross effect on SG assembly (assessed by SG specific marker, eIF3η) when compared to control siRNA treated cells. Neither did depletion of Nup214, another nucleoporin present on the cytoplasmic face of NPC, show any effect on SG assembly. Interestingly, treatment of HeLa cells with Nup358 siRNA, but not control or Nup214 specific siRNA, led to dramatic impairment of P body assembly as assessed by Dcp1a staining. We also confirmed the disruption of P bodies upon Nup358 depletion using another P body marker, Xrn1. These results suggest a specific requirement for Nup358 in aspects of P body formation and function.

Nup358 is essential for miRNA-mediated translation suppression

Previous studies have shown that mRNAs suppressed by miRISC localize to P bodies, and disturbances in miRNA pathway could lead to disruption of

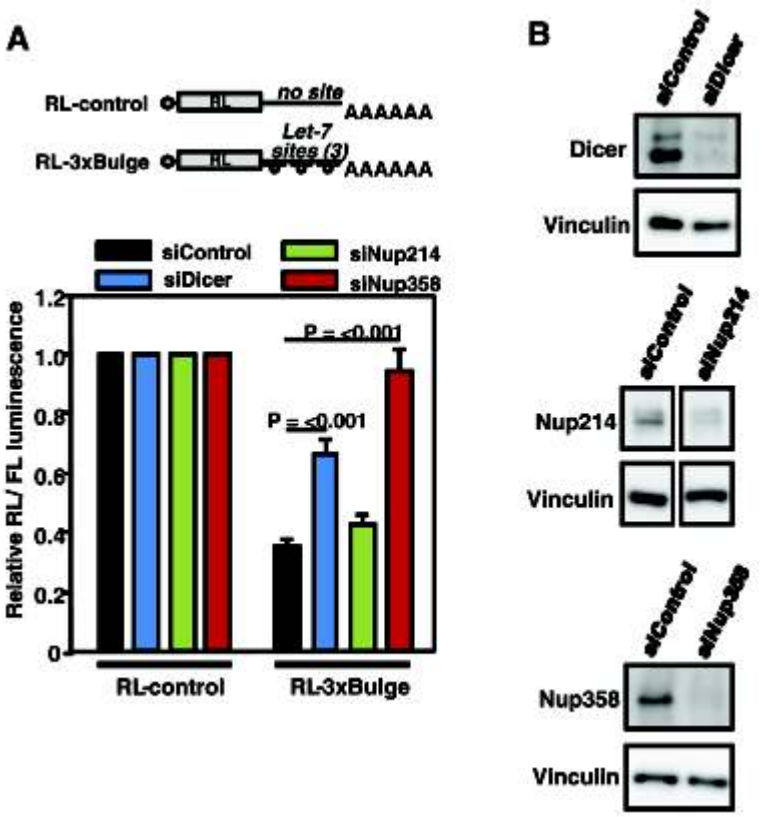

Fig. 2: Nup358 is required for miRNA function. (A) HeLa cells were initially transfected with indicated siRNAs, followed by RL-control (no let-7a binding site in the 3'UTR) or RL-3xBulge (3 imperfect let-7a binding sites in the 3'-UTR) reporter constructs along with firefly luciferase (FL) as internal control. The data was derived from three independent experiments, and the ratio of RL/FL luminescence was plotted, with error bars indicating mean ± SD. (B) Cells were treated with siRNAs for Dicer, Nup214 or Nup358 and the lysates were subjected to western blot analysis for assessing the extent of protein depletion using specific antibodies, as indicated. Vinculin was used as loading control.

microscopically visible distinct P body structures. We sought to find out if Nup358 depletion affected the miRNA pathway. As HeLa cells have higher levels of endogenous let-7 miRNA, a renilla luciferase (RL) reporter construct that expressed mRNAs containing three imperfect let-7a binding sites in the 3'-UTR (RL-3xBulge) was used to monitor let-7a mediated translation suppression in HeLa cells. Compared to control renilla luciferase (RL-control) mRNAs that did not have any let-7a binding site (RL-control), RL-3xBulge generally showed ~60% suppression mediated by endogenous let-7a (Figure 2). We measured the RL activity in cells depleted for Dicer, Nup214 or Nup358 and found that similar to Dicer knockdown, Nup358 depletion caused significant relieval of miRNA-mediated suppression. Cells with Nup214 knockdown, however, showed no discernible change in the reporter activity as compared to that in the control siRNA treated cells (Figure 2). These results demonstrate that Nup358 is specifically required for miRNA function. Our results also suggested that Nup358 depletion does not cause a gross effect on miRNA and mRNA export (Figure 3).

Thus, our results indicate that Nup358 positive AL associates with miRNA machinery and its depletion specifically affected P body assembly.

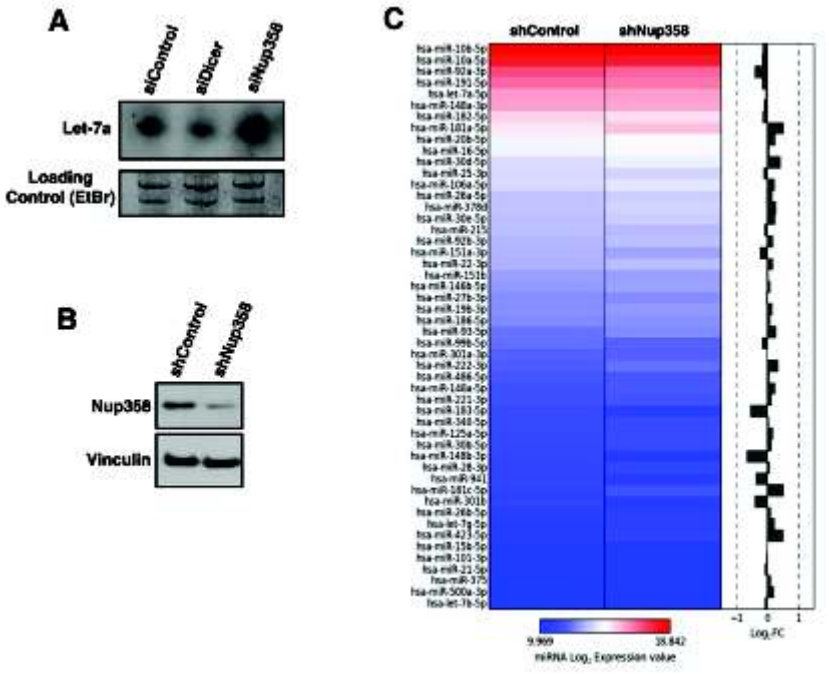

Fig. 3: Depletion of Nup358 does not affect mature miRNA levels. (A) HeLa cells were transfected with control, Dicer or Nup358 specific siRNAs, as indicated. Total RNA was isolated and analysed by northern blotting for let-7a using radio-labeled probe. Ethidium bromide (EtBr) stained gel indicates equal loading of RNA samples. (B) HEK293T cells were transfected with shRNA control (shControl) or shRNA against Nup358 (shNup358) and the cell lysates were analyzed for the levels of Nup358 by western blotting. Vinculin was used as loading control. (C) Heatmap showing relative levels of 50 most abundant miRNAs in HEK293T cells after transfection with control shRNA (shControl) or shRNA against Nup358 (shNup358).

Concomitantly, miRNA mediated suppression was impaired in Nup358 depleted cells indicating that this nucleoporin plays a specific role in miRNA pathway. Further studies will be performed in this project to unravel the molecular mechanism by which Nup358 functions in this regulation. As the Nup358 positive AL structures could originate from NE and were physically interacting with P bodies in the cytoplasm, we hypothesize that the AL could be cytoplasmic platforms for exported mRNAs to be coupled to miRNA machinery.

Future Research Plans

- Understand the molecular details of how Nup358 functions in miRNA pathway.
- Characterize the role Nup358 and / or other components of AL in the regulation of SG function.

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Identification of aging-induced epigenetic changes causing hematopoietic stem cell dysfunction: Rescue using *in vitro* niche (IVN) technology

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Background

Hematopoietic Stem Cells and Niche

Hematopoietic system consists of many cell types with specialized functions viz. red blood cells (erythrocytes), platelets (derived from megakaryocytes), granulocytes (neutrophils, basophils and eosinophils) and macrophages - (collectively referred to as myeloid cells) and B- & T- lymphocytes, NK cells etc. Most of these blood cells are short-lived and are continuously replenished. The continued production of these cells critically depends on the presence of Hematopoietic Stem Cells (HSCs), which are the ultimate, and the only, source for the production of all these cells.

Although the HSCs possess cell-intrinsic properties responsible for these functions, the regulation of hematopoiesis also depends on the external signals provided by the complex microenvironment. Schöfield coined a term 'Niche' for this microenvironment, in the early seventies. The multi-cellular complex composition of the marrow microenvironment makes defining of the HSC-niche in molecular terms a difficult endeavor. In the marrow, the bone marrow stromal cells derived from mesenchymal stem cells (MSCs) are believed to provide the basis for the physical structures of the niche. As such, bone marrow stromal cells are thought to regulate self-renewal, proliferation and differentiation of the HSCs through production of cytokines and intracellular signals that are initiated by cell-cell contact, cell-ECM contact or via soluble mediators. As niche is made up of different types of cells working simultaneously producing different regulatory and signaling molecules, niche cannot have static properties; it has to constantly undergo changes in response to micro-environmental signals which consequently reflects in HSC compartmental changes.

Today, Niche-biology is advancing at a rapid pace! Recent studies have shown that in addition to osteoblast and endothelial cells, sympathetic nerve fibers, perivascular MSCs and $CD169^{+}$ macrophages also participate in the stem cell regulation. Marrow adipocytes have been shown to be the negative regulators of hematopoietic microenvironment. These reports have led to the formulation of a provisional conclusion that the niche acts as a complex cellular unit, rather than as a single cellular entity, to regulate stem cell functions. But the molecular mechanisms involved in bringing about the cellular cross talk within this unit to achieve a well-orchestrated stem cell regulation remain largely unknown.

The knowledge about the niche-function is being generated not only to gain insight in the stem cell regulation in the body, but also to facilitate the studies on the pathological HSC niches allowing development of novel therapeutic strategies for targeting the niche. Development of such strategies will be further accelerated if appropriate bio-mimetic platforms are created. Although these platforms are developed to replicate the physiological context, it may be advantageous to generate functional artificial systems like in vitro niches (IVNs) mimicking the in vivo HSC-niche that are capable of modulating specific context-dependent properties of the stem cells to promote tissue regeneration. Some of the most exciting breakthroughs in regenerative medicine have been achieved by integrating stem cell science with material science. A new focus is now needed to use such bioengineered tools to gain insights into the niche-mediated regulation of stem cells, disease processes and development of new modalities for regenerative medicine.

Stem Cell Aging: The hematopoietic system experiences several age-associated alterations with profound consequences for health, including reduced immune competence, anemia, and an increased propensity for hematopoietic cancers. The increased frequency of cycling HSCs in the older marrows may be partly responsible for acquisition of mutations and subsequent development of malignancy. In addition, graft age is by far the most significant negative parameter in bone marrow transplantation, which represents perhaps the best example of how age can compromise and influence therapeutic approaches based on regenerative medicine. A recently identified hallmark of HSC aging is accumulation and striking dominance within the candidate HSC pool of a defined myeloid-biased subset of HSCs. Although cell-autonomous changes clearly underlie altered stem cell behavior during aging, recent studies have demonstrated that a decline in niche function, including a decreased production of local self-renewal factors, also contributes to reduced tissue homeostasis and repair in a number of systems. Restoration of the niche function can counter the aging-related changes in stem cell behavior, indicating that the strategies to rejuvenate or expand the niche may enhance

the efficacy of the stem cell-based therapies in regenerative medicine. Since direct in vivo modulation of niche or HSCs is not feasible, one needs to examine whether in vitro treatments of the "old HSCs" with "young niches" can restore, at least partly, the functional and epigenetic changes that have occurred during aging. Such approach is eminently dependent on developing an *in vitro* system that mimics the HSC-niche - an aspect where we have already gained a technical advantage.

Epigenetic regulation of aging stem cells: The function of adult stem cells, including the HSCs, declines with age contributing to the physiological decline in the tissue homeostasis. Old stem cells can be rejuvenated by environmental stimuli raising a possibility that at least some of the changes are regulated by reversible mechanisms. The age-related changes could be at the level of the genome, the epi-genome and the proteome. Epigenetic regulators provide versatile checkpoints to mediate reversible changes in the gene expression. Epigenetics is the dynamic regulation of gene expression by sequence-independent mechanisms, including the changes in DNA methylation and histone modifications. It is proposed that control of chromatin state is a pivotal means by which stem cells integrate environmental stimuli to trigger appropriate cell fates. Changes in chromatin are thought to be reversible and are thus ideally suited to be the molecular effectors of the stem cell rejuvenation. Along with the epigenetic regulatory genes, specific genes whose expression levels are governed by factors associated with aging can be used to generate gene expression profiles for young and old HSCs so that a quick molecular assessment of rejuvenation of old HSCs could be made after their culture in modified niches. The change in gene expression taken together with the results of functional assays would help in determining whether the reversal at gene level is translated at functional levels. Such correlative studies are lacking in the literature and we propose to fill up this lacuna. If such reversal of age-related changes can be accomplished *in vitro*, it may be a helpful approach for regenerative medicine.

Aims and Objectives

- To determine whether it is possible to rejuvenate "old" HSCs by exposure to "young" IVNs (In vitro niches) and to elucidate the mechanisms involved.
- To identify the signaling pathways operative in young vs. old IVNs and to determine whether it is possible to rejuvenate "old" HSC by exposure to specific signaling IVNs.
- To determine whether it is possible to improve the impaired functionality of old HSCs by their *in vivo* or *in vitro* treatments with specific pharmacological modulators

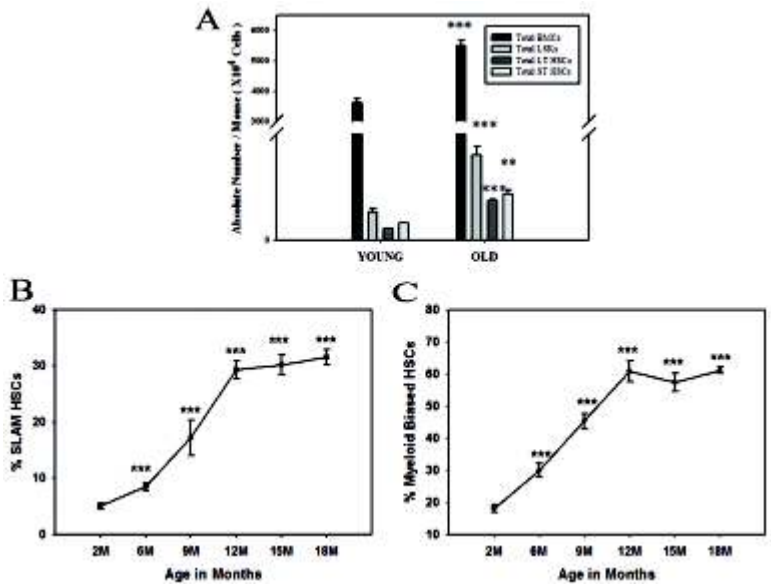

Fig. 1: Old mice harbor higher number of myeloid-biased HSCs in their marrow. A. Flow cytometric analyses of marrow cells from young (6-8 weeks) and old (12 months) mice reveal that old mice harbor significantly more number of LSK ($lin^-Sca\text{-}1^+c\text{-}Kit^+$)-, LT-($LSKCD34^-$) and ST- ($LSKCD34^+$) HSCs in their marrow. ** P <0.01, *** p<0.001. Data are plotted as mean ± SEM. N=6. **B.** Age-dependent increase in SLAM HSCs ($Lin^-Sca\text{-}1^+c\text{-}Kit^+CD150^+CD48^-$). **C.** Age-dependent increase in myeloid-biased SLAM HSCs ($Lin^-Sca\text{-}1^+c\text{-}Kit^+CD150^+CD48^-CD41^+$). N=3 at each time point. Data are plotted as mean ± SEM. *** p <0.001.

Work Achieved

Old mice harbor higher number of HSCs in their marrow and exibit myeloid bias

In these experiments we analyzed the HSC numbers and their lineage commitment in the marrow of young (6-8 weeks) and old (12 months) mice using multi-color flow cytometry. The bone marrow mononuclear cells (MNCs) were isolated and immuno-stained with various antibodies, after depletion of red blood cells. The cells were acquired on FACS Canto II and the data were analyzed using BD DIVA software. We found that the old mice harbored significantly more number of LSK (Lin-Sca-1+c-Kit^+) HSCs in their marrow. The numbers of LT-HSCs (long-term-engrafting HSCs, LSK CD34-) and ST-HSCs (short-term-engrafting HSCs, LSK $CD34^+$) were also significantly high in the marrow of old mice (figure 1A).

Numbers of SLAM HSCs and myeloid-biased HSCs increase with age

Aging is known to increase myeloid bias of the HSCs; however, the precise onset of this bias is not known. In this set of experiments, we determined the frequency of HSCs and myeloid-biased HSCs in the marrow of mice as a function of age. Bone marrow cells from mice of 2 to 18 months of age were analyzed for frequency of SLAM HSCs ($Lin\text{-}Sca\text{-}1^+c\text{-}Kit^+CD150^+CD48^-$) and myeloid-biased SLAM HSCs ($Lin\text{-}Sca\text{-}1^+c\text{-}Kit^+CD150^+CD48^-CD41^+$) in them. As seen in the figure 1B and 1C, the frequency of SLAM HSCs and myeloid-biased SLAM HSCs increased as a function of age. These data suggest that if any interventional strategy to delay the aging process is to be applied then it should be initiated at a very early stage.

Old HSCs exhibit defective engraftment

Here we examined whether the higher number of HSCs present in the marrow of old mice is reflected in the transplantation assays. We intravenously infused young (6-8 weeks) or old (12 months) bone marrow cells ($1X10^5$, $2X10^5$ or $5X10^5$ MNC/mouse) in lethally irradiated adult mice (6-8 weeks, CD45.2) and assessed the engraftment of donor cells (CD45.1) after 16 weeks by flow cytometric analyses of the bone marrow cells. We found that in spite of the increased numbers of HSCs present in the marrow of old mice, these cells exhibited significantly lower engraftment potential (Data not shown). The mice injected with $1X\ 10^5$ or $2\ X10^5$ old MNCs failed to show any engraftment in the recipients' marrow after 16 weeks. The cells engrafted in primary recipients were sorted (only the set where $5\ X\ 10^5$ MNC/mouse were infused could be used in these experiemtns as other sets did not show engraftment) and infused in irradiated secondary recipients to assess their long-term engraftment ability. The old engrafted HSCs sorted from the primary recipients failed to engraft into the secondary recipients, underscoring their severe defect in long-term functionality.

These data show that the marrow of old mice harbor a large number of functionally compromised HSCs. The reason behind this accumulation of dysfunctional HSCs is not understood.

Co-culture of old HSCs in young IVNs controls their excessive proliferation and reverses their myeloid bias

We then determined whether a short-term co-culture with young IVNs has any effect on the proliferation and the myeloid bias of old HSCs. We performed cross co-culture experiments to examine the issue. Old HSCs were co-cultured with young IVNs (experimental set) or old IVNs (self-control), and young HSCs were co-cultured with old IVNs (experimental set) or young IVNs (self-control). After 36 hours of co-culture, the output cells were harvested and subjected to flow cytometry analyses. The data showed that young IVNs control the excessive proliferation of old HSCs (Figure 2A). On the other hand, old IVNs promoted an excessive proliferation of young HSCs, showing that excessive proliferation of old HSCs is extrinsically controlled.

The myeloid bias of HSCs is defined by the expression of CD41 (Integrin αIIβ) on them. $CD41^+$ HSCs are largely quiescent and exhibit myelo-erythroid and megakaryocyte gene priming, governed by Gata1, whereas $CD41^-$ HSCs are more proliferative and exhibit lymphoid gene priming. When the co-cultured cells were analyzed for the frequency of $CD41^+$ LSK HSCs in them, we found that old HSCs grown on young IVNs had a significantly reduced frequency of LSK-

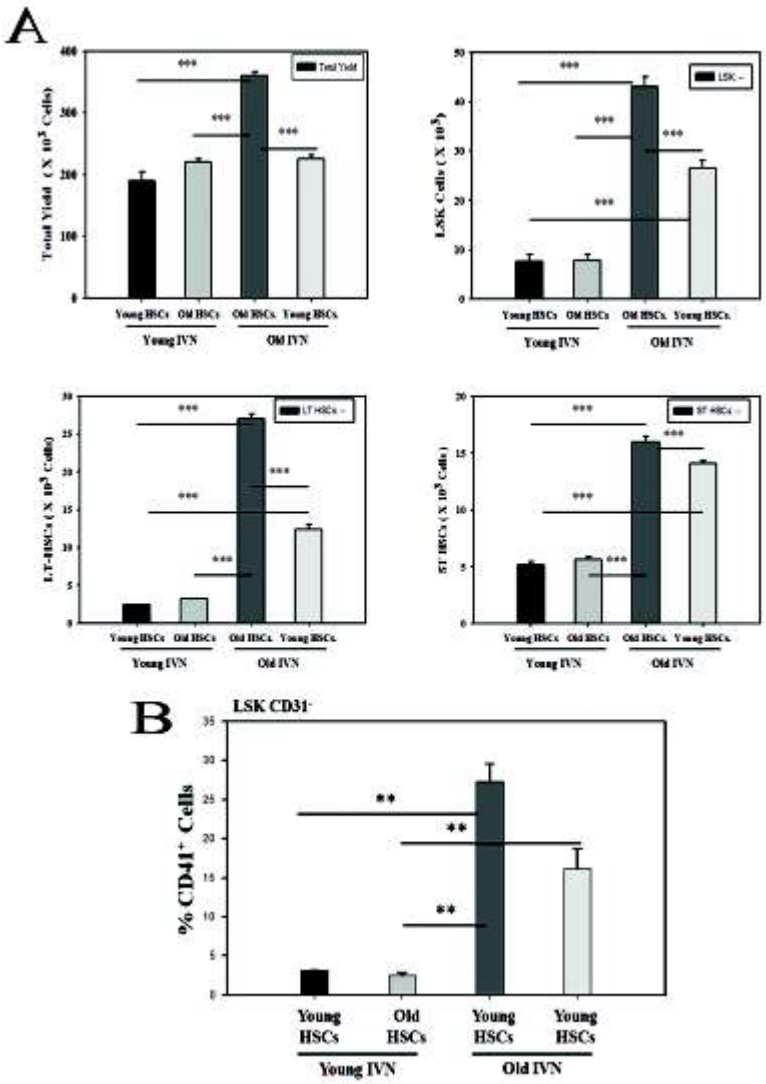

Fig. 2: Proliferation and frequency of myeloid-bias of HSCs are regulated by niche. A. Co-culture of old HSCs on young IVNs controls their proliferation while co-culture of young HSCs on old IVNs induces their proliferation. Effect on total cell yield, LSK HSCs, LT-HSCs and ST-HSCs has been separately depicted for clarity. N = 3. B. Co-culture of old HSCs with young IVNs reduces the frequency of CD41$^+$ HSCs in them. Likewise, co-culture of young HSCs on old IVNs leads to an increase in the frequency of CD41$^+$ HSCs in them. N=3. ** p< 0.01, *** p< 0.001.

CD41$^+$ cells in them (Figure 2B). Likewise, young HSCs co-cultured on old IVNs had a significantly higher frequency of LSK-CD41$^+$ cells in them. These data demonstrated that the CD41 antigen expression on the HSCs is regulated by the niche. It is also possible that the old niche supports expansion of CD41$^+$ HSC subset, while young niche doesn't. Whether CD41 expression is a cause of aging or a consequence of it remains to be determined.

Co-culture of old HSCs with young IVNs increases their long-term engraftment potential and reverses their myeloid bias in *in vivo*

In this set of experiments, we examined the engraftment potential of old HSCs after briefly culturing them with young IVNs. The HSCs from young (6-8 weeks) and old (<12 months) CD45.1 mice were co-cultured with young or old IVNs for 36 hrs. The co-cultured HSCs were then infused in lethally-irradiated adult (8-

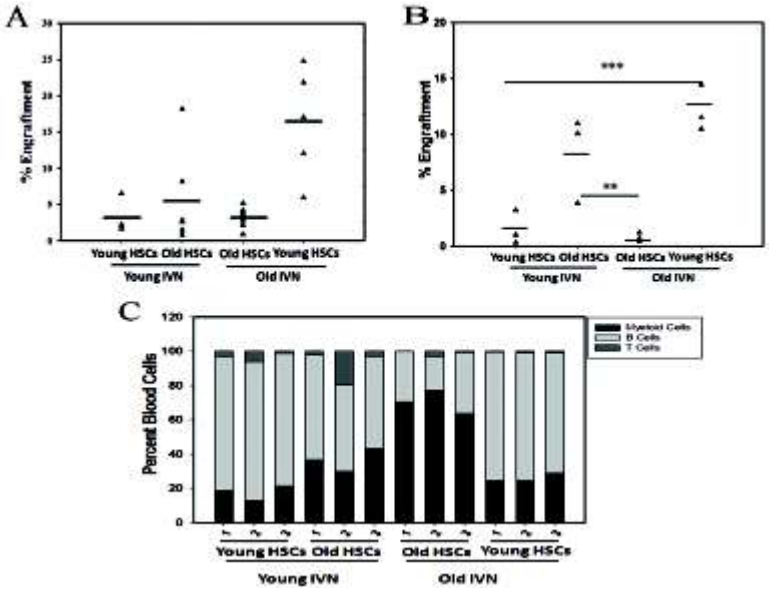

Fig. 3: Co-culture of old HSCs on young IVNs increases their long term engraftment potential and reverses their myeloid bias in *in vivo* Chimera Mouse Model. Results of competitive engraftment studies are depicted. Young or old HSCs cultured with young or old IVNs were intra-venously infused in irradiated recipients. The percent engraftment of donor cells in recipients' peripheral blood was analyzed after 4 weeks. N=6 (short term engraftment potential) **A.** or after 16 weeks (long term engraftment potential) **B.** N=3. **C.** Co-culture of old HSCs with young IVNs reduces their myeloid bias whereas co-culture of young HSCs in old IVNs increases their myeloid bias. These data suggest that expression of CD41 on the HSCs is regulated by the niche.* $p<0.05$, ** $p<0.01$, *** $p<0.001$

10 weeks) recipient (CD45.2) mice at a concentration of $1X10^6$ cells per mouse. The chimerism in peripheral blood was assessed at 4 weekly intervals by flow cytometry. The data at 4th week post-transplant did not reach significance between various groups, (Figure 3A), but 16 weeks post-transplant the old HSCs cultured with young IVNs showed a significant increase in the engraftment levels (Figure 3B). Surprisingly, the young HSCs cultured in old IVNs showed better engraftment compared to their counterparts cultured in young IVNs. A significant decrease in myeloid cell out-put in the peripheral blood was also seen (Figure 3C). These preliminary data show that co-culturing old HSCs with young IVNs rescues their engraftment defect and also reduces their myeloid bias. Further experiments with larger cohorts of mice are in progress.

These data demonstrated that it is possible to improve the defective functionality of the old HSCs using IVN technology. Further experiments are in progress to understand the mechanistic involved.

Future Research Plans

- To develop and use the gene expression profile to examine the effect of "young" niche on the "old" HSCs and correlate this gene expression profile with the HSC functionality.
- To develop specific signaling IVNs by using specific pharmacological modulators and to examine their effects on the HSC functionality.
- Identification of signaling mechanisms operative in the niche cells of old vs. young mice.

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Modulation of Phagolysosome Maturation by Bacterial tlyA gene product

Background

Mycobacterium tuberculosis (M.tb) uses novel mechanisms for survival within mammalian cells, typically macrophages, to evade host immune responses. Among the strategies it orchestrates, the bacteria, post macrophage invasion, inhibit the acidification of phagosomes, and modulate several key events that are vital for phagolysosome maturation.

During this process, the *M.tuberculosis* gets trapped in 'phagosome', which fails to mature into a successful phagolysosome through interaction with endosomes and finally fuse with lysosomes to form the phagolysosome with degradative characteristics. Several proteins including Early Endosomal Antigen-1(EEA-1), Lysosome Associated Membrane Protein-1 (LAMP-1), Rab5, Rab7, play pivotal role in phagolysosome maturation. Many studies on mycobacterial phagosome have suggested that Rab5, Rab7 and LAMPs localizes to phagosomes containing avirulent phenotype, but not with the virulent phenotype. Immediately after phagocytosis, Rab5, an early endosomal marker associated with phagosome, recruits EEA1, which drives the phagosome towards further maturation. As the phagosome matures into more advanced stage, another prominent member of late endosomal marker, Rab7 is recruited to serve as a docking site for RILP (Rab7-interacting lysosomal protein). Other Rab GTPase *viz*. Rab14 was also demonstrated to be important in the process since maintenance of Rab14 on mycobacterial phagosome confers early endosomal characteristics to it and has the potential to escape from components of late endosome or degradative lysosomes. Overall M. tb disturbs the activity of key Rab GTPases and LAMPs which regulate the

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phagolysosome maturation to survive within macrophage. Among the proteins that have been shown to modulate the phagolysosome maturation, the *tlyA* gene product of M. tb (Rv1694; MtbTlyA) is not known to aid to the survival strategies of the bacterium for establishment of the disease either by genome wide analyses or by individual studies.

Hence, we questioned whether or not TlyA protein can aid to the intracellular survival strategies by preventing acidification of the compartments and modulate the maturation of phagoloysosome?

We have, therefore, investigated the role of TlyA during the initial stages of infection for intracellular survival with the help of TlyA expressing bacteria viz. *M. smegmatis* (a surrogate host) and *E. coli* (a heterologous host). We have chosen to study the status of LAMP-1, Rab5, Rab7 and Rab14 GTPases of the infected macrophages. Our findings, for the first time, provide an important direction that mycobacterial TlyA can act as a virulence factor.

Aims and Objectives

- To understand the contribution of TlyA to the intra-cellular survival of mycobacterial species.

Work Achieved

TlyA expression interferes with LAMP-1 co-localization

An examination of the status of LAMP-1, an accepted marker for lysosomal compartments, revealed about ~65% of MtbTlyA expressing *E. coli* were devoid of positive LAMP-1 signal. At the same time about ~85% of non-TlyA expressing *E. coli* showed strong co-localization of LAMP-1. A similar approach using the *M. smegmatis*/TlyA showed LAMP-1 positivity to the extent of ~35% while more than 85% of non-TlyA expressing *M. smegmatis* (*M. smegmatis*/pMyNT) exhibited positive staining of LAMP-1. These observations are consistent with a previous study which has shown that LAMP-1 co-localized with only 22.5% of the phagosomes containing *M. marinum*. In contrast, a much higher percentage of phagosomes containing *M. smegmatis* (40.5%) co-localized with LAMP-1.

These observations suggest that the TlyA has the ability to disrupt fusion of lysosome with phagosome. In order to verify this hypothesis, we have also used the latex beads coated with purified TlyA protein and infected the macrophages to examine the effect of TlyA on acidification since latex beads have been

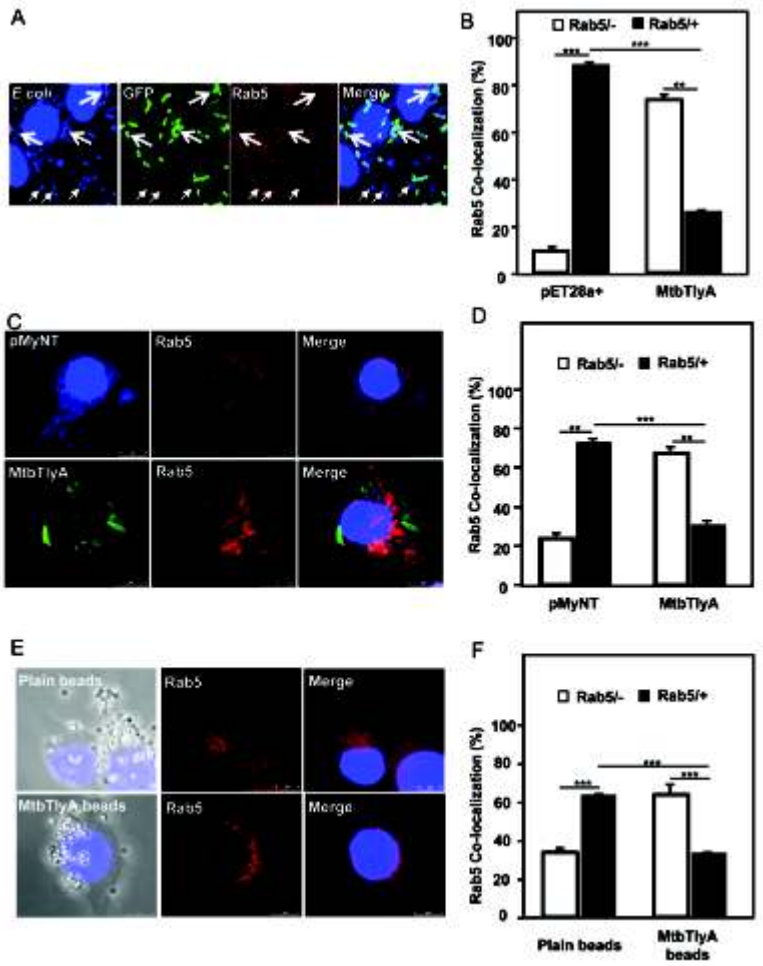

Fig. 1: Rab-5 co–localization in TlyA expressing *E. coli* (A & B) and *M. smegmatis* (C & D): Macrophages (RAW264.7) were infected with (A) MtbTlyA-GFP expressing *E. coli* (C) pMyNT and pMyNT-MtbTlyA transformed *M. smegmatis* at MOI = 10 for 60 minutes. The Rab-5 was visualized with the help of Rhodamine-mouse-anti-Rab5 monoclonal antibody. In panel C, TlyA expressing *M. smegmatis* was visualized with immune rabbit serum specific to TlyA and detected with anti-rabbit-IgG-FITC after permeabilization. Macrophage nucleus and bacteria were stained with DAPI. Thin and thick arrows respectively represent TlyA negative and TlyA positive bacteria. The bar graphs labeled with B, D and F represent the quantitative data i.e. Rab-5 positive or negative bacteria/beads identified in the confocal images shown in A, C and E. The *, ** and *** respectively represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ between the connected samples under the same conditions.

extensively used to understand the phagocytosis mechanisms operative in mammalian cells. Our results results showed ~70% plain beads were uniformly surrounded with red fluorescent vesicles indicative of co-localization with LAMP-1. In contrast, the MtbTlyA-coated beads showed only ~ 35% co-localization and other 65% did not show co-localization indicating the flaws in maturation process. In support of this, the BSA coated beads had exhibited LAMP-1 co-localization on nearly all beads. This result implies that the cell-surface TlyA can, in principle, compromise the integrity of the membrane compartments that it comes into contact with.

TlyA expression excludes Rab5

It is well known that Rab-GTPases are excellent markers for identifying the stage at which the maturation has progressed. For examble, Rab5 is a key regulator of early endocytosis and it plays an essential role in phagosome-endosome fusion for proper regulation and restriction of intra-cellular organisms within the

macrophage. In our attempt, the TlyA expressing *E. coli* showed no significant co-localization with Rab5 (~25%) in comparison to non TlyA expressing *E. coli*, which showed >85% co-localization with Rab5 as seen in Fig. 1. This observation has paralled with MtbTlyA expressing *M. smegmatis*, which showed a mere 30-35% co-localization with Rab5 while remaining 65-70% show no co-localization whereas non-TlyA expressing *M. smegmatis* (*M. smegmatis*/pMyNT) exhibited >75% positive staining of Rab5 (Fig. 1). These observations are also consistent with the observations known in the literature. For example, the Rab5 pattern showed 15% positivity in case of *M. tb* which is consistent our observations i.e. ~25% of *E. coli*-MtbTlyA and ~30% of *M.smegmatis*-TlyA showed Rab5 co-localization. Once again as mentioned ealier, the results obtained from quantitative confocal visualization showed a strong co-localization (65%) of Rab5 with plain beads compared to 35% with MtbTlyA coated beads while they showed 70% positive EEA1 staining in comparison to 34% TlyA coated beads. It is well known that EEA1, a crucial marker for maturation pathway, also exhibits a similar pattern. These observations indicate that the TlyA alone can modulate the phagosome-endosome fusion by modulating the Rab5 and EEA1 during the initial stage of infection.

TlyA expression excludes Rab7

Rab7 has been shown to have an important role in late endocytic pathway and is reported to co-localize with late endosomes. It recruits effectors responsible for tight membrane interactions and can play a -direct or -indirect role for subsequent homotypic and heterotypic fusion of late endocytic structures, thereby providing a basis for the intermixing of membrane and contents. It is required for the final maturation of late, autophagic vacuoles, possibly for fusion with lysosomes. Rab7 shows more or less similar pattern as Rab5. The TlyA expressing *E. coli* shows ~15% co-localization with Rab7 compared to non-TlyA expressing *E. coli* (>80%). In case of MtbTlyA expressing *M. smegmatis*, which show mere 35% co-localization with Rab7 while remaining 65% show no co-localization. Similarly mock vector (pMyNT) transformed *M. smegmatis* exhibited 80% positive and 20% negative staining. These observations are also consistent with the observations known in the literature. For example, the Rab7 was observed in about 30% of *M. tuberculosis* bacteria, 6 hours, post infection, while our study showed about 15% after 1 hour post infection of *E. coli*-MtbTlyA and 35% after 3 hour post infection of *M. smegmatis*/TlyA. Once again, to verify role of TlyA during the final maturation of phagosome, we treated macrophages with plain and MtbTlyA coated beads as

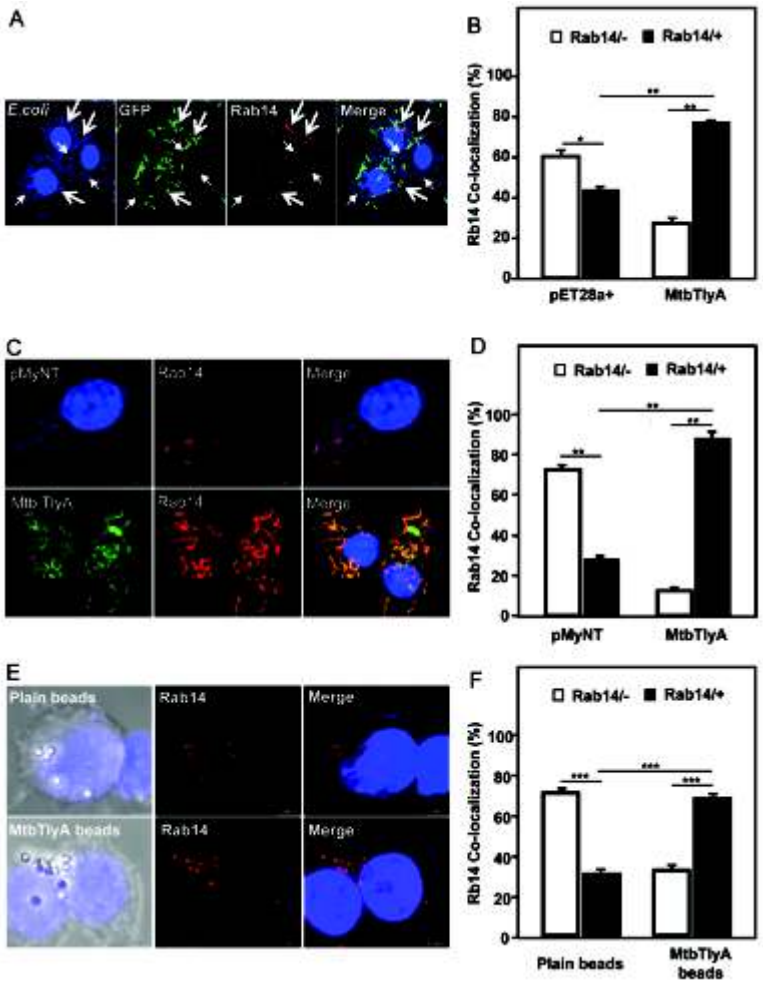

Fig. 2: Visualization and quantitation of Rab-14 co-localization in TlyA expressing *E. coli* (A & B), *M. smegmatis* (C & D) and TlyA-Coated beads (E & F). Rab-14 was visualized with the help of rabbit anti-Rab14 antibody and Rhodamine conjugated anti-rabbit-IgG. The bar graphs labeled with B, D and F represent the quantitative data i.e. Rab-14 positive or negative bacteria/beads identified in the confocal images shown in A, C and E. The *, ** and *** respectively represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ between the connected samples under the same conditions.

done before. We have also observed that nearly >58% MtbTlyA coated beads did not show any positive staining whereas, more than 65% of plain beads exhibited positive staining of Rab7. This data also suggests that TlyA's presence on the extra-cellular surface of bacteria/beads can modulate the fusion events centering on the phagosomes and late endosomes.

TlyA expression maintains Rab14

Many observations in the literature suggest that, in addition to Rab5 and Rab7, Rab14 also plays an important role in blocking of phagosomal maturation in *M. tb* infected macrophage. Rab14 enables mycobacterial phagosomes to maintain an early endosomal characteristic which may help in avoiding the late endosomal/lysosomal degradative components. Our results show ~75% MtbTlyA expressing *E. coli* co-localized with Rab14 (Fig. 2) and nearly >80% co-localization with *M. smegmatis*/TlyA. Whereas, mock vector transformed *E. coli* (pET28a+) and *M. smegmatis* (pMyNT) showed about 40% and 25% co-

localization with Rab14 respectively. This Rab14 staining pattern is again consistent with the literature. For example, Deretic and colleagues observed that the live BCG showed about 75% positive staining for Rab14, while dead BCG exhibited about 20% staining. In comparison, our TlyA expressing *E. coli* showed about 75% positivity for Rab14 and mock vector transformed *E. coli* showed about 40%. It must be noted that TlyA expression has been found to be true in case of live BCG. In comparison, latex bead coated TlyA exhibited about ~70% positive staining with Rab14 whereas plain beads showed very weak co-localization with Rab14 (~30%). This result also supports our hypothesis that TlyA's expression results in interference in phagosomal maturation process. This suggests that the TlyA expressing entities attract the Rab14 containing vesicles, post phagocytosis, to avoid their degradation by lysosomal compartments.

Overall, our work, for the first time, revealed that presence of TlyA on bacterial surface can aid to the survival strategy of its host, especially during the initial stages of infection. The TlyA expressing *E. coli*, *M. smegmatis* and TlyA coated beads have all exhibited the characteristics that are similar to the characteristics observed for live BCG/H37Rv within macrophages reported in the literature with high degree of consistency.

Future Research Plans

To determine:

- What is the underlying mechanism of TlyA protein which does not use any classical pathway to reach the cell-surface?
- What is the significance of the expression and role in establishment of the long-term infection?

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Molecular Mechanisms for Regulation of Ionotropic Glutamate Receptors by their Auxiliary Subunits

Background

Ionotropic glutamate receptors (iGluRs) form the cornerstones of fast excitatory neurotransmission in the mammalian central nervous system. They are essential for many basic nervous system functions, including learning and development, and are involved in a remarkable range of neuronal diseases. Despite their physiological importance, our understanding of these receptors is hampered by a lack of insight into their complex structures and working mechanisms. Recombinant iGluRs on their own form functional channels and are capable of eliciting glutamate-evoked currents in heterologous expression systems, but recent evidence indicates that rather than existing as independent units, native iGluRs are part of a signaling complex that involves transmembrane auxiliary proteins. Till date several of these transmembrane auxiliary subunits have been discovered, which regulate the native iGluRs gating properties, pharmacology, distribution and trafficking to synapses. However, there has been essentially no progress towards understanding the structural basis of how this expanding family of glutamate receptor auxiliary membrane proteins modulates receptor function.

Aims and Objectives

- Structural studies on iGluR auxiliary proteins and their complexes with cognate iGluR receptors.
- Determination of binding site and interaction hotspots of an iGluR-auxiliary subunit complex using electrophysiological and biochemical assays.

Participants

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Work Achieved

We have successfully cloned/obtained 9 out of 13 identified auxiliary proteins. Suitable overexpressing clones for few of these auxiliary proteins for the soluble extracellular domain and the TM domain containing full-length protein has been identified. We have overexpressed and purified one of the auxiliary subnuits extracellular domains from transiently transfected mammalian cells to homogeneity. Size exclusion profile of the purified protein revealed monodispersed homogenous protein preparation suitable for crystallization trials. Our crystallization trials are in process and we have identified potential conditions that may be optimized to yield crystals suitable for X-ray diffraction and data collection.

Future Research Plans

- Interaction analysis and stabilization of auxiliary protein complexes with their cognate receptors.
- Structure determination of auxiliary protein and its complexes with cognate receptor.
- Determination of binding site and interaction hotspots of auxiliary protein-iGluR complex using electrophysiological and biochemical assays.

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Functional Characterization of $CD133^+$ Cancer Stem-like Cells in Regulation of Melanoma Growth, Angiogenesis and Metastasis

Background

Malignant melanoma is a highly metastatic cancer which is responsible for majority of skin cancer-related deaths. Increasing evidences of cancer stem cells (CSCs) in melanoma and other cancers suggest that CSCs play major role in metastasis and angiogenesis. Several groups have shown that epithelial to mesenchymal transition (EMT) generates cancer stem like phenotypes in breast cancer through downregulation of epithelial marker (E-cadherin) and upregulation of mesenchymal markers (N-cadherin, Vimentin and Fibronectin). Earlier it has been reported that melanoma contains tumor-initiating cells that have distinguished $CD20^+$, $CD133^+$ and side-population cells with high efflux activities. Moreover, whether these cells have distinct or overlapping population is the subject of intense investigation. Recent data demonstrated that breast cancer and melanoma cells contain heterogeneous subpopulation and have distinct molecular signatures and display functional stem-like properties. $CD133^+$, $CD20^+$, $CD271^+$, $ABCB5^+$ and $ALDH1A^+$ subpopulations from melanoma cell lines and/or patient's biopsy, have been identified as CSCs. Further reports revealed that Notch signaling plays crucial role in regulation of cell fate and differentiation of stem cells in brain and glioblastoma (GBM). In CSC-derived from GBM, Notch pathway is also involved in CD133 dependent glioblastoma growth in xenograft mice model. Notch signaling has been found in many cancers including melanoma and promotes proliferation and/or survival of cancer cells. It is known that self-renewal and differentiation properties of normal neuronal stem cells are regulated by Notch pathway. Moreover, MAPK signaling plays important role in cervical and other cancers through MEK3/6, p38 and c-Jun activation. Interestingly, MAPK was found to be

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activated in $CD133^+$ stem cells derived from colon cancer and controls tumorigenic activity. Moreover, in liver cancer, $CD133^+$ cells play vital role in regulating tumorigenesis through MAPK signaling pathway. However, the molecular mechanism by which Notch regulates CD133 expressions and controls MAPK activation leading to angiogenesis and tumor progression in CSCs derived from melanoma are not well defined.

In this study, we provide evidences that $CD133^+$ cells behave as stem-like cells and exhibit high tumorigenicity, angiogenesis and metastasis. Blockade of Notch pathway by -secretase inhibitor results in depletion of $CD133^+$ cells suggesting that this pathway is involved in this process. However, $CD133^+$ cells preferentially exhibit MAPK activation and controls MMP-2, -9 and VEGF expressions. Andrographolide (Andro) attenuates $CD133^+$ cell migration and inhibits angiogenesis, melanoma growth, metastasis in mice models. Andro blocks expression of Notch1, p-38 MAPK, c-Jun and c-Fos in these tumors. Our clinical data further supports the *in vitro* and pre-clinical animal data emphasizing the expression of NICD1, CD133, p-p38, p-MEK3/6 and c-Fos with malignant melanoma progression. Thus, targeting Notch1 and its regulated signaling network may have potential therapeutic implication for management of CSC-mediated melanoma progression.

Aims and Objectives

- To isolate and characterize cancer stem like cells (CSCs) from mouse and human melanoma using multiple markers including CD133 and CD20.
- To investigate whether $CD133^+$ CSCs in melanoma is responsible for maintaining long term tumor heterogeneity.
- To study the gene expression profile of $CD133^+$ and $CD133^-$ subpopulations using microarrays and to delineate the specific signaling mechanism involved in CD133 dependent melanoma growth, angiogenesis and metastasis.

Work Achieved

To investigate the heterogeneity in melanoma, the expressions of stem cell markers such as CD133, CD20, CD45, CD271, CD44, CD166, VEGFR1, ABCG2 and ABCG5 from mouse (B16F10, B16F1) and human (A375) melanoma cell lines were analyzed. The results revealed that CD133, CD20, CD166, VEGFR1, ABCG2 and ABCG5 are expressed in these cells in a heterogeneous manner, whereas CD44 is expressed in a homogenous manner in B16F10 cells (Fig. 1).

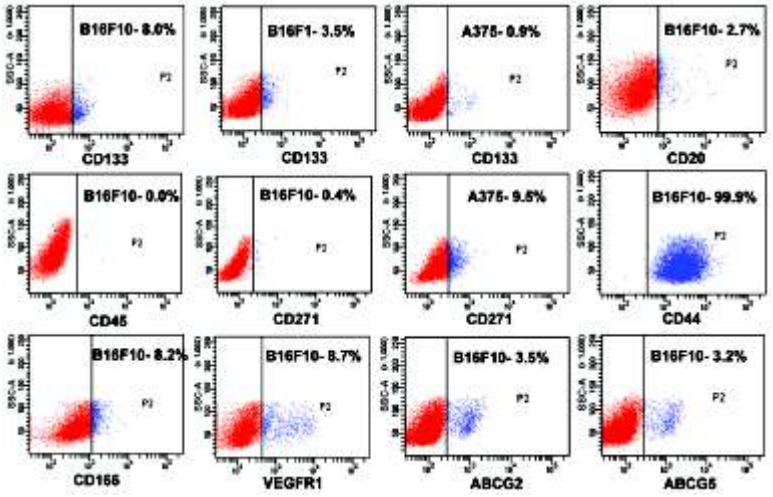

Fig. 1: Melanoma cells exhibit heterogeneous subpopulations. Flow cytometry analyses of heterogeneous expressions of CSC specific markers (CD133, CD20, CD45, CD271, CD44, CD166, VEGFR1, ABCG2 and ABCG5) in melanoma. Each vertical line represents corresponding isotype matched control.

For further characterization, we have examined the levels of CD45 and CD271 in B16F10 cells and observed that these cells do not express these markars. In contrast, CD271 expression in A375 cells was 9.5%. Other hematopoietic stem cell marker, CD166 showed heterogeneous expression (8.2%) in B16F10 cells. Furthermore, we have analyzed the expression of VEGFR1, ABCG2 and ABCG5 and the results depict that B16F10 cells express 8.7, 3.5 and 3.2% of these markers respectively (Fig. 1). CD133 is a cell-surface pentaspan plasma membrane glycoprotein, is being used as marker for both normal and CSCs in multiple tissue lineages and therefore we used CD133 as paramount marker to characterize the CSCs in mouse melanoma cells for further studies.

The differentially regulated global gene expression profile of $CD133^+$ cells was analyzed by microarray (Illumina Mouse WG6 Bead Chip). A total of 411 genes were upregulated and 355 genes were downregulated. These data are represented in the form of heat map analysis (Fig. 2A). We further used Gene Ontology (GO) to annotate genes that were differentially expressed in $CD133^+$ cells. The results suggested that many of the upregulated genes in $CD133^+$ cells were associated with angiogenesis, adhesion, differentiation and metastasis. However, several downregulated genes were linked with apoptosis (Fig. 2B). These findings prompted us to functionally validate these genes in the process of EMT, tumor growth and metastasis in melanoma. We next evaluated the long-term tumorigenic potential of $CD133^+$ subpopulation by serial implantation of $CD133^+$ cells into NOD/SCID mice. Accordingly, $CD133^+$ and $CD133^-$ cells isolated from B16F10 were subcutaneously injected into NOD/SCID mice independently. After this, tumors were excised and primary cultures were established and both these cultured cells were independently re-

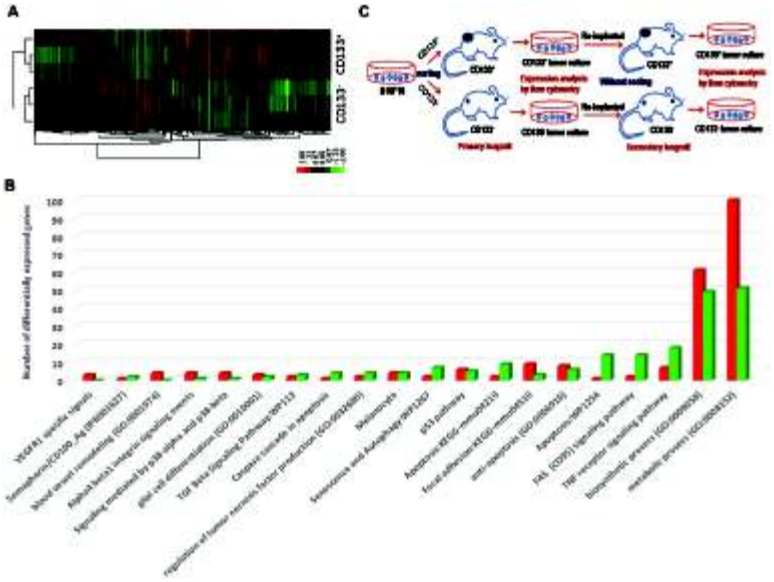

Fig. 2: CD133$^+$ subpopulation exhibits distinct molecular profile and maintains long-term tumorigenic potential. **A)** In CD133$^+$ and CD133$^-$ cells, microarray was performed and differentially regulated genes in CD133$^+$ vs CD133$^-$ cells were analyzed and represented as Heat map plot. **B)** Gene Ontology of differentially regulated genes associated with cancer progression in CD133$^+$ vs CD133$^-$ cells. **C)** CD133+ and CD133$^-$ cells were independently injected s.c. into NOD/SCID mice and primary isografts were generated. The primary cultures established from these isografts were further re-implanted into NOD/SCID mice and secondary isografts were developed. Both primary and secondary isografts were represented schematically.

injected into the NOD/SCID mice. The results revealed that CD133$^+$ cells have higher tumorigenic potential in primary as well in secondary isografts as shown in Fig. 2C.

We have further studied in depth molecular mechanism by which CD133$^+$ cells regulate melanoma progression. Our data show that Notch1 augments CD133 expression and blocking Notch1 function by its inhibitors (GSI-IX or GSI-X) or silencing by its siRNA or using Andrographolide (Andro), substantially attenuates CD133 expression. Overexpression of Notch1 intracellular domain (NICD1IC) in B16F10 cells enhances CD133 expression suggesting that Notch1 is involved in this process. Our findings also suggest that p38 MAPK pathway is highly upregulated which preferentially activate AP-1 and upregulate MMP-2, -9 and VEGF expression in CD133$^+$ cells.

Our study demonstrates that blockade or silencing of Notch1 pathway inhibits CD133 dependent p38 MAPK activation and preferentially attenuates CD133$^+$ cell migration and tumor-endothelial cell interaction that ultimately suppresses melanoma growth, angiogenesis and metastasis

Future Research Plans

- To study the role of bone marrow derived mesenchymal stem cells (MSCs) and MSC specific genes in regulation of breast tumor growth and metastasis.

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

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Background

Inflammation is a complex reaction involving a set of cytokines, chemokines and adhesion molecules. There are several secreted chemokines, cytokines and its specific ligands expressed into the inflamed microenvironment, and a joint venture of pro- and anti-inflammatory functions are initiated together by infiltrating leukocytes, tissue fibroblasts, epithelial and endothelial cells. Chemokine receptors and cell adhesion molecules present on the cell surface are known to be involved in the migration of immune cells into the inflamed tissues. These chemokines and cell adhesion molecules are well studied in the migration of cells. However, whether intrinsic signaling from these chemokine receptors perturb the cell differentiation and functions is not well understood.

Most of the chemokines and some of the adhesion molecules are G-protein coupled receptors (GPCRs). G-proteins are heterotrimer consist of α-, β-, and γ-subunits and transduce signals from surface receptors to intracellular effectors. Upon receptor activation, G-protein complex dissociate into α and βγ-subunits which in turn recruit various signaling components at the inner surface of the plasma membrane followed by production of array of intracellular second messengers such as IP3, DAG, Ca^{2+}, cAMP and IP3. G-protein signaling regulates number of cellular events such as induction of cell polarity, actin polymerization, reversible cell adhesion, myosin dynamics, transmigration, cell activation, differentiation and functions.

CCR6 is a GPCR, expresses on various immune cells and interacts with its specific chemokine CCL20. CCR6 play an important role in various diseases

such as experimental autoimmune encephalitis (EAE), inflammatory bowel disease, psoriasis, chronic hepatitis, rheumatoid arthritis, chronic pulmonary sarcoidosis, cancer metastasis and graft-versus-host disease. How does CCR6 signaling affect differentiation and function of the CD4 T cells is not known?

Aims and Objectives

To determine:

- How does chemokine activation together with co-stimulatory molecules affect the differentiation and function of CD4 T cells?
- How does chemokine receptor signaling perturbs the epigenetic marker in regulatory elements of the genes?

Work Achieved

Dextran sodium sulphate (DSS; 2% w/v in drinking water) treatment induced colitis and bloody diarrhea in C57BL/6 mice within 8 days (n = 5 mice/group, p = 0.004). Immunohistochemical (IHC) analysis of colon showed increased CCL20 expression and infiltration of CD4 T cells. DSS treated mice showed significantly increased $CD4^{+}CCR6^{+}$ cells (spleen 7.7% vs 1.69%, p = 0.001; mesenteric lymph nodes 10.5% vs 5.0%, p = 0.03), CD4+RORγτ^{+} (spleen 20.3% vs 1.61%, p = 0.03; mLN, 3.91% vs 0.75%, p = 0.02), and down-regulated $CD4^{+}Foxp3^{+}$ Treg in spleen and lymph nodes compared to untreated mice. Furthermore, DSS treatment significantly increased RORγτ^{+}T-bet$^{+}CD4^{+}$ and RORγτ$^{+}Foxp3^{+}$ CD4 T cells into spleen and mLN compared to control mice. Activation of CCR6-eGFP^{+} Jurkut cells with CCL20 induced phosphorylation of Akt, STAT3 and p38MAPK. CCL20 treatment enhanced the intracellular expression of ROR (30.6% vs 12.3%, p = 0.008) and IL-17A (30.3% vs 6.1%) in CD4 T cells cultured under Th17 differentiation condition. Wild-type CD4 T cells treated with p38MAP kinase inhibitor SB203580 or genetic deficiency of CCR6

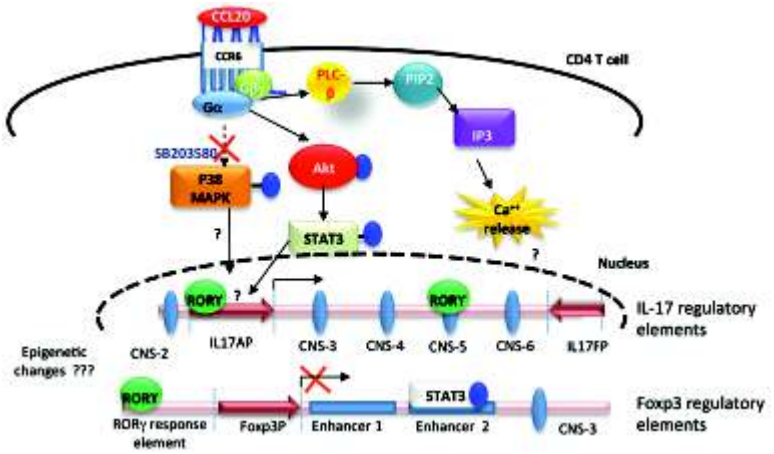

Fig. 1: Schematic representation of CCL20-CCR6 signaling in CD4 T cells.

in CD4 T cells prevented CCL20 induced Th17 differentiation. Luciferase reporter assay showed that CCL20 significant increased IL-17A promoter activity through binding of RORγτ on CNS5 region of IL-17A regulatory elements. These results suggest that CCL20-CCR6 signaling enhances the p38MAPK, pAKT and p38MAPK dependent differentiation of inflammatory Th17 cells leading to increased inflammation into colon (Figure 1). Understanding the molecular mechanism of CCR6 signaling may provide a novel therapeutic target to control autoimmune diseases.

Future Research Plans

- To investigate the role of CCR6 in the antigen-specific differentiation of Th17 and Treg.
- Structural biology and phage-display library approach to development of agonistic and antagonistic CCR6 ligand to control the inflammation and autoimmunity.

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Identification and characterization of cell fate modulators using ES cell model

Background

Embryonic stem cells (ES cells) possess the inherent characteristics of indefinite self renewal and pluripotency that are tightly regulated and any perturbation in the same may lead to alterations in the behaviour of these cell types. Hence, investigations are ongoing worldwide towards gaining better understanding of the selective activation and repression of regulatory networks governing ES cells properties. Although identifications of various pluripotency associated factors, epigenetic modifications and miRNAs provide evidences regarding the existence of multiple genetic and epigenetic modulators that may influence ES cells differentiation, a thorough understanding of the same is far from being complete. Hence, determination of the guiding cues directing specific cell fates and what leads to their altered physiology, is essential for gaining insight into the molecular basis of cell commitment and that would in turn facilitate determining the causative factors underlying human disease processes. Accordingly, we have used murine ES cells to address the intricate fate decision machinery operational during early embryonic development. Since loss of function studies by gene disruption reckons the function of specific genes and their downstream relevance, we have used an insertional mutagenesis approach and tried to identify and elucidate the chromatin and epigenetic regulatory complexes that underlie commitment and specification of undifferentiated ES cells to specific lineages. Our strategy has yielded a couple of known and hither-to unknown candidates. An in-depth knowledge of these key factors and their regulatory pathways may offer new strategies to direct the differentiation of cells into specific cell types of interest as well as to reprogram differentiated cells.

Participants

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Aims and Objectives

- Generation of loss-of-function mutants in ES cells.
- Isolation and mapping of the trapped genes.
- Validation of loss-of-function mutants and their characterization during ES cells maintenance and differentiation.

Work Achieved

Insertional mutagenesis by loss of function genetic screens has emerged as a powerful tool to identify novel genes that may regulate the innate characteristics of a biological system. We have used murine ES cells model to generate loss of function genetic mutants. Considering the advantage of LINE1 (L1) retrotransposon in its capability to insert its sequence to a new genomic location by a 'copy and paste' mechanism, the same has been used to bring about random disruption of genes at a high frequency. The vector used had contained S/MAR region for maintaining stability, neomycin cassette for stable ES cells clones' selection and GFP for easier identification of mutant clones based on its expression only upon L1 insertion. This strategy has yielded 21 GFP positive clones (Fig. 1A) out of ~ 200 G418 resistant clones, indicating not all ES cell clones had undergone L1- insertion. Out of these 21 GFP+ clones, 5 had shown integration in noncoding intergenic region, 5 had within the gene. Notably, ~80% of the clones had single gene disruption as determined by FISH.

Three genes such as Actr6, GATA4 and Zfp 57 amongst the five identified targets have been proven to have known functions in ES cells. Hence, we proceeded with the other two; one belonging to Toll interacting protein group (Tollip) and the other to one of the deubiquitinase family member having hitherto no known functional annotation being made in both the cases in ES cells. While the former is known to act in the immune response to invading pathogens by controlling IRAK phosphorylation in the TLR and IL-1R signaling pathways, the latter is presumed to be having ubiquitin thiolesterase activity. Hence, the ease of using this insertional mutagenesis approach and simplicity of identifying the cells with disrupted genes by GFP expression has made this L1 system a promising tool for ES cells gene discovery.

To delineate the functional significance of both the genes we generated a number of stable shRNA clones of the respective targets using the existing Nes-EGFP (EGFP under the regulatory control of nestin enhancer) ES cells in our laboratory. The clones showing puromycin resistance following transfection were picked up and monitored for m-cherry expression. A couple of clones

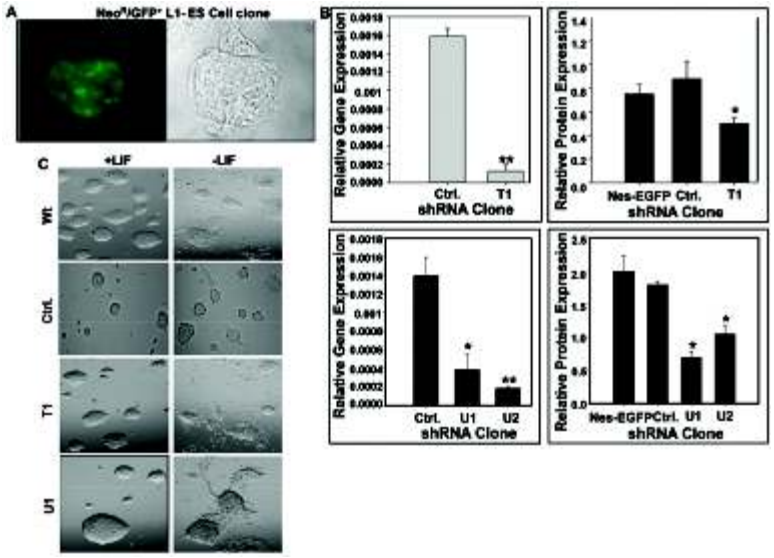

Fig. 1: **(A)** The G418 resistant L1 ES cell clones showing GFP expression. **(B)** Assessment of knockdown efficiency in the Tollip (T) and deubiquitinase (U) member knockdown clones both at transcriptional and translational levels when compared with parental (Nes-EGFP) and control shRNA (Ctrl.) clones. **(C)** Morphology of ES cell clones under maintenance (+LIF) and spontaneous differentiation inducing (-LIF) conditions.

from each were further considered for assessing their knockdown efficiency for respective genes at both transcriptional and translational levels by comparing with control shRNA and the parental Nes-EGFP clones (Fig. 1B). The knockdown efficiency in different clones varied from 60-85%. Almost all the clones showed proper ES cell-like colony morphology when maintained in the presence of the cytokine LIF similar to parental Nes-EGFP cells. Notably, some of the selected Tollip knockdown clones retained the undifferentiated morphology even when grown in the absence of LIF (Fig. 1C) suggesting the importance of Tollip during ES cells differentiation, the knockdown of which prevented ES cells from undergoing differentiation. In contrast, the deubiquitinase member knockdown clones showed differentiating morphology under –LIF condition similar to that of the control shRNA clones and parental ES cells (Fig. 1C). This was further validated by monitoring pluripotency associated markers such as Oct4, Nanog and Sox2 expression by immunocytochemistry and their quantification at transcriptional and translational levels.

We further investigated their pluripotent differentiation potential both *in vitro* and *in vivo*. Successful generation of teratoma upon injection of the respective cells in SCID mice and their subsequent immunohistochemical characterization revealed their pluripotent ability *in vivo*. Similarly *in vitro* differentiation into neural lineage revealed similar neural differentiation potential in these clones when compared with control and Nes-EGFP cells. Strikingly, the smooth muscle differentiation was much more pronounced in Tollip knockdown clones while there was a delayed differentiation into cardiomyocytes when compared with that of control and Nes-EGFP. Together our investigation suggested a positive

regulatory influence of Tollip on cardiomyogenesis and a negative influence on smooth muscle differentiation. Further investigations are underway for the mechanistic underpinning of the same.

Future Research Plans

- We will be investigating the mechanistic basis underlying Tollip mediated ES cells maintenance and differentiation. Further screening of L1 clones as well as the characterizations of the remaining insertional mutant clones will be undertaken to identify newer targets and their functional relevance during either ES cells maintenance or differentiation.

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Studies on Expansion, Cryopreservation and Differentiation of Hematopoietic, Mesenchymal and Induced pluripotent Stem cells isolated from Umbilical cord tissues

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Background

Out of the five different ongoing projects from this research group, the project on "in depth functional studies of dendritic cells" is described in details below.

The development of dendritic cell based vaccines is a promising approach in cancer immunotherapy. For their successful use in the clinics, the propagation and functionality of dendritic cells (DCs) is crucial. We earlier established a two-step method for the large scale generation of DCs from umbilical cord blood derived MNCs/CD34+ cells. The present data aimed at improving their functionality based on the following observations: *in vitro* generated DCs can be less efficient in migration and other functional activities due to lower eicosanoid levels. The production of eicosanoids from Arachidonic Acid (AA) can be hampered due to suppression of the enzyme phospholipase A2 by IL-4, an essential cytokine required for the differentiation of DCs. We hypothesized that exogenous addition of AA to the culture media during DC generation may result in DCs with improved functionality.

In another set of experiments we wanted to study the potency of cord blood derived DCs in comparison with the standard source i.e.PBL monocyte derived DCs particularly with reference to CTL characterization and *in vitro* and *in vivo* activity.

Aims and Objectives

- Improvement in functionality of DCs by adding Arachidonic acid at the differentiation stage.
- Comparison of CTLs generated from Umbilical Cord Blood (UCB) and Peripheral Blood (PBL) DCs.

Work Achieved

DCs were generated with and without AA at the differentiation step. The two DC sets were compared by morphology (Fig.1a) and phenotypic analysis (Fig.1b) and were found to be similar. Among the functional assays like antigen uptake, no significant differences were seen between the AA^+ and AA^- DCs (Fig.1c). However the migration towards CCL19 (Fig.1d) and MLR capability(Fig.1e) of AA^+ DCs was significantly higher than that of AA^- DCs. Higher transcript levels of COX-2 in AA^+ DCs (Fig.1f) also indicate restoration of eicosanoids levels through COX2 mediated pathway there by enhancing the functionality of these cells to be used as a potent cellular vaccine. *In vivo* homing to bone marrow in NOD /SCID mice (Fig.1 g) confirmed the superior nature of DCs generated with arachidonic acid addition. Taken together, these findings will be helpful in the better contriving of DC based vaccines for cancer immunotherapy.

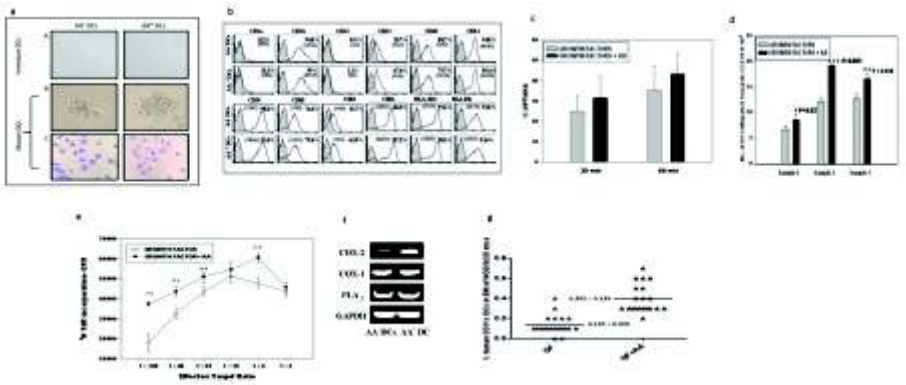

Fig. 1: Characterization of AA^+ and AA^- DCs: DCs were generated from the expanded DC precursor cells with and without AA. **(a)** Morphology of DCs along with Wright-Giemsa staining. **(b)** Phenotype profile of a representative experiment. Filled histograms show the isotype control and open ones show the specific CD marker with respective MFI values inside the brackets. **(c)** Receptor mediated uptake of Dextran-FITC. **(d)** Chemotaxis and **(e)** MLR of the two types of DCs **(f)** Gene expression profile of three key enzymes associated with AA pathway along with the housekeeping gene GAPDH. There is a substantial up-regulation of transcript level of key enzyme COX-2 in the DCs cultured in presence of AA. **(g)** Scatter plot showing higher percentage of DCs (human CD11c positive cells) in bone marrow of mice infused with AA^+ DCs

Next we addressed the issue whether DCs generated from UCB are equivalent in potential to the standard source i.e. PBL monocyte derived DCs in terms of CTL generation. CTLS generated from these two types of DCs were characterized by expression of activation markers like CD25/CD69 (Fig. 2a), presence of Granzyme A and B (Fig.2 b) along with tetramer staining for MUC1 specific peptide positive TCR(Fig.2c). Secretion of interferon gamma by the activated cells was estimated by ELISA (Fig.2d). From the data it is clear that CTLs from UCB DCs were not only equivalent but had an upper edge over the

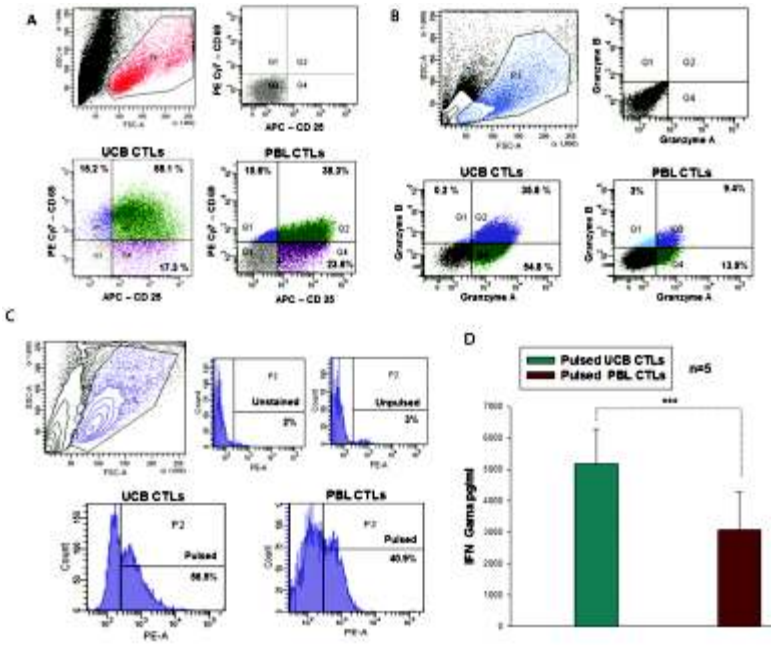

Fig. 2: Characterization of Cytotoxic T Lymphocyte (CTL) generated from UCB and PBL samples. Scatter plots showing **A)** the presence of activation markers like CD69 and CD25 on cell surface of CTL. **B**) the presence of serine proteases like Granzyme A and Granzyme B present inside the cell. **C)** Histogram plots for tetramer staining showing the presence of MUC1 specific CTLs in the total population of CTLs generated in the co-culture. **D)** IFN gamma secretion by the CTLs quantitated by ELISA.

PBL DCs. In order to study the killing activity of the two CTLs we performed *in vivo* CTL assay. The NOD /SCID mice were infused with MCF-7 LUC cell line sub cutaneously and once visible tumors (5 mm) were formed they were imaged on In vivo imaging system (IVIS). Then the mice were divided in three groups were i.v. infused with PBS (control), UCB and PB CTLs respectively. After 10 days the

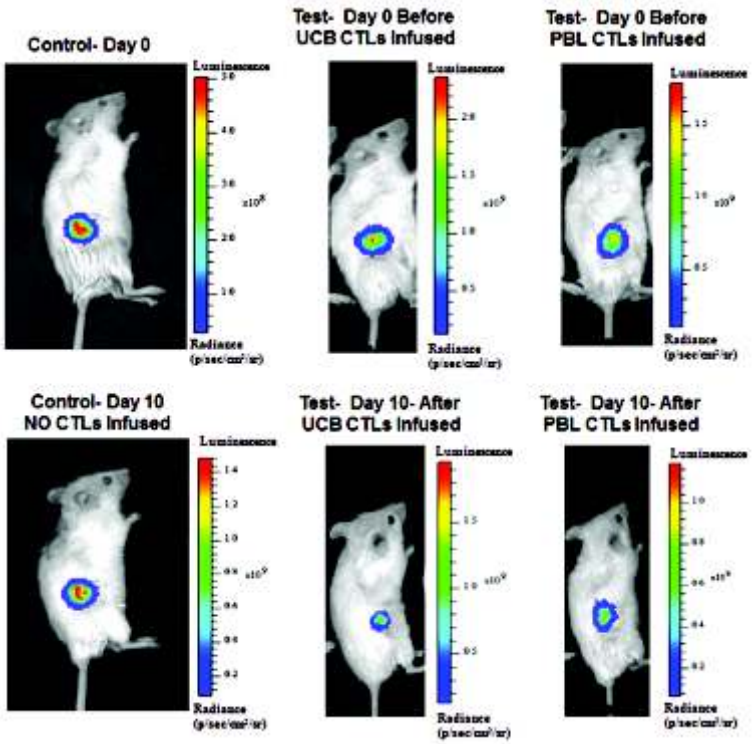

Fig. 3: IVIS images showing the regression of tumor as analyzed by average radiance of MCF-7 Luc positive xenograft in NOD/SCID mice. Mice were imaged before and 10 days after infusion of PBS(Control) / CTLs specific for the MCF-7 luc tumor derived from PBL and UCB DCs respectively. Regression of tumors is seen in the CTL infused mice.

mice were again imaged on IVIS and the regression in tumor was analyzed by reduction in tumor size by calculating the radiance emitted by the Luc positive cells. As depicted in Fig. 3, it is evident that these CTLs are effective in killing the target cells in the xenograft model. Our data conclusively prove that UCB-derived DCs can serve as an alternate source of allogeneic DCs for their future use as cancer vaccines in clinics.

Future Research Plans

- We will attempt to generate DCs by our two step method from apheresis samples obtained from clinics.This will have a direct application in clinics.

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Structure function properties of *M. tuberculosis* proteins

Background

Our laboratory has been involved in two broad areas of work, namely, (1) determining and analyzing structures of important protein derived from M*ycobacterium tuberculosis* and (2) developing novel computational methodologies in the analysis of large scale data. In the former, our focus has been on heat shock proteins of *M. tuberculosis* and those involved in redox processes. In the latter, we are attempting to develop computational methods to understand the dynamics and evolution of protein interaction networks. The networks are typically predicted using in-silico methods developed in our laboratory, or are from the published experimental work.

The heat shock protein family that is of our interest is the GroEL Chaperonin family. Conformational changes and allosteric transitions are hallmarks of chaperonin mechanism. We have exploited the inability of *M. tuberculosis* GroEL2 to functionally complement *E. coli groEL* mutant to address the importance of allostery and hinges. Our work points to the significance of conservation at the hinge regions, which stands out as the prominent feature of GroEL mechanism.

Redox reactions form the basis of most biochemical reactions. *M. tuberculosis* is known to survive harsh environmental conditions where it successfully manages to overcome oxidative stress. We believe that understanding these redox reactions mechanistically will help us come up with better therapeutics against tuberculosis. Previously we have structurally and functionally characterised the genes involved in the Thioredoxin pathway of *M. tuberculosis*. We are currently focussing our efforts in the electron transport mechanism of the ribonucleotide reductase, and redox reactions involved therein.

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Aims and Objectives

- Genetic analysis of *M. tuberculosis* - *E. coli* GroEL chimeras in GroEL depletion strain of *E. coli*.
- Biochemical and structural analysis of the these chimeras using normal mode analysis.
- To structurally and functionally characterise the redox pathway in *M. tuberculosis*, especially with regards to electron transfer to Ribonucleotide reductases (RNR).
- Structural and functional studies on *M. tuberculosis* Rv0081. Purification of the recombinant protein followed by structural and biochemical characterization.

Work Achieved

Different GroEL chimeras were created and characterized. The properties of these chimeras are summarized in the following table. Nomenclature used is as follows: The first letter indicates the source of GroEL, *E. coli* or *M. tuberculosis*; A and I refer to Apical and Intermediate domains sourced from these GroEL's; 1 and 2 refer to hinge variations in these chimera. Oligomerization of T, H and D refer to tetradecamer, heptamer or dimer, respectively.

	Chimera	Hinge Variations		GroES	Complementation	GroES Interaction	Oligomerization	Prevention of Aggregation
		1a or 2a	1b or 2b					
Hinge 1	EAI1	:: G	Δ	Mt	+ + + +	+ + +	T + D	+
				Ec	+ + + +	+ + + + +		
	EAI2	-	-	Mt	-	+ + +	T	+ +
				Ec	+ +	+ + +		
	MAI1	Δ	:: EG	Mt	+ + + +	+ + + +	T	+ + +
				Ec	+ + + +	-		
	MAI2	-	-	Mt	+ + +	+ + +	T	+ + +
				Ec	+ + +	+		
Hinge 2	EA1	:: EG	Δ	Mt	-	+ + + +	T + D	+ + +
				Ec	-	+ + +		
	EA2	-	-	Mt	+ + +	+ + +	T	+ +
				Ec	+ +	+ +		
	MA1	Δ	:: VA	Mt	-	+ + + +	T	+ + + +
				Ec	-	+		
	MA2	-	-	Mt	+	+ + +	T + H	+ + +
				Ec	-	+ +		

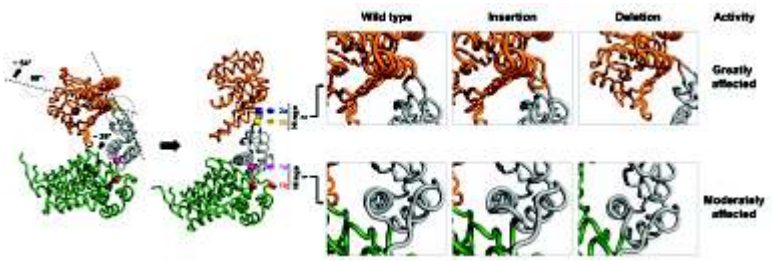

Fig. 1: Effect of Hinge Variations on GroEL Activity: Homology models of M. tuberculosis GroEL2 representing the T and R conformational states. The apical, intermediate and equatorial domains are presented in gold, silver and green, respectively while the color-coded arrows represent the hinges. Effect of the hinge indels are compared to the wild-type as represented. Variations at the hinge 2 have been shown to greatly affect the activity.

In the past one year we have purified relevant complexes, namely TrxR-NrdH and the RNR complex. The two subunits of RNR, coded by the *nrdE* and *nrdF2* genes, co-purify only under reducing conditions, suggesting that redox status of environment controls the complex formation between these two subunits. In collaboration with Dr. Ashish from IMTECH we used Small Angle X-ray Scattering to probe large scale shape changes in the large subunit of RNR under different redox conditions. With these experiments, we are now beginning to get a glimpse of what happens when RNRs undergo various steps in its redox cycle.

The major operon which is overexpressed under hypoxic conditions is known to be under transcriptional control of Rv0081. In the past one year, we have expressed, purified and crystallized Rv0081. X-ray diffraction data has been collected at 3.2 Å resolutions. Understanding finer structural features using crystallographic analysis is in progress.

Future Research Plans

- We intend to undertake comprehensive structural studies on the GroEL chimeras using X-ray crystallography and Cryo-EM. The purified chimeras are already available, and crystallization attempts are being initiated on these proteins. Simultaneously, we also wish to undertake genetic studies on the hinge variations in *E. coli* GroEL
- We anticipate to make significant progress on the crystallographic study of Rv0081. We hope that it will lead to better understanding of the structural features of Rv0081 and its complex with DNA.

Report of DST-INSPIRE Faculty from
Dr. Shekhar Mande's research group:

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Project: Small non-coding RNA mediated defense and counter-defense in the antiviral immune system of silkworm

Background

Unlike vertebrates, invertebrates lack adaptive immunity and possess very primitive innate immune responses against pathogens, especially viruses. Nevertheless, invertebrates are able to combat viral infection very effectively, suggesting that they rely on alternative mechanisms for antiviral defense. One of the antiviral defense mechanisms whose understanding has gained momentum in the recent decade is the small non-coding RNA mediated interference (RNAi). RNAi is a mechanism of eukaryotic posttranscriptional gene silencing that relies on ~22 nucleotides long non-coding RNAs binding to their complementary sites on target mRNA(s). Two of the key players of RNAi are small interfering RNAs (siRNAs) and micro RNAs (miRNAs). Due to the close resemblance between innate immune responses of insects and mammals, antiviral immune mechanism of insects has aroused considerable interest in the recent years. Small non-coding RNA molecules engaged in sequence-specific interactions to inhibit gene expression by RNA silencing have emerged as one of the key players in virus defense and provide another layer to innate immune responses in insects.

My lab is interested in understanding the role of small non-coding RNA mediated defense and counter-defense in the antiviral immune system of the domesticated silkworm, *Bombyx mori* against its viral pathogen, *Bombyx mori nucleopolyhedrosis virus* (BmNPV). *Bombyx mori* is a genetic model system of the largest and the most diverse insect orders, Lepidoptera, which includes many devastating agricultural pests.

Participants

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Collaborators

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Aims and Objectives

- Identification and characterization of small non-coding RNAs and their mRNA targets encoded by the host, *B. mori* as well as its viral pathogen, BmNPV.
- Analysis of miRNA(s) role in resistance/susceptibility of *B. mori* against BmNPV infection.

Work Achieved

Key players of small RNA biogenesis: When we started our study, many of the important key factors of RNA silencing machinery were not identified and characterized in *B. mori*, so we first checked the expression of these important factors using RT-qPCR. All the important genes of small non-coding RNA biogenesis found to be expressed in *B. mori*, and their expression were upregulated upon viral infection (Figure-1).

Small RNA Sequencing: The fifth instar larvae of resistant (SBNP-1) and susceptible (CSR2) strains of *B. mori* were orally fed with the purified BmNPV suspension. Total RNA was then isolated from fat body and midgut tissues of infected and control larvae of these two strains of *B. mori*. The small RNA libraries of these samples were constructed and sequenced using Illumina.

Computational analysis of small RNA sequences: We have received the small RNA sequence libraries from all the samples and presently, we are mapping these sequences on to the host as well as viral genomes to characterize them into different classes of small non-coding RNAs.

Future Research Plans

- Characterization of mapped small RNA sequences into different classes of small non-coding RNAs.
- Experimental validation of the differentially expressed small non-coding RNAs in the resistant and susceptible strains of *B. mori*.

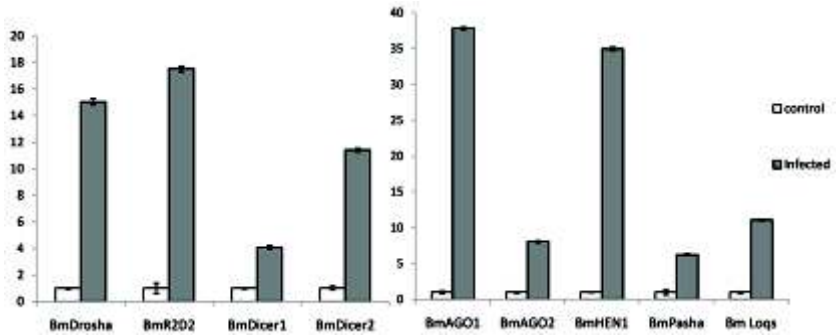

Fig. 1: All the important components of non-coding small RNA biogenesis pathways upregulated upon BmNPV infection. In **(A)** and **(B)**, RT-qPCR was done to quantify the expression of all the selected genes in uninfected (control) and infected samples. Three independent experiments were performed in triplicates, and the results were normalized against endogenous 18S rRNA.

Publications

- Singh, C.P., Singh, J.* and Nagaraju, J. (2014). bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in *Bombyx mori*. Insect Biochemistry and Molecular Biology 49:59-69 (*corresponding author).

Conferences attended

- Talk given on: 'BmNPV encodes miRNA for modulating the expression of its own late genes to establish infection in *Bombyx mori*'. 23rd International Congress of the International Sericultural Commission on Sericulture and Silk Industry Conference from 24-27 November 2014 at Bengaluru, India.

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

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Background

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS). The hallmark of the disease is gradual depletion in the number of CD4+ T cells leading to the onset of opportunistic infections. The incidence of HIV infection has reached pandemic levels worldwide including India. The therapeutic regimen being used at present can reduce the viral load remarkably but are not the ultimate answer to AIDS patients. Our group has been working on different aspects of HIV pathogenesis, related to host-virus interactions, immune response and drug discovery. The primary objective is to gain more understanding of the virus and its interaction with the host cell, which may lead to new antiviral strategies.

Aims and Objectives

- Role of viral regulatory proteins Tat and Nef in HIV pathogenesis.
- Differential gene expression studies to identify molecular mechanism of HIV-1 induced T-cell apoptosis.
- Identification of novel molecules with anti-HIV activity and their potential for use as microbicides.

Work Achieved

Role of viral regulatory proteins Tat and Nef in HIV pathogenesis

HIV-1 Tat protein is one of the most important regulatory proteins for inducing viral gene expression in the host cell. It functions primarily by binding to initial short transcript of HIV genome named transactivation response element (TAR), which results in recruitment of positive transcription elongation factor B (pTEFb) complex to the LTR promoter. The pTEFb complex then hyper-phosphorylates

the C-terminal domain of RNA polymerase II increasing the processivity of polymerase, which leads to elongation of transcription. There are convincing evidences that Tat also functions independently of TAR element to activate the LTR promoter. A previous report from our lab has shown Tat binding to the NFB enhancer sequences on the LTR promoter as one of the mechanisms underlying TAR independent transactivation. Thus, DNA binding activity of Tat could be also one of the potential mechanisms of TAR independent Tat mediated regulation of cellular gene expression. Recent studies by two independent groups have shown association of Tat with cellular gene promoters in Tat transfected cells using ChIP and related techniques. However, as Tat transfection cannot generate the same physiological microenvironment as in case of HIV-1 virus infection, thus we have studied the genome wide occupancy of Tat protein on host cell chromatin by ChIP-on-chip analysis in

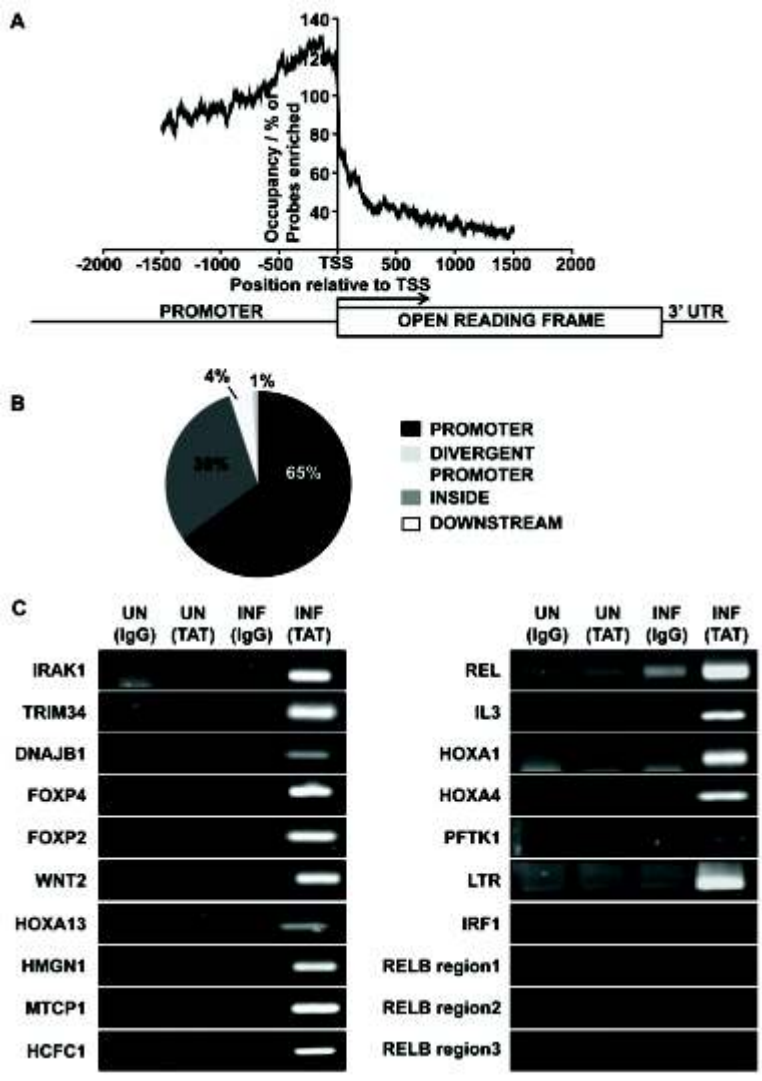

Fig. 1: HIV-1 Tat is majorly recruited on cellular gene promoters in HIV-1 infected T-cells. (A) Positioning of enriched probes around the transcription start site (TSS). The summation of log enrichment ratios was calculated across the TSS (-1500 to +1500 bp) and divided by the percentage of total probes occupied and plotted against the position with respect to transcription start site (TSS). **(B)** The pie chart depicts the percentages of genes showing Tat recruitment on their promoters (-5.5kb upstream from the TSS), divergent promoters (upstream from two genes that are transcribed in opposite directions), inside (inside a gene) and downstream (+2.5 kb beyond the end of the gene). **(C)** Validation of selected Tat targets identified by ChIP-on-chip using ChIP PCR. Fifteen targets were selected for validations of ChIP-on-chip. IRF1 and REL B region 1, 2 and 3 are the four negative controls. PCR encompassing the NFκB sites of the LTR promoter was taken as the positive control.

HIV-1 infected T cells to look for a potential role of Tat on cellular gene expression in a more biologically relevant situation. We have identified 568 genes (1629 probes), which show significant recruitment of Tat protein in HIV-1 infected T-cells. In agreement with the pattern of the probes, 65% of the genes showed recruitment of Tat protein on their promoters (Fig-1). Validation of some selected genes using ChIP-PCR confirmed the recruitment of Tat on these genomic regions in HIV-1 infected CEM-GFP T-cells (Fig-1).

Tat protein has well defined domains or motifs for its versatile functions. It is encoded by two exons, where the first exon extends from 1-72 residues. Amino acids 1-48 frame the activation domain. Amino acids 49-72 define the TAR binding region and the nuclear localization signal. Amino acids 73-101 of Tat protein are encoded by the second exon and contain the highly conserved RGD motif involved in cell adhesion. Tat is not only known as an activator but also as a repressor. Tat represses a number of cellular gene promoters namely major histocompatibility complex I, mannose receptor, bone morphogenic protein receptor-2, beta 2- microglobulin and manganese superoxide dismutase etc. Though repressive activity of Tat has been reported on a limited number of cellular promoters but so far no domain or motif of Tat has been assigned repressive function. Tat was identified as a repressor of c-Rel in the present study as it down regulates expression of c-Rel in HIV-1 infected cells. We also show that Tat down regulates c-Rel promoter activity by interacting with its specific NFκB sites (Fig-2). This repression potentially involves the 68-72 motif of Tat protein. Thus, we have not only identified the genome wide recruitment of Tat protein in HIV-1 infected cells but also provide c-Rel promoter as a case study, which is down regulated by Tat using specific NFκB enhancer sites in the promoter. Down regulation of an NFκB family transcription factor C-Rel by Tat could also be a viral strategy to induce persistent infection in T cells.

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 in viral replication and infectivity remains to be elucidated. We have now initiated a comprehensive study of all the HSP protein family members during HIV infection. Each family is represented by different HSP members and their isoforms, encoded by different genes. In order to identify various HSP family members that are

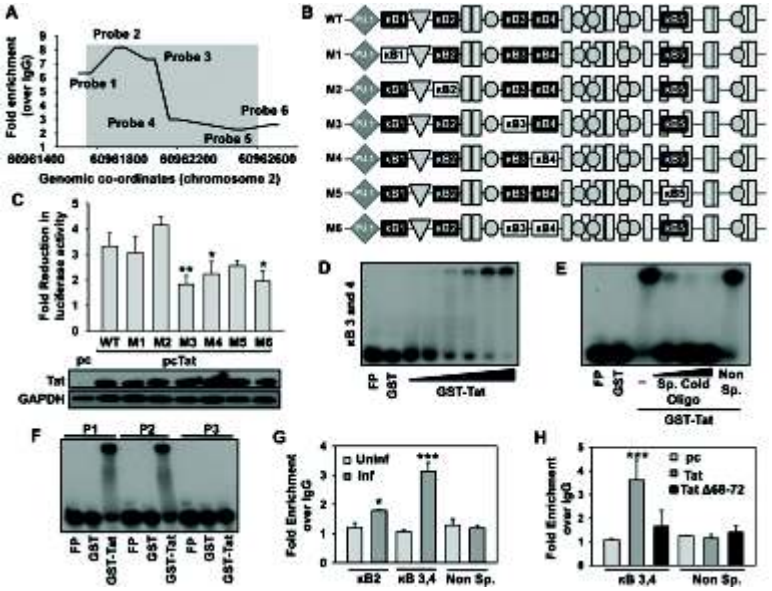

Fig. 2: Tat down regulates c-Rel promoter activity by interacting with specific NFκB sites in the *c-Rel* promoter. (A) The *c-Rel* gene enrichment in ChIP-on-chip analysis in infected CEM-GFP cells. The light grey shaded region is the characterized *c-Rel* promoter which was cloned and used in the study. Probe 1, 2, 3, 4, 5 and 6 are the six probes that got significantly enriched in the Tat immunoprecipitated sample. (B) Schematic illustration of wild type (WT) *c-Rel* promoter and its NFκB site mutants. NFκB sites in white boxes are the mutated sites. Grey rectangle and octagon represent AP2 and SP1 sites respectively. (C) The promoter activity of wild type and mutant *c-Rel* promoters in presence of Tat. WT and mutant *c-Rel* promoter luciferase constructs were co-transfected with Tat and luciferase activity was measured. Tat expression was checked by immunoblotting. (D) Dose dependent binding of GST-Tat protein with the κB 3 and 4 sites of the *c-Rel* promoter (-262 to -305) in EMSA; FP, Free Probe (E) Specificity of Tat binding to κB 3 and 4 probe in EMSA as analyzed by competition with specific and non-specific cold oligo; FP, Free Probe. (F) Binding of GST-Tat with single and dual κB 3 and 4 mutants of *c-Rel* promoter. Probe 1 (P1), κB3 mutant; Probe 2 (P2), κB4 mutant; Probe 3 (P3), dual κB 3 and 4 mutant; FP, Free Probe (G) Recruitment of Tat on κB 3 and 4 sites of c-Rel promoter as analyzed by ChIP using Tat immunoprecipitated chromatin of Day 7 infected CEM-GFP cells. Occupancy of Tat was also tested on κB2 site. (H) Recruitment of Tat in CEM-GFP cells transfected with Tat and TatΔ68-72 as analyzed by ChIP. Pulled chromatin was used for qPCR using primers encompassing *c-Rel* promoter κB 3 and 4 sites.

modulated during HIV-1 infection, we have performed PCR array analysis for HSP family members using RNA isolated from HIV-1 infected CEM-GFP cells. Our results indicate that a significant number of genes belonging to HSP40 and HSP70 family are differentially expressed during infection. We have now cloned many of these isoforms and further characterization of the individual role of these isoforms in HIV-1 infection is currently in progress. We have also looked at the role of different HSPs in viral replication and infectivity by knocking down individual HSPs and analyzing viral production and infectivity. Our preliminary results suggest that HSPs play an important role in viral replication and infectivity.

We have also been studying the role of HSP70 binding protein; HspBP1, a co-chaperone molecule of HSP70. HspBP1 negatively affects the binding of substrate to HSP70 by accelerating nucleotide exchange of ATP domain. It has also been reported that HspBP1 antibody levels increase in the serum of HIV-1 infected individuals. HSP70 is associated with various phases of HIV-1 life cycle and HspBP1 can regulate various HSP70 activities; so it is worth studying the role of HspBP1 during HIV-1 infection, if any. Expression of HspBP1 was down-modulated during HIV-1 infection in T-cells. Furthermore, silencing of HspBP1 seems to increase HIV-1 gene expression whereas its over-expression leads to inhibition of HIV-1 replication. Our results also indicate that over-expression of HspBP1 significantly reduces LTR-driven gene expression while silencing enhances it. Our recent studies also suggest that HspBP1 might restrict viral replication by interacting with the LTR promoter. Further studies are in progress to elucidate the mechanism.

The major problem in defining Nef function has been its possible involvement in multiple pathways and its pleiotropic role in HIV-1 life cycle. Nef directed regulation of transcription can be best visualized when HIV infection is allowed to progress in presence or absence of Nef. Definite role of Nef in enhancing viral replication is evident from our results where Δnef HIV infected cells produced less virus as compared to WT infected cells. Genome wide comparison of gene expression in presence and absence of Nef in virus infected cells revealed perturbations in cellular transcriptome due to Nef. These differentially expressed genes were then subjected to transcription factor search and all such genes being regulated by specific transcription factors were identified. YY1 was found to be one such transcription factor which seems to regulate several differentially expressed genes in presence of Nef. Based on the previous preliminary studies in our lab and available literature about YY1's role in HIV-1 infection, it was an obvious choice to pursue further to study Nef's important role in HIV-1 transcription and pathogenesis. Our preliminary results indicate that Nef might inhibit YY1 mediated suppression of viral gene expression. Further characterization of the role of YY1 during HIV-1 infection is in progress.

Differential gene expression studies to identify molecular mechanism of HIV-1 induced T-cell apoptosis

Studies done till date to elucidate the pathways involved in HIV-1 induced T cell depletion has revealed that apoptosis underlie the etiology; however, a clear molecular understanding of HIV-1 induced apoptosis has remained elusive. Furthermore, HIV-1 infection is known to be associated with the hijacking of a number of cellular factors including the cell cycle associated molecules. The subversion of the host cell cycle during HIV-1 infection progression includes arresting of the normal cell cycle at mitosis, specifically at the boundary of the G2-M phase. Although many studies validate that Vpr and Vif is involved in causing the G2-M arrest associated with HIV-1 infection, not much information is available related to other viral and cellular protein interactions that might be crucial in the background. We have now initiated studies intending to look into the role of cell-cycle associated proteins in HIV-1 pathogenesis. In this direction, we have performed differential gene expression analysis using PCR array specific for cell cycle associated genes. Our results show differential gene expression of a significant number of cell cycle associated genes. One such identified gene, Cyclin-F, is being currently studied to identify its role in HIV infection.

Identification of novel molecules with anti-HIV activity and their potential for use as microbicides

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 anti-HIV therapeutic strategies. We have been also involved in identification of novel anti-HIV molecules and study of their potential use as microbicides. We have initiated screening of a library of pharmacologically active bio-molecules which are known to target cellular pathways for identification of novel anti-HIV molecules, with ultimate objective to identify novel cellular targets for inhibition of HIV-1. We have been successful in identifying several promising bioactive molecules from the screening of the library and further characterization of some of these molecules are currently in progress. Identification of such novel targets may lead us to a novel therapeutic strategy to inhibit the virus.

Future Research Plans

- Our results till date indicate that heat shock proteins play an important role during HIV-1 infection. We now intend to elucidate the role of individual heat shock protein isoforms in HIV-1 replication and pathogenesis, with specific reference to HSP70 and HSP40 isoforms. We are continuing characterization of several new Nef interacting host cell proteins identified previously, for their functional relevance in HIV life cycle. Identification of differentially expressed genes and their relevance to HIV induced cell death will be continued, with a focus on cell cycle associated proteins and autophagy. Finally, studies to identify novel anti-HIV molecules will be continued with the objective to identify novel lead molecules with cellular targets and molecules with potential for use as anti-HIV microbicides.

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In silico Analysis of Ebolavirus Genome Sequences and Glycoprotein Gene

Background

Ebola virus disease (EVD) is a severe haemorrhagic fever caused by ebolavirus (genus Ebolavirus, family Filoviridae) with a very high case-fatality rate ranging from 25 to 90%. EVD was first identified in 1976 with few cases restricted to sub-Saharan Africa followed by sporadic reports in the ensuing years. However, in 2014, WHO confirmed the worst EVD outbreak with heavy mortality and large morbidity. The ebolavirus genome is a linear, negative-sense, singlestranded, ~19 kb RNA containing seven protein-coding genes. The terminal ends of the genome have short non-transcribed 30 'leader' (Ldr) and 50 'trailer' (Tlr) sequences that contain the signals for replication, transcription and encapsidation. The genus Ebolavirus has five members namely, Taï Forest virus (TAFV; formerly Côte d'Ivoire), Reston virus (RESTV), Sudan virus (SUDV), Bundibugyo virus (BDBV) and Ebola virus (EBOV; member of Zaire ebolavirus species).

Ebola virus [EBOV; member of Zaire ebolavirus species] has caused at least 12 major outbreaks since 1976 including the recent epidemic of 2013-15. During the present epidemic a large amount of data has been generated by doing complete genome sequencing of EBOV isoles from several patients from geographically different locations. In the light of the rapid accumulation of genomic data, intelligible investigations of the sequence data are of importance and crucial to study the epidemiology of the virus.

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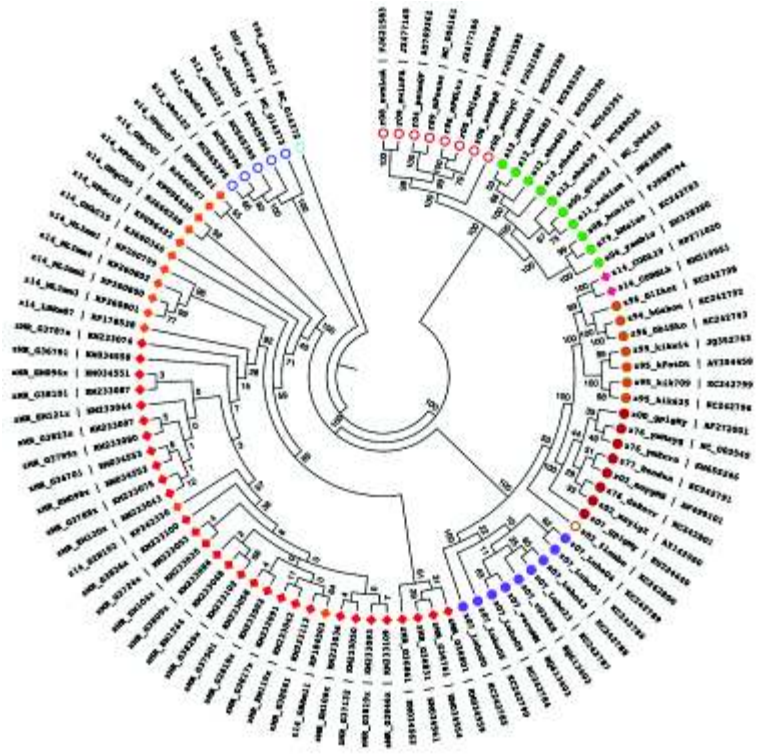

Fig. 1: Consensus phylogenetic tree of different genomes of ebolavirus isolates. Phylogenetic analysis was performed on aligned genomes of 94 unique hepta-CDS sequences; alignment was done using MAFFT v7.186. The aligned sequences were further analyzed by the 'seqboot.exe' (1,000 bootstraps), 'dnadist.exe', 'neighbor.exe' and 'consense.exe' programs of the Phylip v3.695 package. The tree shown is the consensus of a 1,000 randomly assorted bootstrap replicates.

Aims and Objectives

- To compare all complete genome sequences of ebolavirus available until 2014 to demonstrate the overall variation in the genome.
- To investigate the glycoprotein [*GP*]-gene and its three protein products for their sequence variation and glycosylation potential.

Work Achieved

As it is difficult to obtain the exact DNA sequence of the terminal ends of the ebolavirus genome, we have excluded the terminal non-coding 'Ldr' and 'Tlr' sequences in the present analysis. This study utilized the intermediate hepta-CDS region (~17.7 kb), which includes sequence from the start codon of the first gene (NP gene) till the stop codon of the rearmost 7th gene (L gene). An initial alignment of ebolavirus genomes showed that many genomes had identical hepta-CDS sequences. Such identical sequences may arise from close neighbors in a transmission chain and are meaningful in terms of temporal context in epidemiology. Inclusion of a large number of identical sequences in phylogenetic construction often leads to spurious results. Therefore, we have taken only 94 unique hepta-CDS sequences out of 169 ebolavirus genomes for phylogenetic analysis. The phylogenetic tree constructed using the hepta-CDS region for different ebolaviruses clearly indicated that various ebolavirus

members have split during evolution and isolated genetically from one another evolving separately.

The set of 113 genomes of Makona variant from the recent epidemic of 2013-15 in western Africa form a very close cluster and a separate clade with a common ancestor. Interestingly, this ancestor is also shared by the viral isolates from 2007 Luebo outbreak. Maximum divergence seen amongst the members of the Makona variant of 2014 is .001353 with corresponding 24 SNPs observed between the most distant isolates. Whereas, the combined set of genomes isolated from 43 Makona and 7 Luebo variants showed a maximum divergence of .028525, with a corresponding 483 SNPs amongst them.

The ebolavirus GP-gene contains a polyadenosine stretch, which is susceptible to transcriptional slippage. Unedited transcripts (seven adenosine residues at the editing site) of the GP-gene, encode a soluble form of the glycoprotein (sGP, 364 amino acids in EBOV). The unedited transcript (þ1 Frame) encodes sGP protein containing 364 amino acids in EBOV, 365 amino acids in TAFV, 366 or 373 amino acids in BDBV and 372 amino acids in SUDV. In EBOV, the stop codon for sGP is either 'taa' or 'tga'. However, the 1,093rd residue (1st residue of the stop codon) is mutated (t1093c in TAFV and BDBV; t1093a in SUDV) resulting in the variable length of sGP protein. When the slippage occurs in the +2 frame, the 886th adenosine residue is not transcribed (1..885, 887..895) encountering a stop codon prematurely, leading to an early termination of transcription. This mRNA translates to a smaller ssGP protein with a highly variable length of 297 (EBOV), 302 (TAFV and BDBV), 318 (SUDV) and 331e395 (RESTV) amino acids (Table 2). When the slippage occurs in -1 frame, with the 885th adenosine residue transcribed twice (1..885, 885..2030), a 2031 bases mRNA is formed, which encodes for 676 (¼ 295 þ 381) amino acid long protein (preGP or GP1,2; or virion spike protein). This membrane form of GP has a constant length of 676 amino acids in all non-RESTV members (RESTV preGP: 677 amino acids). Each monomer of preGP protein contains two subunits, GP1 and GP2. The GP1 subunit contains the core of the glycoprotein, its receptor binding domain, a glycan cap, and a large mucin-like domain. Although there are 43 homologs for GP-gene at DNA sequence level, the resulting protein homologs are 38 for preGP (GP1,2), 27 for sGP and 26 for ssGP proteins. In the case of Luebo 2007 isolates, the three GP-gene representatives encode identical GP proteins.

This study includes genome sequences reported for isolates from most outbreaks and geographical regions. The phylogeneticanalysis performed using automated alignment and SNP-based manual alignment showed that hepta-CDS based analysis yields proper linkage between different isolates showing a spatial variation among the different members of ebolavirus. Nonetheless, the EBOV isolates fall correctly into separate clades, not only with respect to their geographical location, but also with respect to their temporal origin of outbreak. The variants from each outbreak, namely Yambuku (1976), Kikwit (1995), Gabon (1996), Luebo (2007) and the latest Makona (2013e15) variants, group into distinct clades.

A recent study showing the presence of virus in the ocular fluid from an EVD recovered patient indicated the viral existence inunanticipated tissues of the human and animal body. Therefore, EVD demands a constant surveillance by isolation or molecular detection of ebolavirus from different tissues of humans and animals residing in geographical niches that are hotspots for EVD.

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Studies on murine Experimental Cerebral Malaria

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Background

Malaria affects approximately one-half of the global population, which lives in malaria-endemic regions, and it has been reported that there are about 4.5 million new cases of malaria reported every year, with more than 5 lakhs succumbing to the disease. Earlier reports indicated that infection with the parasite, *Plasmodium falciparum*, leads to neurological impairment in malaria patients. It has been hypothesized that differential gene expression contributes to phenotypic variation of parasites, which results in specific interactions with the host, leading to different clinical features of malaria. The mechanisms underlying the heterogeneity of clinical malaria remain largely unknown. Studies in children with *P. falciparum* asymptomatic cases showed lower levels of hemoglobin, lymphocyte and platelet counts, high monocyte counts, and the absence of cells producing inflammatory cytokines. *P. falciparum* asymptomatic malaria was found associated with increased TNFRII expression on T regs and lower levels of Th2 cytokine in children and these conditions might help parasite survival in malaria asymptomatic cases. However, other reports state that no such relationship between T reg cells and symptomology was observed and suggested that other immune mechanisms are involved in the asymptomatic *P. falciparum* cases. It has been reported that *Plasmodium* berghei ANKA (PBA)-infected mice (5dpi) showed anxiety symptoms, histopathological alterations in the brain and secretion of proinflmatory cytokines IL-1β and TNF-α. Further, it has been found that murine ECM animals showed significant impairment of object-recognition and loss of visual memory (7dpi). The cognitive dysfunction correlated with hemorrhage and inflammation-associated morphological changes detected throughout the

brains of infected mice. The present study focus on parasitemia, histopathological changes and serum cytokines levels in different phases of ECM-developing C57BL/6 mice infected with *P. berghei* (ANKA) parasites.

Aims and Objectives

- Studies on host-parasite interactions.
- Studies on murine malaria pathogenesis.
- Studies on the effect of immune modulators on ECM.
- Studies on parasite-macrophage interactions.

Work Achieved

The C57BL/6 mice and *P. berghei* ANKA murine malaria model is a well-accepted animal model for studying Cerebral Malaria (CM) as it shares many common pathological features with human CM. We infected C57 mice with 10^6 PBA parasites for Experimental Cerebral Malaria (ECM) studies. More than 90 percent animals succumbed to ECM on Day 6/7 after infection in most of the experiments. The remaining animals developed severe malaria and died after 3-4weeks with high parasitemia (>15%). The ECM-developing mice were without any symptoms for 3-4 dpi (asymptomatic). These animals became symptomatic, with ruffed fur, hunching and dullness, on 4-5 days after infection. The animals became paralyzed, developed coma and become moribund 6-7 dpi, and died with typical ECM-like characters. Parasitaemia was first detected on day 2. The parasitemia levels were around 2 % on day 3, when all the animals were normal, without any symptoms and as active as controls. The animals became weak with ruffled fur and slight hunching symptoms on day 4-5 (dpi), and had parasitemia between 3 -5 percent. The animals that died with typical ECM had 8-10 percent parasitemia on day 6 & 7. The animals which survived for more than 15 days had >15 percent parasites. The brain histopathological studies of C57BL/6 mice infected with PBA did not shown any detectable malaria-parasitized red blood cells (pRBC) in the tissue of animals 1 day and 2 days post-infection, and no changes in the histology were observed, as compared to naïve animals. However, 2 days post-infection, the animals showed pRBCs in the blood vessels. 3-days post-infection, the animals showed some pRBCs, with mild alteration in the brain tissue.

More pRBCS were found in the brain tissue 4 & 5 days after parasite infection. Dilated /congested blood vessels with parasites were detected in the cerebrum/cerebellum with odema. Day 6 and 7-post infection, the ECM animals showed a further increased in pRBCs, collapsed blood capllaries,

odema and multifocal hemorrges. One day post-infection, the spleen tissue in parasite-infected mice showed normal distribution of red and white pulp with no changes in the tissue, as compared to controls. However, some changes in the white and red pulp were observed 3 days post-infection. The spleen tissue of these animals showed an increase in the red pulp with pigmented macrophages. White pulp with pRBCs was markedly increased in the animals 5 days post-infection. The white pulp was prominent and the red pulp increased markedly. The spleen tissue of these animals showed a lot of malaria pigment in the red pulp, mainly within macrophages. The tissue sections show pigmented hemorrhages. The gross color had changed to black. The size of the spleen was grossly increased because of overall enlargement of the red pulp. The liver tissue in infected animals without symptoms showed no changes in the tissue architecture and no parasites in the tissue sections 1 and 2 days post-infection. 3 days post-infection, infected animals showed the presence of parasites and mild inflammation of tissue. Infected mice showed enlarged livers loaded with malaria pigment 6 & 7 days post-infection. The tissue of these animals showed large vessels contained malaria pigment. No changes were observed in the lung tissue samples of infected mice 1 and 2 dpi, as compared to controls. But pRBCs were observed in the tissue of animals 3 dpi, without any tissue changes. An increased in parasites was observed in the animals 4 & 5 dpi, but not much change was observed in the tissue histology. Lymphocyte accumulation was seen in the tissue. A large increase was observed in the pRBCs with pigment-hemozoin deposits and congested-dilated blood vessels, in infected C57BL/6 mice, 6 and 7 dpi.

Serum cytokines were estimated in PBA-infected ECM-developing C57BL/6 mice using the CBA kit. These animals showed higher levels (5 fold increase) of interferon gamma 4 & 5 days post infection, as compared to 1-3 days post-infection. Similarly, the animals also showed increased cytokine levels 6 & 7 days post-infection. Interleukins-6 and IL-10 levels were found to be very high in ECM mice, as compared to non-ECM mice. TNF-α, a proinflammatory cytokine, increased gradually from day 1 post-infection to ECM development in the mice. Levels of IL-2, IL-4 and IL-17 cytokines in the mice developing ECM were very less and differences between infected and control mice were non-significant.

Future Work

- Studies on interactions of parasitized RBCs with macrophages.
- *In vivo* antimalarial screening.

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Identification of novel potential biomarkers for breast cancer towards early diagnosis and prognosis using proteomic and metabolomic approaches

Background

In women, breast cancer is the most common malignancy and the second most common cause of cancer-related mortality. It accounts for 23% of all the cancer cases and 14% of all the cancer deaths. Breast cancer incidences in India are increasing at a rate of 3% per year and by 2020 the cases may increase to 100,000. Three subtypes of breast cancer are identified ER and PR positive, HER2 positive and triple negative. As yet very few drugs are available for breast cancer, which remains a major cause of morbidity and mortality in women. Early diagnosis of breast cancer improves the likelihood of successful treatment and can save many lives. However, current techniques like mammography to detect breast tumor has intrinsic limitations. There is an urgent need to discover novel biomarkers of breast cancer for early detection and diagnosis. Quantitative proteomic and metabolomic profiling of body fluids, tissues, or other biological samples to identify differentially expressed proteins/metabolites represents a very promising approach for discovering novel potential biomarkers. Proteins and metabolites associated with breast cancer identified through proteomic and metabolomic profiling technologies could be useful as biomarkers for the early diagnosis, assessment of prognosis, prediction of therapeutic effect and treatment monitoring. In this work, we plan to identify novel potential biomarkers for breast cancer using high throughput mass spectrometry based proteomic and metabolomic approaches in Indian scenario. In addition, identified biomarkers will be subjected to bioinformatic tools to understand the association of various physiological pathways.

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Aims and Objectives

- Identifying novel potential biomarkers for Luminal B HER2 positive and HER2 enriched breast cancer subtypes using proteomic approaches.
- Identification of non-invasive urinary proteomic biomarkers in breast cancer using multipronged quantitative proteomic approaches.
- Metabolomic profiling towards novel potential biomarkers for breast cancer.

Work Achieved

Identifying novel potential biomarkers for Luminal B HER2 positive and HER2 enriched breast cancer subtypes using proteomic approaches

Although biomarker discoveries through various proteomics approaches have been studied in breast cancer, a limited number of studies have explored the invasive ductal carcinoma (IDC) with Luminal B HER2 positive (LB) and HER2 enriched (HE) subtypes. In this study, we investigated to compare the tissue proteome profiling of LB and HE subtypes against normal breast tissue in order to identify differentially expressed proteins which are unique to these two subtypes. To achieve this goal we used high-throughput quantitative proteomic approaches such as 2-D DIGE (gel-based approach) and iTRAQ (gel-free approach) in combination with LC-MALDI-TOF/TOF. Representative 2-D DIGE images of control vs. LB and control vs. HE are shown in Fig. 1A and 1B. A total of 67 differentially expressed proteins were identified using 2-D DIGE and iTRAQ approaches. Further, we also identified 69 differentially expressed proteins that could discriminate early and late stages using similar proteomic approaches.

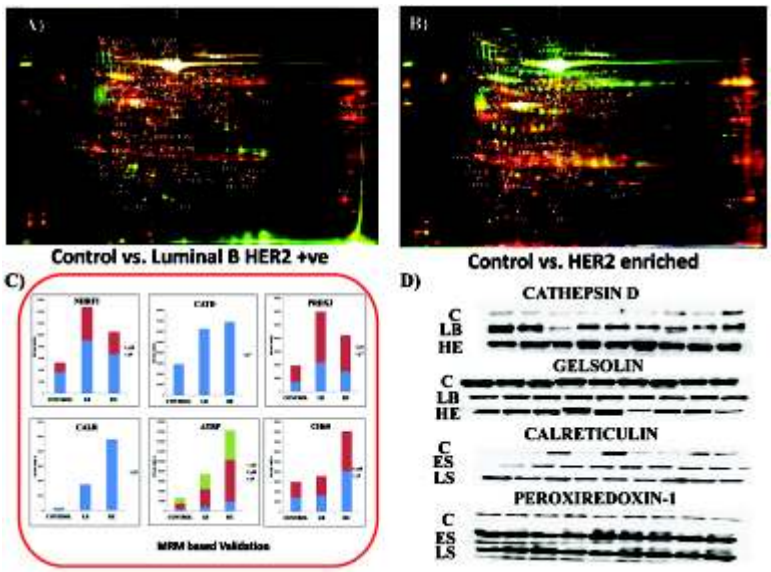

Fig. 1: Representative 2-D DIGE images **A)** control vs. LB, and **B)** control vs. HE, Validation of selected differentially expressed proteins using **C)** LC-MRM-MS/MS and D) Western blotting.

The identified differentially expressed tissue proteins were subjected to functional pathway analysis for better understanding of the biological context of the identified proteins and their involvement in various physiological pathways. Functional pathway analysis suggested the modulation of multiple physiological pathways including ATP synthesis, blood coagulation, cytoskeletal regulation by Rho-GTPase, glycolysis and cadherin, EGF, FAS, FGF and integrin signaling pathways. Multivariate statistical methods like PCA, OPLS-DA shown clear discrimination between control and subtypes. Validation experiments using western blot and MS based MRM in a separate cohort of patients revealed that HSP90, EF1A1, Cathepsin D, Gelsolin, and PRDX3 can be considered effective tissue markers which can discriminate LB and HE subtypes (Fig. 1C and 1D). For early and late stage diagnosis Calreticulin, Transferrin, CAPG, HSP60, PRDX1 proteins seemed promising.

Identification of urinary proteomic biomarkers in breast cancer using multipronged quantitative proteomic approaches.

Biofluids such as urine may serve as an excellent non-invasive source of easily available protein markers for cancer diagnosis. In the present study, we aim to investigate such protein markers in early grades of breast cancer urine using 2-D DIGE, iTRAQ and label free approaches. Representative 2-D DIGE images of control vs. malignant and control vs. benign including hierarchal clustering analysis is shown in Fig. 2. A total of 78 differentially expressed proteins were identified using multipronged proteomic approaches that could discriminate healthy and malignant patients. Gene ontology analysis suggested the modulation of multiple physiological pathways including blood coagulation,

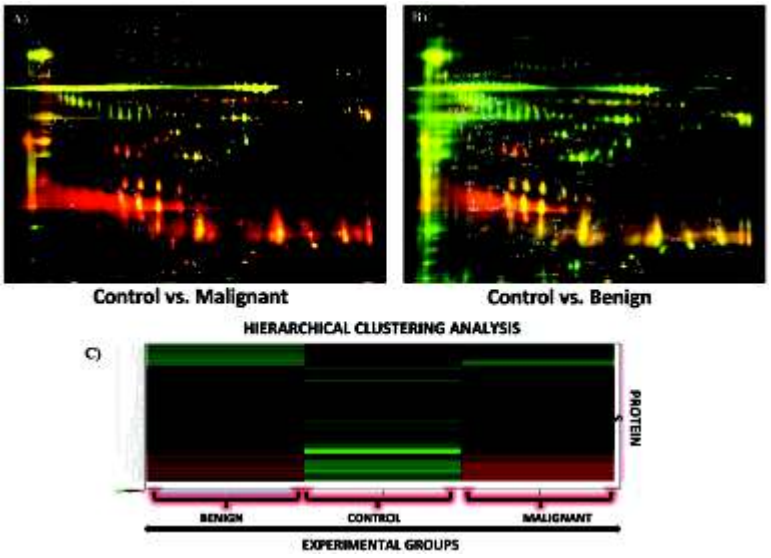

Fig. 2: Representative 2-D DIGE images **A)** control vs. Malignant, **B)** control vs. Benign, **C)** Hierarchical clustering analysis of breast cancer urine proteome using DeCyder extended data analysis for protein vs. experimental groups.

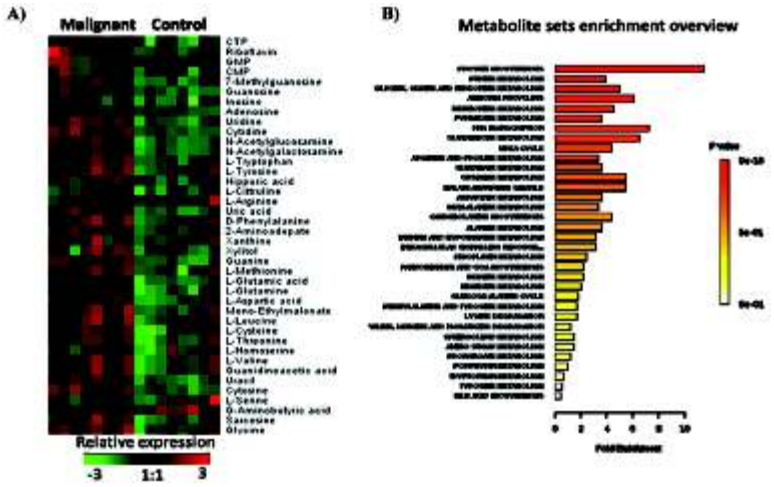

Fig. 3 : A) Heat map constructed based on values obtained in serum and tissue samples of breast cancer and normal. B) Pathway enrichment analysis performed using Metaboanalyst.

glycolysis, cadherin, Wnt, Toll receptor, EGF, FAS, TGF-beta, integrin signaling pathway. Multivariate statistical analysis exhibited clear discrimination between control and malignant samples. Validation experiments using western blot and MS based MRM in a separate cohort of patients brought out Clusterin, Gelsolin, GM2A, ZAG, LGALS3, ANXA1, Cathepsin B, and Transferrin to be a panel of potential urinary markers which can discriminate healthy controls and breast cancer patients.

Metabolomic profiling towards novel potential biomarkers for breast cancer

Although there is a lot of research done in the field of genomics and proteomics in breast cancer, metabolomics is less explored. In this study, we have performed a comparative analysis of metabolome of normal, benign and malignant serum and tissue samples for the understanding of the molecular events involved in tumor development that are essential for early detection and diagnosis. We followed two approaches for metabolic profiling, untargeted approach using NMR and GC-MS as well as targeted approach using LC-MS. We applied multivariate statistical analysis on data obtained from these three techniques and observed that Adipic acid, 2-Aminoadepate, L-Methionine, L-Cittruline, Propanoic acid, Allantoin, Ectoine, 11-trans Octadecenoic acid, Guanine were significantly up-regulated and L-Arginine, N-Acetyl-D-glucosamine, L-Glutamic acid, L-Glutamine, 8-Aminooctanoic acid were significantly down regulated in malignant patients compared to healthy individuals and benign samples. OPLS-DA analysis showed a trend for supervised separation between breast cancer malignant, benign and healthy control categories. Heat map is constructed based on values obtained in serum

and tissue samples of breast cancer and healthy controls (Fig. 3A). Pathway enrichment analysis performed using Metaboanalyst showed that Purine metabolism, Pyrimidine metabolism and Glycine, Serine and threonine metabolism pathway were among significant pathways (Fig. 3B).

Future Research Plans

- Validation of differentially expressed proteins in a large cohort of patients using western blotting and MRM based LC-MS/MS.
- Identification of phospholipids involving in breast cancer using lipidomic approach.
- Integration of metabolomic data with proteomics data for understanding the various physiological pathways and disease pathogenesis using bioinformatics.

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The role of T-cells and non- T cells in anti-leishmanial immunity

Background

Intracellular parasites like Leishmania manipulate the host cell signaling to promote their survival within the cells. Macrophages act as both host to and sentinels against Leishmania parasites and play a vital role in establishment or elimination of infection. We have shown earlier that instead of abrogating the host cell signaling completely, these intracellular parasites manipulate CD40 and TLR2 signaling in macrophages to shift the module from the anti-parasitic p38-IL12 pathway which leads to a Th1 response to the pro-parasitic ERK 1/2-IL10 module which results in a Th2 bias and establishment of infection. Thus, the antigen presenting cells play a key role in establishing the type of T-cell response that would lead to either disease progression or parasite elimination.

Aims and Objectives

- The aim of our project was to examine the role of macrophage differential signaling in *Leishmania major* infection and the corresponding T-cell response.

Work Achieved

Leishmania major is a parasite that resides and replicates in macrophages. We previously showed that the parasite enhanced CD40-induced Raf-MEK-ERK signaling but inhibited PI3K-MKK-p38MAPK signaling to proleishmanial effects. As Raf and PI3K have a Ras-binding domain but exert opposite effects on Leishmania infection, we examined whether Ras isoforms had differential roles in Leishmania infection. We observed that L. major enhanced N-Ras and H-Ras

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expression but inhibited K-Ras expression in macrophages whereas only N-Ras activity was enhanced by the parasite. TLR2 short hairpin RNA or anti-TLR2 or anti-lipophosphoglycan Abs reversed the L. major–altered N-Ras and K-Ras expressions. Pam3CSK4, a TLR2 ligand, enhanced N-Ras expression but reduced K-Ras expression, indicating TLR2-regulated Ras expression in L. major infection. Because reduced N-Ras expression or activation resulted in host protection, its role in Ag-specific T-cell response was examined. Therefore, we cloned, expressed, and purified L. major MAPK10 (M10). Peritoneal macrophages from CD40+/+ mice were treated with the lentivirally expressed N-Ras shRNA or control shRNA, pulsed with M10, and injected s.c. in the hind footpad of CD40-deficient (CD40-/-) mice. Treatment of mice with N-Ras shRNA but not the control shRNA resulted in increased IFN-κ and decreased IL-4 production from the M10-treated lymph node cells and higher M10-specific IgM but lower IgG1 and insignificant IgG2a production indicating N-Ras regulation of Ag-specific immune response. We observed that priming of CD40-/- mice, which were more susceptible to Leishmania than were CD40+/+ mice, with the N-Ras shRNA-treated, M10-pulsed macrophages resulted in significantly lower parasite burden and higher M10-specific IFN-ϒ production and Th1 response than for the CD40-/- mice that received the control shRNA-treated, M10-pulsed macrophages, demonstrating a novel N-Ras isoform–targeted host-protective immunoprophylaxis. Treatment of macrophages with lentivirally expressed N-Ras shRNA but not control shRNA reduced CD40-induced phosphorylation of ERK-1/2 but augmented phosphorylation p38MAPK, accompanied by enhanced antileishmanial effects of CD40. However, K-Ras and H-Ras silencing enhanced the infection both in macrophages in vitro and in C57BL/6 mice. As N-Ras is activated by Sos, a guanine nucleotide exchange factor, we modeled the N-Ras–Sos interaction and designed two peptides from their interface. Both the cell-permeable peptides reduced L. major infection in BALB/c mice but not in CD40-deficient mice. These data reveal the L. major–enhanced CD40-induced N-Ras activation thereby leading to anti-inflammatory IL10 production and Th2 response as a novel immune evasion strategy.

Future Research Plans

- To examine the role of Ras isoforms in antileishmanial immunotherapy and mmunoprophylaxis.

Report of DST-INSPIRE Faculty from
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Project: Analysis of the CD40 signalosome complex built-up in macrophages

Background

Receptor-ligand interactions are the basis of most cellular processes. CD40 is a costimulatory receptor expressed on the surface of many cells of the innate and adaptive immune systems including macrophages and B lymphocytes. CD40-CD40 ligand interactions are known to play crucial role in the maintenance of immune homeostasis during invasion of pathogens. In case of infection by intracellular parasites like Leishmania CD40-CD40L interaction and eventual downstream signalling play a determining role in the establishment or elimination of infection. It has been shown that the CD40 receptor can signal from two different membrane domains, namely the detergent resistant (DRM) or rafts or the detergent soluble (DSM) non-raft regions leading to distinct effector functions in macrophages. Such differential signalling of CD40 is a function of strength of its ligand binding. The signalosome complexes thus formed are also believed to be constitutively different. However, the mechanism of built up of the CD40 signalosome in macrophages as a function of dose and duration of ligand binding is yet unclear and needs to be elucidated.

Aims and Objectives

- Elucidating the identity of the signalling molecules involved in the building up of the CD40 signalosome complex.
- Determining the kinetics of recruitment of the intermediates as a function of strength and duration of ligand stimulation.

Collaborators

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Dr. Bhaskar Saha, *Scientist G, NCCS, Pune, India*

Work Achieved

We have completed standardization of the procedure for identification of signalling intermediates using LC-MS/MS. The identity of the intermediates recruited at different time points after stimulation with three different doses CD40L is being determined at present.

Future Research Plans

- Validation of the identified intermediates by western blotting.
- Identification of role of different signalling intermediates by analyzing their expression profile as a function of dose and time.

Publications

Mukherjee D, Ghosh AK, Dutta M, Mitra E, Mallick S, Saha B, Reiter RJ, Bandyopadhyay D. Mechanisms of Isoproterenol-induced cardiac mitochondrial damage- protective actions of melatonin. **J Pineal Res. 2015; 58: 275-290**.

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Understanding the molecular basis of viral complement regulation

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Background

The complement system is the central component of innate immunity that controls invading pathogens directly - by lysis or inactivation, and indirectly - by recruiting and boosting the pathogen-specific adaptive immunity. Although the system efficiently targets the invaders, it is equally deleterious to the host cells and therefore, an effective regulation is needed to control complement activation on the cell-surface. This regulation is primarily achieved by a family of proteins termed regulators of complement activation (RCA), which are located on the cell-surface such as decay-acceleration factor (DAF; CD55), membrane cofactor protein (MCP; CD46) and complement receptor 1 (CR1; CD35) and in solution like factor H (FH) and C4b-binding protein (C4BP). The RCA proteins are formed by 4 to 59 complement control protein (CCP) modules, that are separated by small linkers, and regulate complement by inactivating C3-convertases by two distinct mechanisms dubbed as 'cofactor activity' and 'decay-accelerating activity' which work in concert to achieve the robust regulation. It is therefore not inexplicable that mutations and polymorphisms in RCA proteins are linked to various diseases like age-related macular degeneration (AMD), atypical hemolytic uremic syndrome (aHUS) and dense deposit disease (DDD).

The large DNA viruses such as herpes and poxviruses have a proclivity for gene piracy. It is therefore not surprising that these viruses have pirated genes that encode for proteins structurally similar to the human RCA proteins. Our laboratory is utilizing these viral complement regulators as model proteins to further probe the molecular mechanism underlying the complement

regulatory mechanisms – the cofactor activity and the decay-accelerating activity.

Aims and Objectives

- To understand the molecular basis of complement regulation of viral complement regulators.
- To understand the in vivo functioning of viral complement regulators.

Work Achieved

Previously our laboratory has demonstrated that Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) encodes a structural and functional mimic of the human RCA protein and like human RCA proteins regulates complement through its decay-accelerating activity and cofactor activity. We named this protein as Kaposica. In the present exercise we have utilized Kaposica as a model protein to probe the molecular mechanism underlying the cofactor activity.

Molecular basis of cofactor activity of Kaposica

Structurally Kaposica is composed of four CCP modules followed by a serine/threonine (S/T)-rich region and a transmembrane domain for membrane attachment, wherein regulatory activities are provided by the CCP modules. Thus, as a first step towards understanding the structural requirements for its C3b cofactor activity, we sought to map its CCPs critical for this activity. Swapping of its CCP modules with that of the human DAF (CD55), that lacks cofactor activity revealed that the central domains (CCPs 2-3) of Kaposica are critical for providing this activity. Next, we pursued to determine the region(s) within modules 2 and 3 that are decisive for this activity. Each CCP module contains four invariant cysteines therefore, to localize the critical regions within the CCPs, we swapped the inter-cysteine regions of modules 2-3 of Kaposica with the homologous regions of DAF. Of the six mutants generated, two almost completely lacked the C3b cofactor activity, while others retained the activity. The inter-cysteine regions that were swapped in these mutants were the ones adjacent to module 2-3 linker suggesting that these regions are most critical for the cofactor activity.

Having localized the inter-cysteine regions critical for the cofactor activity of Kaposica, we next followed the identification of functional residues within these regions by Ala substitution mutagenesis of the surface exposed residues. Since three-dimensional structure of Kaposica is not available, we built a homology

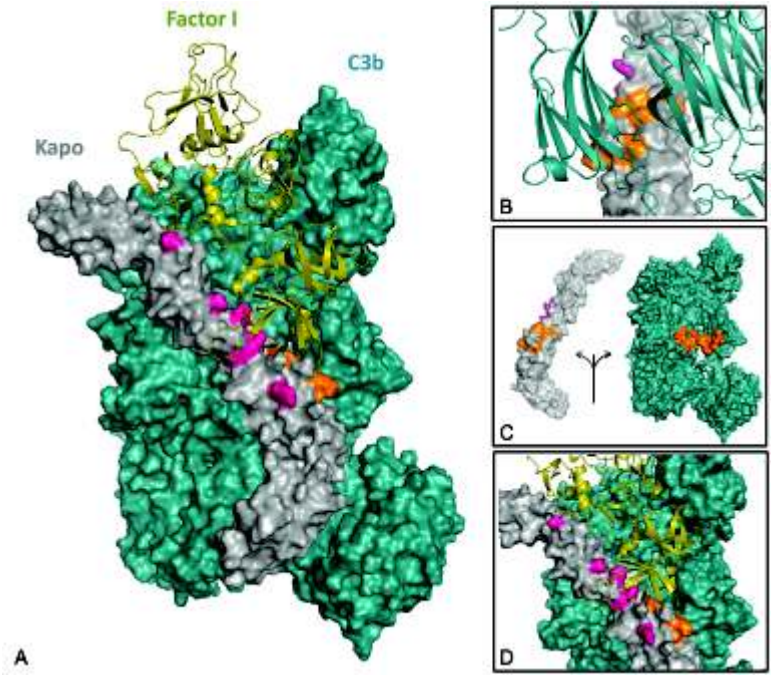

Fig. 1: Mapping of functional sites in C3b: (Kapo): Factor I trimolecular complex. **(A)** Model of C3b:Kapo:FI tri-molecular complex. Kapo model was superimposed with the coordinates of FH in C3b-FH1-4 structure (2WII) and then factor I was docked onto the C3b-Kapo structure to generate the ternary complex. C3b (Cyan) and Kapo (Gray) are represented by solid surface, while factor I is represented by cartoon (olive). Residues of Kapo that affect its activity are labelled in orange and pink colours. **(B)** Zoomed view of C3b interaction sites (orange) of Kapo. C3b domains are represented by cartoon (cyan). **(C)** Footprint of Kapo interaction sites on C3b. The footprints are seen in MG2 and CUB domains in C3b. **(D)** Inset view of factor I contact sites (pink) of Kapo.

model of this molecule using the structure of murine complement regulator Crry as the template and employed it to identify the surface exposed residues. This exercise led to the identification of 25 residues in the functionally critical inter-cysteine regions of CCP 2 and CCP 3 which were then selected for mutagenesis. In addition, we also selected a few other residues outside to these regions based on our and others previous mutagenesis data. Evaluation of the Ala substitution mutants for C3b cofactor activity showed significant loss in eight mutants.

Because cofactor activity is a summation of interaction between Kaposica and C3b as well as Kaposica and factor I, we measured direct binding of the mutants to C3b using surface plasmon resonance to determine whether reduction in the cofactor activity of the substitution mutants is owing to decrease in their ability to bind to C3b. We observed that four out of the eight mutants that displayed reduced C3b cofactor activity also showed substantial reduction in C3b binding. It is therefore apparent that residues mutated in the above mentioned four mutants are involved in binding to C3b. Further, it may also be inferred that the residues that are vital for the cofactor activity but do not participate in binding to C3b, are likely to be involved in factor I interaction.

Next, to obtain a detailed view of the interactions of vital residues with C3b and factor I, we mapped the functionally important residues of Kaposica in the modelled structure of C3b-Kaposica-factor I complex (Fig. 1A). We observed

that as expected, the C3b interacting residues of Kaposica were present at the C3b-Kaposica interface (Fig. 1B) while the C3b non-interacting residues made contact with factor I (Fig. 1D). Interestingly, mapping of the footprints of C3b interacting residues of Kaposica onto C3b showed that these residues interact with CUB and MG2 domains of C3b (Fig. 1C) suggesting that bridging of these two domains of C3b by Kaposica is critical for its cofactor activity.

Viral and human RCA proteins employ a common mechanism to inactivate complement

Because viral RCA proteins are structural and functional mimics of the human RCA we hypothesized that human and viral RCA proteins may inactivate C3b by a common mechanism. To test this, we determined whether cofactor activity can be incorporated into human DAF (lacks cofactor activity) by swapping DAF inter-cysteine regions with MCP (possesses cofactor activity) inter-cysteine regions homologous to that found to be crucial for the activity in Kaposica. The results showed that the chimera indeed gained the ability to inactivate C3b, suggesting that the inter-cysteine regions found critical for imparting cofactor activity in Kaposica are also critical for imparting this activity in MCP.

Based on our data we propose that the bridge formed by CCP3 of Kaposica between MG2 and CUB holds the later in a proper orientation with the core of the molecule which then allows its efficient cleavage by factor I. And that, both viral and human RCA proteins employ a common mechanism for imparting the cofactor activity.

Future Research Plans

- Fine mapping of functional sites in Kaposica critical for its decay-accelerating activity.
- Crystallization of vRCA molecules – alone as well as in complex with target proteins.
- Role of complement during viral infections.

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Protein phosphatase 1 regulatory subunit p90 functions as a tumor suppressor

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Background

Cancer is one of the foremost causes of death worldwide. It manifests due to uncontrolled proliferation of genomically unstable abnormal cells. These are generated due to the transformation of normal cells and it happens either due to the activation of proto-oncogenes and/or inactivation of tumor suppressor genes. In many instances, kinases play pivotal role in accelerating malignant transformation. Furthermore, phosphorylation of these kinases (oncogenes) is essential for promoting malignant transformation. Dephosphorylation of these kinases is an important step to prevent their oncogenic activity. Therefore, dephosphorylation of malignancy promoting kinases by phosphatases may have important role in controlling malignant transformation.

The protein phosphatase 1 (PP1) is a serine/threonine phosphatase and plays an integral role in the regulation of a number of major signaling pathways. Deregulation of PP1 is associated with many human diseases like cancer. It is ubiquitously expressed in all eukaryotic cells and is involved in a wide range of cellular processes, including meiosis and cell division, apoptosis, protein synthesis, metabolism, cytoskeletal reorganization, and the regulation of membrane receptors and channels. Each functional PP1 enzyme consists of 2 parts; one catalytic subunit having the potential of dephosphorylating specific substrates and other is the regulatory subunit which governs the substrate specificity. The specificity and activity of PP1 is mainly regulated through its interaction with number of regulatory subunits. Human genome encodes approximately more than hundred regulatory subunits. Regulatory subunits dictate the substrate specificity for the protein phosphatase complex. These

regulatory subunits may associate with the PP1 catalytic subunit to specific subcellular compartment, modulate substrate specificity, or serve as substrates themselves. Thus, the interactions between the catalytic subunit and specific regulatory subunits are central to the functions of PP1.

Though the human genome codes for approximately 100 regulatory subunits, however, there is limited knowledge about most of the regulatory subunits. Recently, CCDC8/p90 has been shown to be an important regulatory subunit of PP1 and is deleted in human glioma. It is also found to be epigenetically silenced in renal cell carcinoma and its expression is associated with 3M syndrom. The above observations suggested that p90 may have function in malignant transformation; however the exact role of p90 in cancer and its mechanism of action are yet to be known. In this study, we sought to look for the biology of p90 in cancer using breast cancer as a model system.

Aims and Objectives

- Is p90 a potential Tumor SuppressorΔ
- What is the molecular mechanism of functionΔ
- What is physiological role of p90Δ
- How cellular expression of p90 is regulatedΔ

Work Achieved

Is p90 a potential Tumor Suppressorκ

Last year we reported the tumor growth suppressor activity of p90 by overexpressing it in breast cancer cell lines MCF7 and MDA-MB-231. Our cell counting data showed that p90 significant suppressed the growth proliferation of both MCF7 and MDA-MB-231. The growth suppressive activity of p90 is further supported by colony formation assay. Results taken together suggested that p90 has growth suppressive activity. Further, we checked the growth suppressive activity of p90 in vivo using NOD-SCID mice and found that p90 overexpression leads to significant reduction of tumor size as compared to vector control overexpressing cells.

This year, we have studied the effect of p90 on wound healing activity. Wound healing assay measures the potential of any gene to prevent or promote the migration ability of the cells. Migration of the cells is further associated with the metastatic potential of the malignant cells. We overexpressed p90 in MDA-MB-231 cells for 48 hrs and then generated a scratch on the plate by tips edge and

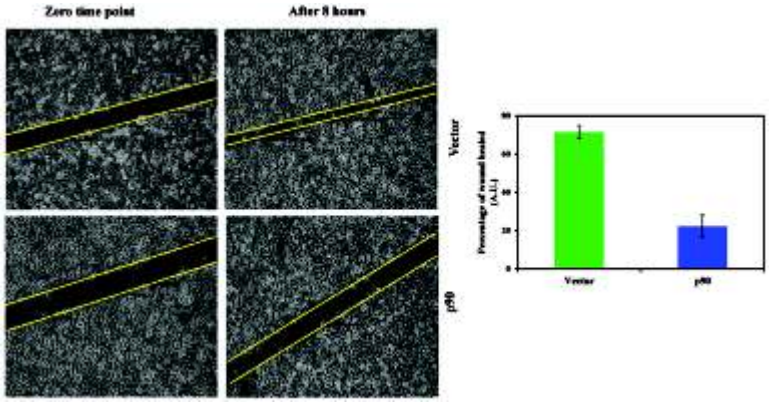

Fig. 1: p90 inhibits cell migration: Measurement of cell migration by wound healing assay. MDA-MB-231 cells were infected with lentiviral vector and p90. Wound healing assay was performed (left panel) after 24 hours of infection and quantitative data were presented in the right panel.

then measured the width of the sratch by live cell imaging technique. We found that overexpression of p90 leads to significant suppression of wound healing Fig. 1. This result further supported by our previous findings that p90 may be a putative tumor suppressor for breast cancer.

What is the molecular mechanism of functionκ

Last year we showed that p90 suppressed the growth by induction of apoptosis using multiple techniques such as FACS and immunoblotting. FACS data analysis demonstrated that population of sub-G1 phase was increased upon p90 overexpression. Further, we showed that p90 overexpression led to induction of known proapoptotic proteins like Bax, Puma. To further understand why p90 induces apoptosis, we observed that p90 overexpression promotes dephosphorylation of MEK, and ERK kinase and thereby inactivated the growth promoting MAPK kinase pathway. Then we ask whether p90 interacting with the components of MAPK pathway. Our results demonstrated that p90 specifically interacts with ERK Fig. 2A. These results taken together suggested that p90 inactivate MAPK kinase pathway through interaction with components of MAPK kinase pathway. To get further insight, we generated stable p90 knockdown MCF7 cells using short hairpin RNA and examined the phosphorylated level of ERK. Results showed that p90 knockdown cells have moderate elevated level of phospho ERK as compared to the normal cells Fig. 2B. This further confirmed the involvement of p90 in the regulation MAPK kinase pathway under physiological condition.

Further, we analyse the patient samples of different grades of breast cancer. We prepared the whole cell lysates of patient samples and check the expression level of proteins by immunoblotting. We found that there is a good inverse correlation between p90 expression and activation of ERK activity in the patient samples (Fig. 2C). This inverse correlation is more profoundly observed in

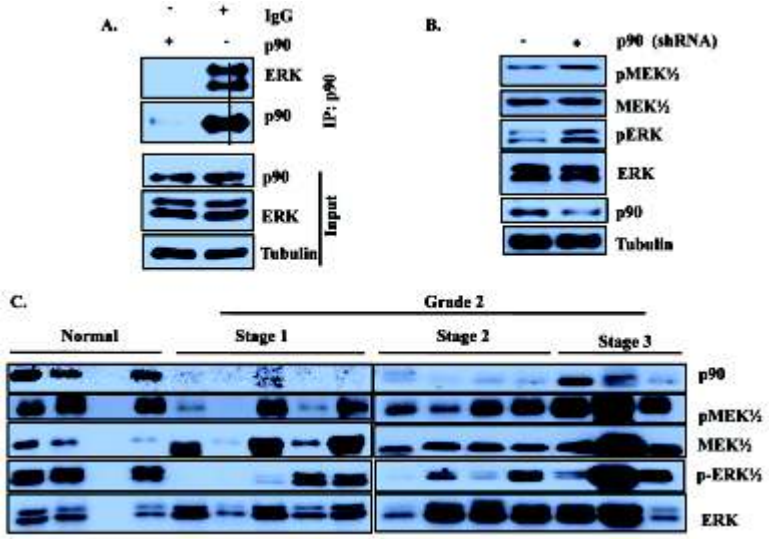

Fig. 2: p90 specifically interacts with ERK to regulate the MAPK-ERK pathway **A)** p90 specifically interacts with ERK. **B)** Expression levels of MAPK-ERK pathway components in p90 stable knock down cells. **C)**.Relative expression level of p90 and MAPK-ERK components in human breast cancer patient samples.

higher grades of cancer patient samples. Results taken together suggest that there is good correlation between patient samples and *in vitro* data.

What is physiological role of p90κ

Our results demonstrated that p90 may be a putative tumor suppressor. Most of the tumor suppressors are found to have role in keeping genomic integrity intact. To understand the role of p90 on genome stability, we generated p90 stable knock down MCF7 cells and scramble shRNA (NS) transfected stable cells were taken as control cells. Last year we reported that p90 knockdown cells were more lethal as compared to the NS cells when exposed to ionizing radiation suggesting that p90 has role in maintaining genome stability. Further, we found that p90 stabilized upon double strand as well as single strand DNA damage. In addition, we found that ATM kinase is essential for the stabilization of p90 upon DNA damage induction. Furthermore, we found that p90 has role in dephosphorylation of γH2AX. Phosphorylation of γH2AX is an early event in DNA damage response pathway. Phosphorylated γH2AX forms the foci on the DNA damge lesions and marked for recruitment of DNA damage repair machinery. Once DNA damage repair machinery assembles at the DNA damage site, dephosphorylation of γH2AX becomes essential for proper repair of damage DNA. We hypothesized that p90 may be involved in dephosphorylation of γH2AX for fruitful repair process of damage DNA. Supporting this hypothesis, we observed that dephosphorylation kinetic of γH2AX is much slower in p knockdown cells as compared to the NS cells. We determined the γH2AX foci under normal as well as DNA damaged condition and found that p90 stable KD cells showed more number of foci compared to

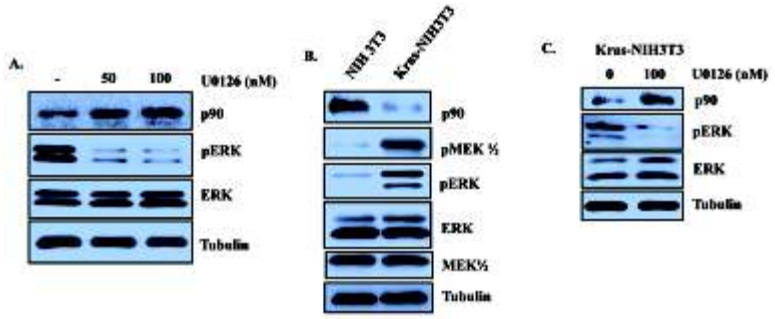

Fig. 3: p90 is regulated through epigenetic silencing via MAPK-ERK pathway **A)** Inhibition of MAPK pathway by chemical inhibitor U1026 leads to increased expression of p90. **B)** Western blot showing p90 expression level in Kras-NIH3T3 cells compared to NIH3T3. **C)** Inhibition of MAPK pathway by chemical inhibitor U1026 in Kras-NIH3T3 leads to derepression of p90 expression.

control cells. We found similar results, that p90 stable KD cells are having more number of γH2AX foci as compared to NS cells as observed in immunoblot study suggesting that p90 might be regulating DNA damage repair machinery through controlling phospho level of γH2AX. Further study will be carried out for detailed understanding how p90 regulates DNA damage repaired through regulation of γH2AX.

How cellular expression of p90 is regulatedκ

Previous report suggests that p90 is epigenetically silenced in renal cancer. Last year we reported that p90 is underexpressed in these highly aggressive metastatic cancer cell line. We also observed that MAPK kinase pathway is highly active in higher grade cancer where p90 is underexpressed. Similar results were also observed in the breast cancer patient samples (Fig. 2C). Then we asked whether MAPK kinase has any role in the expression of p90. We found that inhibition of MAPK kinase activity by kinase inhibitor led to derepression of p90 expression suggesting that MAPK negatively regulates p90 expression (Fig. 3A). Similar results were observed in the NIH3T3 and Kras-NIH3T3 cell lines (Fig. 3B). p90 is highly expressed in NIH3T3 as compared to Kras-NIH3T3. MAPK kinase activity is highly activated in Kras-NIH3T3 as compared to NIH3T3 suggesting that MAPK kinase may plays a crucial role on the expression level of p90 (Fig. 3C). Treatment of Kras-NIH3T3 cells with MAPK kinase inhibitor led elevated level of p90. We also observed the transcriptional activation of p90 in Kras-NIH3T3 with the treatment of DNA methylation inhibitor 5-Aza. However, we did not observe any change in the expression level of p90 when Kras-NIH3T3 cells were treated with histone deacetylase inhibitor. These results taken together demonstrated that MAPK kinase suppresses the expression level of p90 through DNA methylation-mediated epigenetic silencing process.

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Role of RNA-binding proteins in insulin biosynthesis

Background

Insulin is a small peptide hormone secreted by pancreatic β cells and is important for glucose homeostasis in mammals. Insulin expression in β cells is regulated by many nutrients, but mainly by glucose. Interestingly, glucose stimulation results in insulin secretion within minutes and is immediately followed by specific increase in insulin translation. The 5' and 3'un-translated regions (UTR's) of *insulin* mRNA has been shown to have a role in this translation regulation. In mouse, two non-allelic genes encode for insulin and specific splice variants with altered 5'UTR from these genes have also been reported.

MicroRNAs (miRNAs) are short (~22-nt) regulatory RNAs that influence a number of pancreatic events, including the development of pancreatic islets and β cells, insulin secretion, insulin resistance and diabetes. Normally, miRNAs target the 3'UTRs, causing degradation and/or translational repression of the target mRNA; occasionally, miRNAs have also been found to activate translation, however, the mechanism of miRNA-mediated increase in translation is not fully understood.

We have previously shown that mouse insulin2 mRNA undergoes alternative splicing, resulting in a shorter 5'UTR splice variant insulin2-S lacking 12 nucleotides in the 5'UTR. In the present study, we show that miR-196b can specifically target the 5'UTR of *insulin2* mRNA (the longer 5'UTR containing a splice variant) and regulates its translation in an Ago2-dependent manner. Interestingly, miR-196b increases target gene expression without affecting

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insulin2 mRNA levels but by enhancing the size of insulin2 mRNA polysomes, indicating that miR-196b upregulates *insulin2* translation.

Aims and Objectives

- Isolation and characterization of the insulin mRNA UTR binding factors and their partners.
- To understand the basic mechanism of translational regulation of insulin mRNA and the role of trans-acting factors in this regulation.

Work Achieved

Identification of miRNA that target 5'UTR of mouse insulin mRNA

Insulin mRNA expressed in mouse pancreas consists of a pool of transcripts containing different 5'UTRs. The variations in the 5'UTR are due to two non-allelic genes as well as alternative splicing, resulting in at least three different 5'UTRs in insulin mRNA. In light of recent reports that microRNAs can function through the 5'UTRs of target mRNAs, we explored the possibility that the insulin mRNA 5'UTR isoforms could contribute to their differential regulation via miRNA actions. miRNAs that can potentially target insulin 5'UTR were identified by MicroInspector web tool. Four miRNAs (miR-196b, miR-323-5p, miR-338-5p and miR-370) with high complementarity to seed sequences (at least 5 base pairs between nucleotide position 2-8 of the miRNA) and a free energy of less than -22 kCal/Mole were selected for further analysis. The miR-196b target site

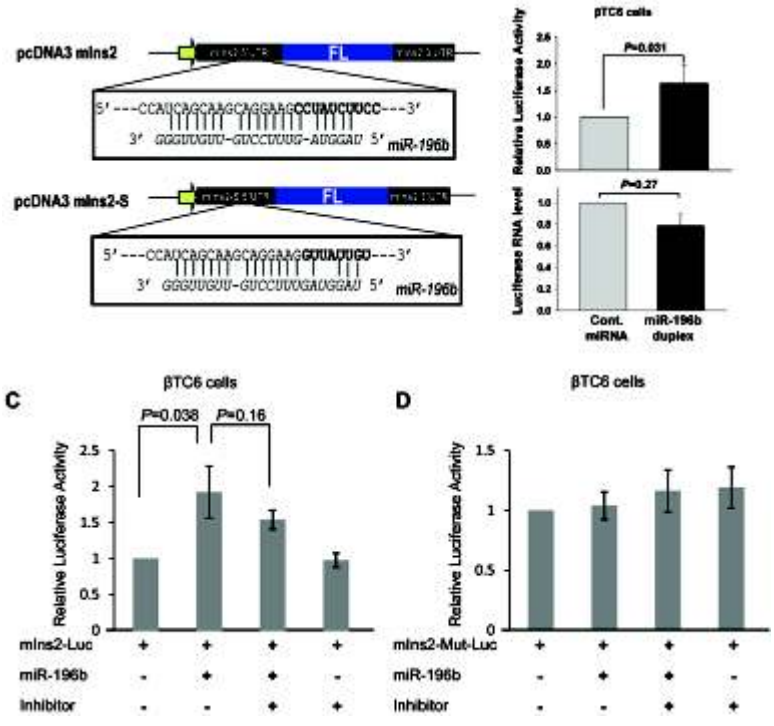


Fig. 1: miR-196b activates insulin2-reporter expression. **(A)** Schematic representation of the reporter constructs for insulin2 and insulin2-S 5'UTR constructs. The bold letters in the RNA sequence represents the start of exon 2 of *insulin2* mRNA and the lower *italic* letters represents the miR-196b sequence. The vertical lines between the sequences denote base pairing. **(B)** The miR-196b duplex/Control siRNA was transfected along with insulin2 reporter construct and Renilla luciferase as internal control in βTC6 cells. The fold change in translation is shown for the insulin2 reporter, with expression levels of control miRNA-transfected cells set to 1. The relative RNA levels as assessed by RT-qPCR are indicated in the bottom panel. The graphs represent the means ± SD of 3-9 independent experiments; *P* values (Student's t-test) are indicated. **(C)** Anti-miR-196b was introduced into cells along with reporter and the miRNA-pSuper; 48 hr later, the effect of the miR-196b inhibitor was analyzed by measuring the relative luciferase activity in cells transfected with insulin2 reporter or **(D)** insulin2 mutant reporter. The graphs represent the means ± SD of 3 independent experiments; *P* values (Student's t-test) are indicated.

is just at the exon1-exon2 junction of the *insulin2* mRNA which makes it very specific to this insulin2 isoform, not the *insulin2*-S splice variant, which lacks the miR-196b seed sequence thus miR196b showed differential binding ability to the two insulin2 splice variants (Fig. 1A).

Mouse miR-196b activates the expression of insulin2-5'UTR reporter construct

The translation regulation ability of these miRNAs was tested using a luciferase reporter system. Insulin 5'UTR corresponding to mouse *insulin1*, *insulin2* and *insulin2*-S were cloned at the 5'UTR of a luciferase reporter gene. The luciferase reporter plasmids, the control renilla plasmids, and the microRNAs were transfected 48 hours later, luciferase activity was measured. Transfection of miR-196b resulted in an increase in luciferase activity from constructs containing the target insulin2 5'UTR by about 50% (Fig. 1B). Luciferase mRNA

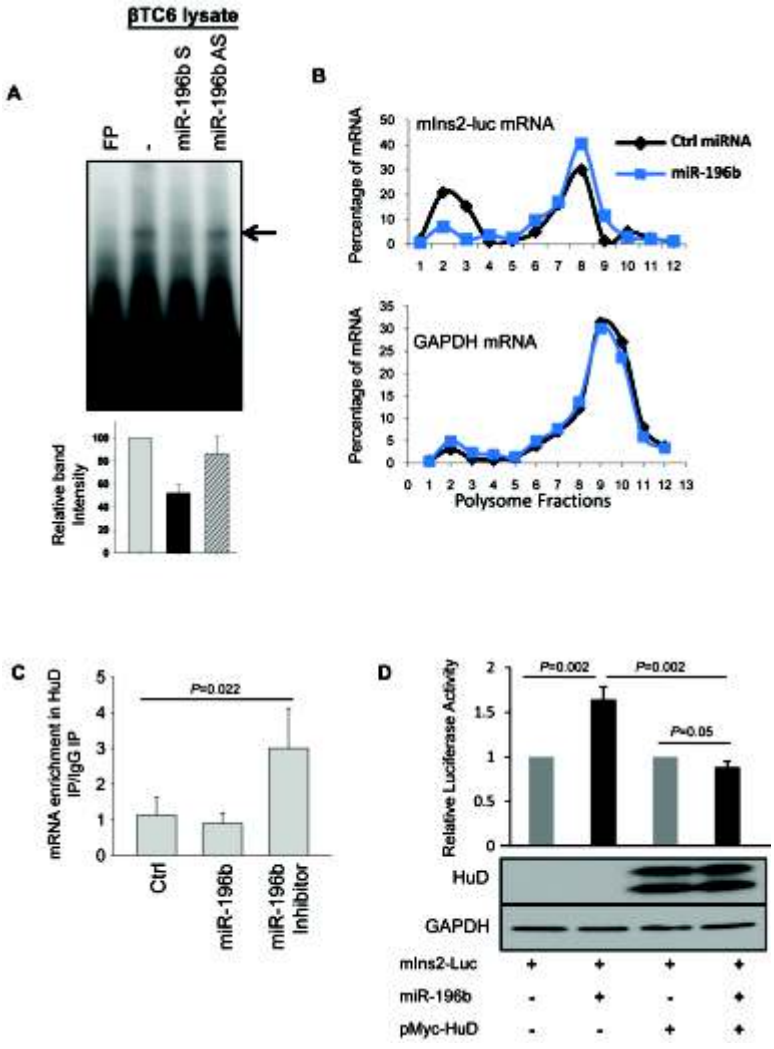


Fig. 2: miR196 inhibits the insulin 5'UTR interaction with protein factors (A) RNA-EMSA using the radiolabeled *insulin2* RNA as probe and extracts from βTC6 cells in the presence of miR196b or control antisense miR196b. The arrow indicates the shifted RNP complexes. The lower panel shows the RNA-protein complex intensity as measured by densitometry. The graphs represent the means ± SD of 3 independent experiments; *P* values (Student's t-test) are indicated. **(B)** The relative distribution of Insulin2 reporter mRNA and *GAPDH* mRNA on polysome gradients was studied by RT-qPCR analysis of the RNA present in each of 12 gradient fractions, and represented as percentage of total mRNA. One of the representative experiments is shown here. **(C)** Interaction of HuD with *Ins2-reporter* mRNA in βTC6 cells transfected with control, mature miR-196b or miR-196b inhibitor, was studied by mRNP IP analysis using anti-HuD or control IgG antibodies. The RNA in the IP material was isolated, and *Ins2*-reporter mRNA levels were measured by RT-qPCR analysis and normalized to *PGK* mRNA levels. **(D)** Translation upregulation of insulin2 reporter with miR-196b in cells transfected with either control or Myc-HuD plasmid. Lower panel shows the immunoblot for the over expression of Myc-HuD.

levels, assessed by quantitative PCR, showed that miR-196b transfection did not result in increased luciferase mRNA levels, but actually caused a modest decrease in luciferase mRNA levels (Fig. 1B, bottom panel). Thus, the increase in relative luciferase activity upon miR-196b transfection was likely due to an increase in translation and not due to increased mRNA abundance. The expression of miR-196b had no effect on the other 5'UTR luciferase constructs that did not contain the target sequence in the 5'UTR. Similarly expression of other miRNA also did not affect the reporter gene activity.

The translation affect was specific as anti-miR196b blocks the miR-196b mediated activation of insulin2-5'UTR-Luciferase translation while having no affect on the mutant insulin 5'UTR containing reporter (Fig. 1C and 1D). Further we had previously shown that this translation activation by miR196b requires AGO2.

miR-196b inhibits the formation of Insulin2-5'UTR-protein complexes

A specific RNA-protein complex is formed with *insulin2* 5'UTR and cytoplasmic protein factors, but in case of insulin2, the complex formation is increased. In vitro and in vivo translation experiments showed a correlation between the complex formation and reduced translation efficiency, suggesting that the *trans*-acting factor that associates with *insulin2*-5'UTR is likely to be a translation inhibitor. Since the translation efficiency of insulin2 mRNA is the lowest among mouse *insulin* mRNAs, we hypothesized that the miRNA could activate translation by interfering with the RNA-protein interactions at the 5'UTR. The miR-196b sense or antisense strand were incubated with the *insulin2* 5'UTR before the addition of lysate, and the RNA-protein complex formation was assessed by RNA-EMSA. The sense strand miR-196b inhibits the complex formation while the antisense strand had no effect on the complex formation in βTC6 cells (Fig. 2A). These data suggest that the binding of miR-196b disrupts the RNP (ribonucleoprotein) complex formation.

miR-196b causes reduced association of mouse Insulin2 mRNA with HuD

To study the effect of miR-196b on insulin translation, we analyzed the association of Insulin2 reporter with the cell's polysomes in Ctrl siRNA- or miR-196b transfected βTC6 cells. miR-196b did not affect the global translation profile of βTC6 cells. RT-qPCR analysis of polysomal fractions show an increased association of the insulin 2 reporter with the polysome upon miR-196b treatment, suggesting that miR-196b promotes the translation of Insulin2 in βTC6 cells (Fig. 2B).

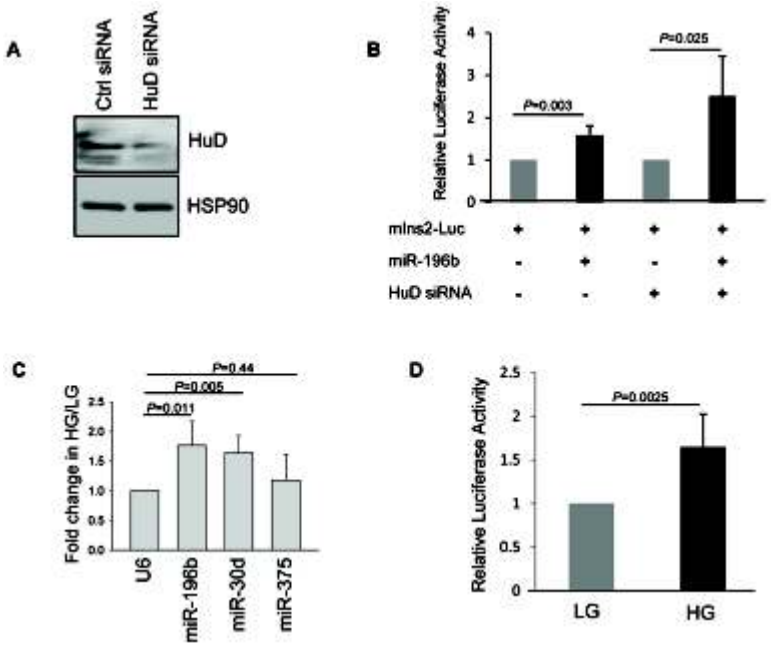
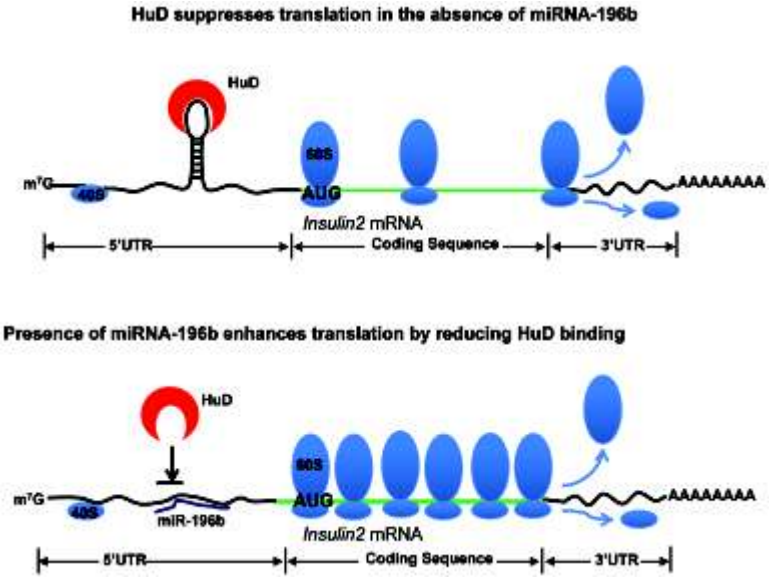


Fig. 3: miR196b displaces HuD from the insulin 5'UTR **(A)** Forty-eight hours after transfection of βTC6 cells with Ctrl siRNA or HuD siRNA, HuD silencing was assessed by western blot analysis. **(B)** Luciferase reporter with *insulin2* 5'UTR along with miR-196b or control miRNA duplex was introduced into βTC6 cells expressing normal or reduced HuD levels The fold change in relative luciferase activity was measured with the activity of the luciferase construct with control miRNA set to 1. The graph represent the means ± SD of 3-9 independent experiments; *P* values (Student's t-test) are indicated. **(C)** The change in expression of various miRNAs in high/low glucose treated βTC6 cells using QuantiMiR RT-qPCR kit. **(D)** Expression of insulin2 reporter in high glucose treated βTC6 cells normalized to renilla expression. The graph represent means ± SD of 3-8 independent experiments; *P* values (Student's t-test) are indicated.

Recently, it was shown that the RNA-binding protein HuD bind to insulin 5'UTR and repressed the translation of insulin mRNA. As miR-196b also targets the 5'UTR of *insulin2* mRNA, we sought to determine if there is any interplay between HuD and miR-196 in controlling Insulin2 translation. Following transfection of control RNA, miR-196b miRNA or miR-196b-as inhibitor into βTC6 cells along with the Insulin2 reporter, the interaction of HuD with the reporter mRNA was assessed by ribonucleoprotein immunoprecipitation (RIP) analysis followed by detection of the reporter RNA in the IP material. We found increased association of insulin 5'UTR with HuD after antagonizing miR-196b function, while expression of miR-196b modestly decreased the association of the insulin 5'UTR reporter mRNA with HuD. These results suggest that HuD and miR-196b might compete for binding to the 5'UTR of *Ins2* mRNA (Fig. 2C).

We analysed the functional interplay between miR-196b and HuD, an RNA binding protein that associates with insulin mRNA 5'UTR. Over expression of HuD in HEK293T cells along with the insulin2 reporter and miR-196b abolished the miR-196b mediated translation up regulation (Fig. 2D). This finding suggests that HuD/miR-196b compete for binding to 5'UTR of insulin2 mRNA. Further, upon HuD silencing in βTC6 cells, we observed even higher insulin2 translation compared with miR-196b overexpression alone (Fig. 3A and 3B).

Fig. 4: Mechanism of miR-196b action. The miR-196b target site is at the 5'UTR stem loop structure of the *insulin2* mRNA. Targeting of miR-196b to the stem-loop region of the *insulin2* mRNA disrupts the secondary structure and prevents binding of the translational inhibitor, resulting in the activation of insulin translation.



Glucose increases the miR-196b expression in βTC6 cells

We analysed the effect of glucose on the expression on miR-196b in βTC6 cells, we incubated βTC6 cells in the absence of glucose or in the presence of 25 mM glucose for 16 h, and prepared total RNA. Analysis of the miRNA expression revealed that miR-196b and miR30d was upregulated by high glucose, whereas miR-375 level did not change significantly (Fig. 3C). The insulin2 reporter expression also increased under these conditions, suggesting a potential positive influence of the heightened miR-196b levels (Fig. 3D). These results suggest that increased miR-196b expression in response to glucose may be an additional mechanism for glucose-stimulated insulin synthesis (Fig 4).

Future Research Plans

- Functional characterization of HuD, PABP and their interaction with miR196 and insulin mRNA

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Oncostain-mediated regulation of mesenchymal and proneural markers in gliomas

Background

Gliomas, the most predominant primary brain tumors in adults and children are a leading cause of cancer-related deaths. Gliomas are divided into low grade glioma (LGG) and high grade glioma (HGG) and based on the WHO classification; Grade I is benign while Grade II and Grade III are categorized as LGG and are associated with the slow growth rate and better survival period (3–8 years), however, have high probability to transform to higher grade. HGG include Grade IV glioblastoma (GBM), which is the most common and aggressive of brain tumors in adults and accounts for nearly 75% of all gliomas. The GBM type is classified on the basis of molecular signature genes using TCGA data into four subtypes- classical, mesenchymal, proneural and neural. The mesenchymal phenotype is associated with greater aggressiveness and low survival in contrast to GBMs enriched with proneural genes. Invasion and neo-angiogenesis are the hallmarks of GBM and contribute to reduction of median survival period (<1 year) post diagnosis. Although most GBMs share similar histological features such as microvascular, proliferation and pseudopalisading necrosis, the patients differ in their response to treatment and survival rates. Several transcription factors including SNAI1, SNAI2, TWIST1, ZEB-1 play important role in the MES differentiation and aberrant activation of transcriptional factors such as STAT3, ZEB-1and NFκB are shown to be responsible for MES-shift in the GBM. STAT3 is activated in response to cytokines and growth factors that results in transcription of diverse genes involved in cell cycle progression, apoptosis, cell survival, angiogenesis, migration, and invasion. More recently, STAT3 along with C/EBPβ has been reported to function as synergistic initiators and master regulators of mesenchymal transformation and persistent activation of STAT3 in GBM.

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Oncostatin-M (OSM), a pleotropic proinflammatory cytokine belonging to the IL-6 family, is secreted into the microenvironment during inflammation and injury. OSM is associated with multiple biological processes and cellular responses including growth, differentiation, and inflammation. OSM induces its biological activity by binding to two distinct heterodimers of gp130 with either OSM receptor (OSMR) or leukemia inhibiting factor receptor (LIFR).OSM-mediated signalling through STAT3 activation is associated with poor prognosis and aggressiveness in GBM and other solid tumors including those of breast and lung.

Aims and Objectives

- While the role of IL-6 cytokines is well studied in GBM, little is explored of the expression of IL-6 cytokine receptor family in progression of glioma and in subtypes of GBM. The study aimed towards understanding the significance of OSMR-mediated STAT3 signalling in association with mesenchymal (MES) subtype of GBM.

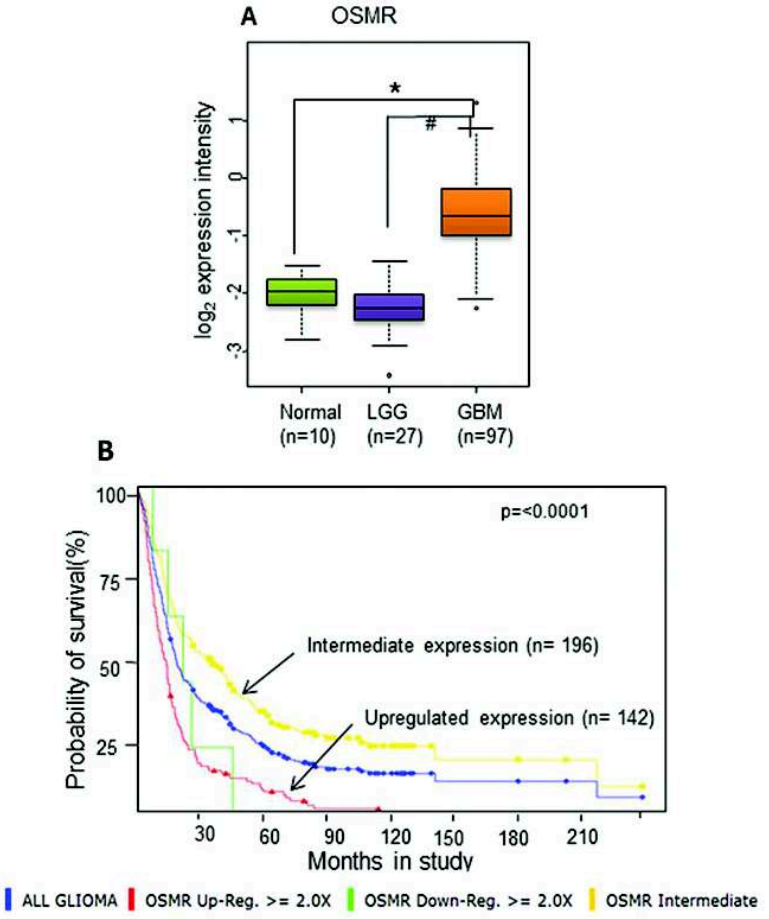

Fig. 1: Expression profile of Oncostatin-M receptor (OSMR) gene and its correlation with glioma patient survival. (A) Box whisker plot for Log2expression intensity of OSMR in normal, LGG and GBM from TCGA database. *p<0.0001, #p<0.0001. **(B)** Kaplan-Meier graph showing probability of survival of glioma patients (REMBRANDT database) in relation to OSMR expression. P value <0.0001 between groups with intermediate and up-regulated levels of OSMR.

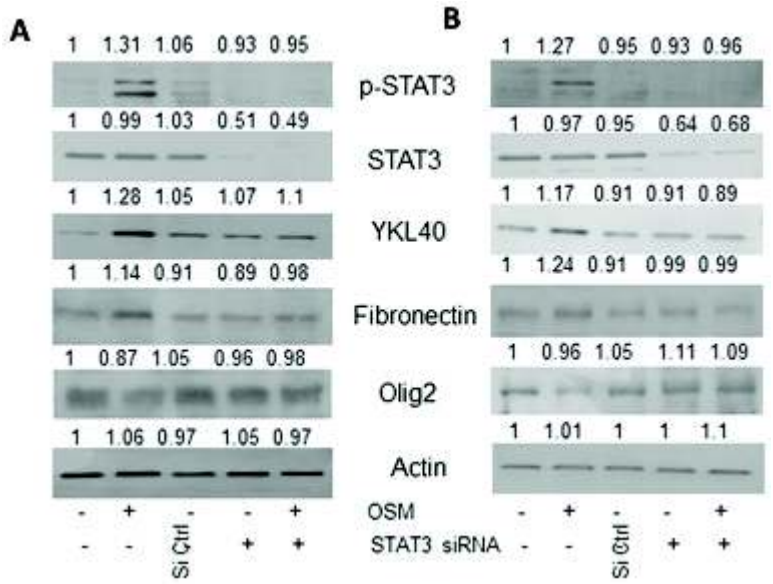

Fig. 2: Role of STAT3 in OSM-induced mesenchymal signature markers. LN18 cells were transfected with STAT3 si RNA (100nM) followed by treatment with OSM (50 ng/ml) and expression at protein level of mesenchymal markers in LN18 (A) and LN229 cells (B)detected by western blot analysis. The blots are representative of three independent experiments.

Work Achieved

Initial studies of analysis of TCGA and REMBRANDT data revealed that the expression of OSMR is upregulated in GBM and has negative correlation with survival (Figure 1). Amongst the GBM subtypes, OSMR level was in the order of mesenchymal > classical > neural > proneural. TCGA data and RT-PCR analysis in primary cultures of low and high grade gliomas showed a positive correlation between OSMR and mesenchymal signature genes-YKL40/CHI3L1, fibronectin and vimentin and a negative correlation with proneural signature genes-DLL3, Olig2 and BCAN. OSM enhanced transcript and protein level of fibronectin and YKL-40 and reduced the expression of Olig2 and DLL3 in GBM cells. The biological activity of OSM is classically mediated by the activation of STAT3. We further observed that silencing of STAT3 using specific siRNA reduced significantly the expression of mesenchymal signature genes- fibronectin and YKL-40 induced by OSM in human GBM cell lines-LN18 and LN229 cells (Figure 2), suggesting that OSM regulated the expression of mesenchymal markers and proneural signature genes through STAT3 signalling. Functionally, OSM-regulated mesenchymal phenotype was associated with enhanced MMP-9 activity, increased cellular invasion and self-renewal potential in LN-18 and primary tumor cultures derived from GBM tumor (Figure 3). Importantly, OSM induced mesenchymal markers and reduced proneural genes in primary cultures of grade-III glioma cells. Collectively, the study demonstrates that OSM differentially regulates the expression of mesenchymal and proneural signatures and contributes to aggressiveness via STAT3 signalling in gliomas.

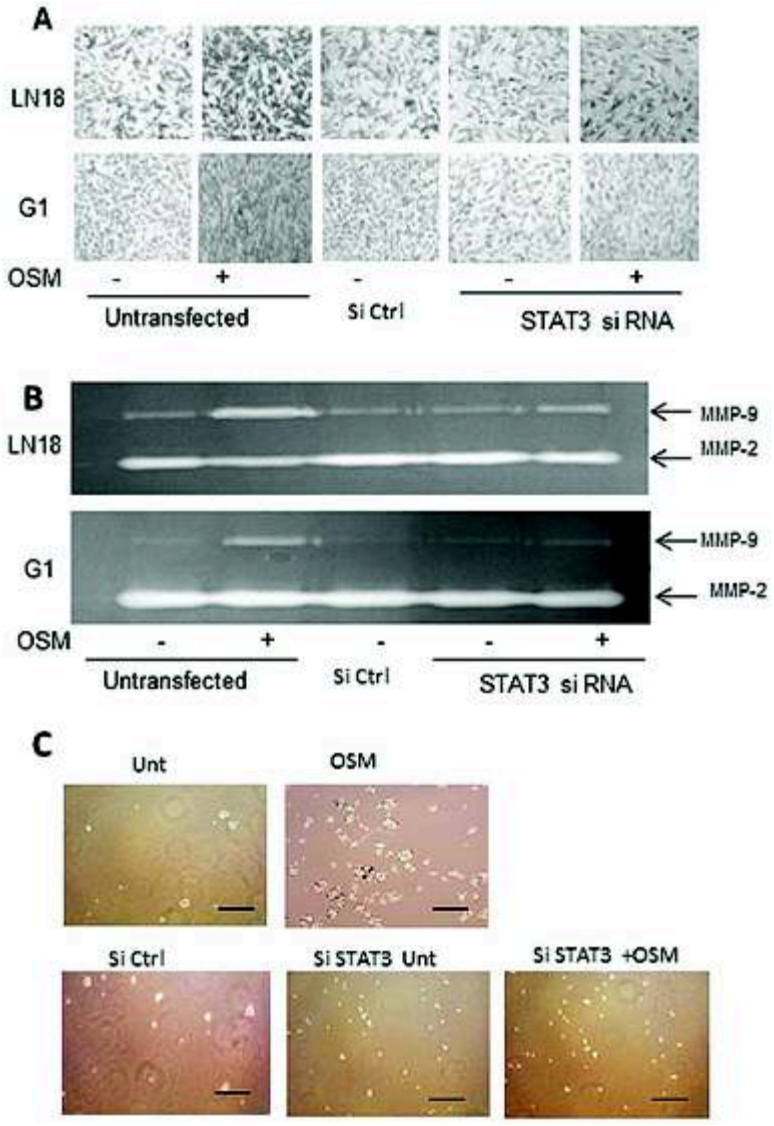

Fig. 3: Role of STAT3 in OSM- induced invasion potential and self- renewal. LN18 cells and primary cultures- G1 cells were transfected with STAT3 si RNA(100nM) followed by treatment of OSM (50 ng/ml). (A) Invasive potential assessed using matrigel invasion assay. The images are representative of two independent experiments. (B) MMP-9 and MMP-2 levels estimated using conditioned medium subjected to gelatin zymography analysis. The zymogram is a representative of three independent experiments. (C) G1 cells were transfected with control or STAT3 siRNA (100 nM) and seeded in low attachment plate for neurosphere assay followed by OSM (50 ng/ml) treatment. The images are representative of two independent experiments. Scale: 50µm.

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

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Background

An increasing number of cellular processes in eukaryotes seem to be regulated by a large and diverse family of RNA molecules categorized as non-coding RNAs. This family besides including transcripts functioning as 'housekeeping' RNAs like rRNA or tRNA importantly includes RNAs with regulatory roles. These regulatory RNAs are classified as per their size into small (<200 bp, e.g. miRNAs, endo-siRNAs, & piRNAs) & long RNAs (>200 bp)[1]. While small RNAs like miRNAs are involved in regulating gene expression through its target proteins, the long non-coding RNAs participate in more varied functions like gene silencing, gene transcription, imprinting, dosage compensation, DNA demethylation, chromatin remodeling to RNA interference. Some of the ncRNAs may be natural antisense transcripts (NATs) that overlap to significant extents by arising from opposite transcription units present in the same locus (cis-NAT) or by arising from distinct loci (trans-NATs) [2]. Till date, the functions of only a limited number of lncRNAs are well characterized and include RNAs like Xist/RepA, KCNQ1OT1, AIR, HOTAIR, Evf-2, H19, MALAT1, and some natural antisense lncRNAs (NATs)[3,4]. The functions of many of these RNAs have been elucidated using different approaches such as genome-wide gene expression screen, genome wide association studies, region-targeted association assay and conventional linkage screen designed lncRNA array, RIP-RNA sequencing as well as transgenic expression and gene knockdown /knockout method. Earlier studies in our lab led to identification of a non-coding RNA termed as Ginir (Genomic Instability Inducing RNA) from mouse cells. This RNA is categorized as long intergenic non-coding RNA (Linc RNA) and is localized to chromosome X in mouse and Chromosome 6 in human. The RNA has an

oncogenic potential and our current efforts are focused on elucidating its functional role in mouse and human cells as well as gaining mechanistic insight about the cellular signalling pathways activated by this RNA in evoking its transforming function.

Aims and Objectives

- To study sub-cellular localization of linc RNA- Ginir in various mouse cells and in Ginir over-expressing cells.
- To identify targets of Ginir at *in vitro* and *in vivo* levels as interacting protein partners or RNA binding proteins in inducing cellular transformation and metastasis in mouse cells.
- To delineate cellular signalling pathways specific through which Ginir mediates its effects on cellular growth and their relationship to various signalling pathways in embryo development, cellular transformation and metastasis.

Work Achieved

Most of the non-coding RNAs are present as a pair of sense and anti-sense over-lapping transcripts. In line with this finding the sense transcript- Ginir has an anti-sense counterpart- Giniras. Both together forms a pair of sense and anti-sense transcripts evoking a mechanism of SAST (Sense-Anti-sense Transcription) that could contribute to cellular homeostasis in normal cells.

Sub-cellular localization of Ginir

To determine localization of Ginir in mouse cells, we performed subcellular fractionation of fibroblast cells into nuclear and cytoplasmic fractions. Our RT-PCR data using gene specific primers for Ginir indicated that in normal fibroblast cells, Ginir was expressed predominantly in the cytoplasm (Fig. 1.b), while in NIH/3T3-Ginir & mouse melanoma cells- Clone M3, which are transformed cells from where Ginir was initially identified, a significant enrichment of Ginir RNA occurred to the nucleus (Fig. 1.a, c). This suggests for a prominent shuttling mechanism of this RNA between nuclear and cytoplasmic compartments that could be playing an important role in cellular transformation. Further, we performed RNA FISH on fibroblasts, Ginir over-expressing fibroblasts and melanoma cells to investigate the physiological state of Ginir RNA in mouse cells. Using LNA-FISH probes, we obtained cytoplasmic localization of Ginir in mouse cells (NIH/3T3) whereas in transformed cells (NIH-Ginir and Clone M3) the presence of Ginir RNA was significantly enhanced in the nucleus (Fig. 1.d, e).

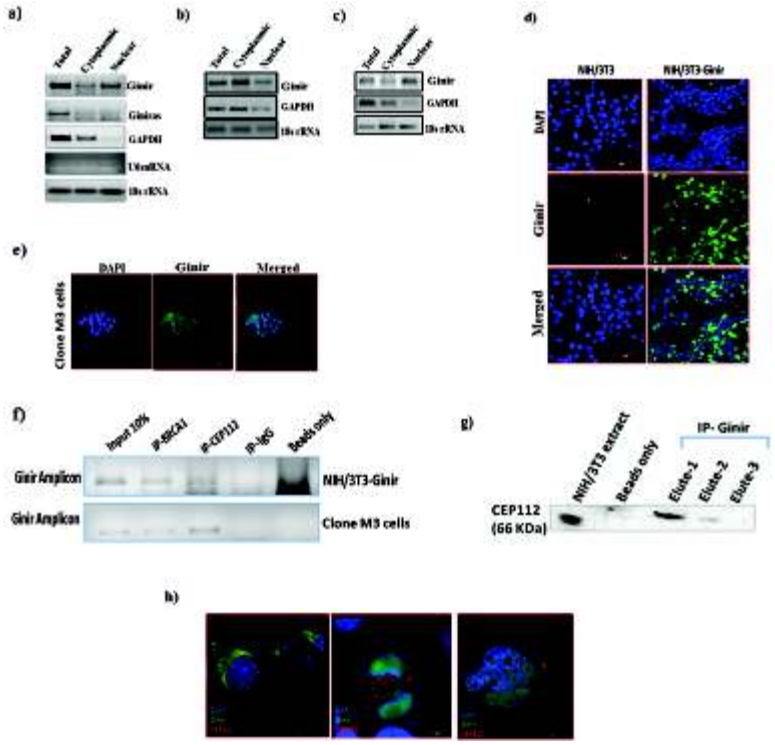

Fig. 1: Sub-cellular localization of Ginir & Giniras transcripts in mouse cells by fractionation followed by strand specific PCR in **(a)** Clone M3 **(b)** NIH/3T3, and **(c)** NIH/3T3-Ginir cells. GAPDH was used as a fractionation control for cytoplasmic RNA & U6 sn RNA for nuclear RNA. 18S rRNA served as internal control for all fractions. RNA FISH (Fluorescent In situ Hybridization) for Ginir RNA (FAM labelled) in NIH/3T3, NIH/3T3-Ginir cells **(d)** & Clone M3 cells **(e)**. Biotin RNA Affinity pull-down for Ginir RNA followed by Western blotting for Cep112 expression in NIH/3T3 cells **(f)**. RNA immuno precipitation with Brca1 & Cep112 followed by RT-PCR for Ginir in NIH/3T3 Ginir & Clone M3 cells **(g)**. IgG was used as negative control for non-specific binding to the antibody. RNA FISH for Ginir (FAM labelled) in NIH/3T3-Ginir cells followed by immunofluorescence for Cep112 to study co-localization of Cep112 with Ginir **(h)**.

Targets of Ginir in Mouse Cells

We aimed to determine whether Ginir mediated its cellular effects on transformation through interactions with specific proteins or whether it bound chromatin modifying complexes like PRC1/2 and then exerted its effects through epigenetic modifiers. For this, we used in-vitro-transcribed biotinylated RNA to retrieve binding proteins from native cell lysates (RNA chromatography) and to investigate RNA-protein interactions. While, a majority of over-lapping proteins were ''nonspecific hits, we retrieved a common set of proteins in all four cell types chosen for study. These cell systems were mouse fibroblasts, mouse embryos, brain derived from mouse embryos and Ginir over-expressing mouse fibroblast cells. Collating data from all four cell systems we initially chose to determine whether a novel centrosomal protein Cep112 was as an interacting partner for mediating Ginir action. Cep112 (Centrosomal protein of 112 KDa) or CCDC46 is a centrosomal protein with ATPase domain. It is reported to be associated with microtubule organizing centre & centrosomes and plays a vital role in centriole biogenesis and cell cycle progression control. Ensembl Genome browser shows Mus musculus Cep112 to have 18 possible splice variants & 12 predicted protein isoforms out of which protein isoforms of 112 KDa & 28 KDa are the predominant ones. Our immunoblotting data shows NIH/3T3 cells to express three predominant isoforms- isoform-1 (112 KDa), isoform-2 (66 KDa) & isoform (28 KDa) which

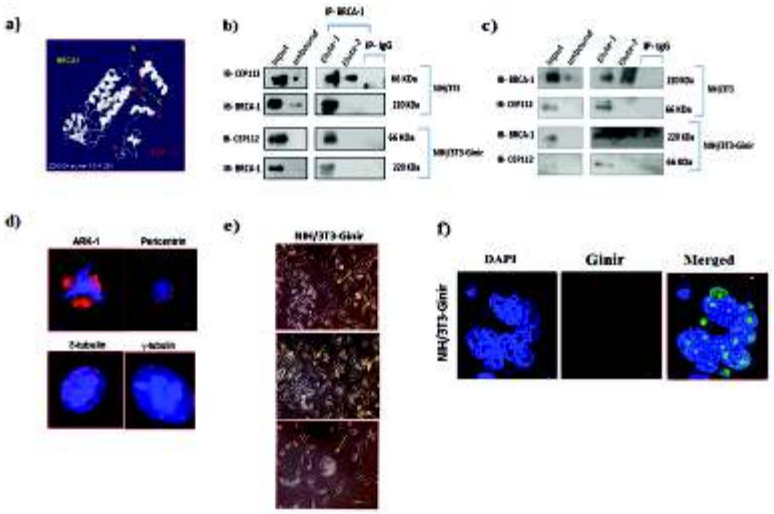

Fig. 2: Interaction of Brca1-Cep112 in mouse fibroblasts & visualization of cytokinetic defects & multinucleated giant cell (MNCs) formation in NIH/3T3-Ginir cells. **a)** Protein docking analysis for Brca1-Cep112 interaction using ZDOCK tool. **b)** Protein immuneprecipitation with Brca-1 followed by immunoblotting for Cep112 in NIH/3T3 & NIH/3T3-Ginir cells. **c)** Protein immuneprecipitation with Cep112 followed by immunoblotting for Brca1 in NIH/3T3 & NIH/3T3-Ginir cells. Isotype IgG served as negative control for non-specific binding to the antibody. **d)** Immunofluorescence for Ark-1, pericentrin, δ tubulin & γ tubulin in NIH/3T3-Ginir cells. **e)** Phase Contrast Micrographs of NIH/3T3-Ginir cells. Arrow heads point to multinucleated giant cells (MNCs). **f)** RNA FISH using LNA probes (Exiqon) for Ginir (FAM labelled) in NIH/3T3-Ginir multinucleated giant cells.

are down-regulated in Ginir over-expressing cells. The RNA-IP as well as biotin affinity capture experiments validated the interaction of Cep112 protein with Ginir (Fig.1.f & g). Our earlier studies have demonstrated interaction of Brca-1 with Ginir. Brca1, a caretaker protein is central player of genome surveillance complex & thus very important for genomic stability. It plays a central role in chromatin re-modelling & regulation of the expression of repeat elements & imprinted genes. It has also been reported to regulate centrosome amplification & cytokinesis by interacting with centrosomal proteins like OLA1 & Nlp. To determine whether Cep112 interacted with Brca1, we began by performing protein docking analysis using ZDOCK tool and determined that there is a stable interaction between the C-terminal of Brca1 with a domain near the C-terminal of Cep112 (Fig.2.a). Later, with help of protein immune-precipitation experiments we demonstrated strong association between Cep112 isoform-2 & Brca1 in both NIH/3T3 & NIH/3T3-Ginir cells (Fig. 2.b & c). Our data confirms interaction of Ginir RNA with Cep112 and its interaction with Brca1 thereby forming a tripartite complex (Ginir with Cep112 and Brca1) that was causal for genomic instability in mouse fibroblast cells.

Cellular signalling pathways mediated through Ginir

The manifestation of GI by Ginir by binding to Cep112-Brca1 complex led to down regulation of both the proteins. Knock-down of Brca1 and Cep112 individually as well as in combination showed that loss of Brca1 in Ginir expressing cells caused defects in DDR pathway along with heterochromatin impairment resulting in de-repression of tandem repeats & up regulation of imprinted genes. Down regulation of Cep112 caused defects in cytokinesis as was evident by presence of multinucleated giant cells (Fig.2.e & f). We believe

that interaction of Ginir with proteins like Cep112 caused defective cytokinesis (Fig.2.d) that contributed to significant increase in multi-nucleation, multipolar mitosis, failed abscission, asymmetric segregation of daughter nuclei, and formation of anucleated daughter cells causing centrosomal amplification predisposing cells towards cellular transformation. The defects in cytokinesis with Ginir over-expression are evident in Fig.2.d

Thus elucidation of detailed mechanism of interactions of non-coding RNAs (ncRNAs) function with associated proteins to effects complex structural and regulatory outcomes may help us elucidate how the engagement with different proteins in a modular and developmentally controlled manner helps coordinate cellular transitions from development to differentiation to disease.

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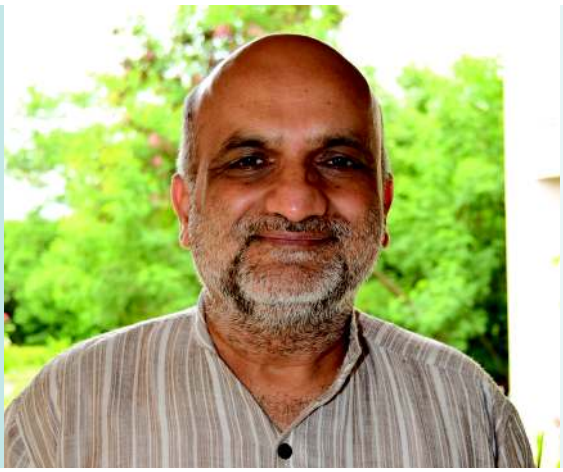
Future Research Plans

- We aim to understand signaling network mediated by non-coding RNA Ginir via interaction with RNA binding proteins, other protein interactors and interactions with various chromatin modifying complexes in mouse and human systems.



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Human Microbiome : Indian perspective

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Background
Eubacterial assemblage together with other microbes like Archea, Microeukaryotes and Fungi is referred to as 'microbiota' associated with the human body. Of all the body sites, the microbiota of the gastro-intestinal tract is found to be denser, richer and mainly dominated by bacteria belonging to the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. All these bacterial phyla are found in specific proportions in healthy individuals and any deviation from the healthy composition is termed as the 'dysbiosis' which means the imbalance in abundance of specific bacterial phyla or bacteria belonging to these phyla. The dysbiosis in the gut microbiota has been associated with important diseases both in and outside the gastro-intestinal tract. Some of the specific diseases includes: IBD, celiac disease, colon cancer, obesity, and diabetes. Interestingly, it has also been noted that individuals residing in different countries harbor population specific microbiota and individual's microbiota can be clustered into different groups based on their gut microbiota compositions, specific groups of bacteria that are responsible for such grouping are termed as 'enterotypes'.

It is thus clear, that the gut microbiota plays an essential role in normal well-being of the human host. It is also crucial for extracting nutrients from diet, regulating host fat storage, stimulating intestinal epithelium renewal, and directing the maturation of immune system. If disturbed, the perturbations in the gut microbiota may lead to the disease condition. Hence, characterizing the gut microbiota is essential to understand the spatial, temporal and disease associated variations in gut microbial communities across the life-span, of individuals of different nationalities and geographic and/or socio-economic

groups. This may generate wealth of information relating the gut microbiota in development of certain diseases.

Aims and Objectives

Indian population is a unique conglomeration of genetically diverse groups having varied dietary habits and residing in vast geographic locations. Apart from the ethnic and genetic differences, Indians have distinctive metabolic and anthropometric features. All these features of Indian population makes it an excellent cohort whose microbiota needs to be assessed for characterizing specific biomarkers and for finding its association with specified diseases in this population. With this in mind, we have specifically aimed to accomplish the following objectives:

1. Quantifying population specific differences in gut microbiota in different geographical regions.
2. Understanding the association of gut microbiota with metabolic disorders.

Work Achieved

Quantifying population specific differences in gut microbiota from different geographical locations

In this part, we provide detailed account of prominent attributes of Indian gut microbial composition and its functions from 34 healthy Indian subjects, of which 26 were from Western India and 8 were from Northern India. Based on

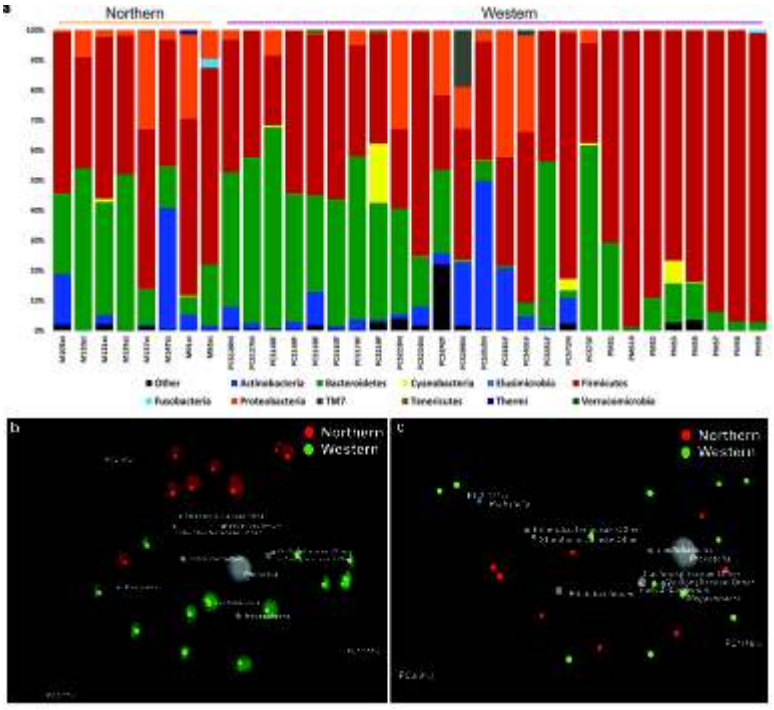

Fig. 1: a) Phylum level abundance of Northern and Western Indian subjects. b) Unweighted and c) weighted UniFrac PCoA bi-plots; in which samples are coloured according to the region. The grey coloured sphere represent a taxonomic group that influence the clustering of samples in particular area of the PCoA plot and its size demonstrate the abundance of that taxonomic group.

abundance of major bacterial phyla and individual specific OTUs; we report substantial variations in gut microbial communities of individuals from Western and Northern Indian population (Figure 1a). To identify the factors explaining differences in microbial communities of these populations, we performed UniFrac analysis (a measure of β-diversity) that uses phylogenetic information to compare the samples. We hypothesize that distinct separation observed on unweighted UniFrac PCoA (Figure 1b) is influenced by less abundant unique OTUs, which was lost on weighted UniFrac PCoA (Figure 1c) because of abundance of dominant OTUs. The distinct separation was also not evident at phyla level abundance. On PCoA bi-plots, we further showed the contribution of dominant taxonomic groups in influencing the segregation of samples between Western and Northern populations. Thus, our results are robust and proves the presence of population specific OTUs; at the same time it confirms that Indian population could not be separated into two groups based on presence and abundance of dominant taxonomic groups i.e. the major contributing phyla.

Comparative analysis microbiota in early age

In collaboration with Functional Food Forum (FFF), University of Turku, Finland comparative analysis of gut microbiota of Indian and Finnish teenagers borne by delivery and caesarian section was carried out. Indian and Finnish cohorts were studied using quantitative PCR for *Bifidobacterium* group which plays a key role in human health, as it carries a wide range of functions like improving intestinal integrity, development of infants' immune system and harboring genes for utilizing the complex oligosaccharides which are prime source of nutrition (breast milk) during the early stages of the infant. The experimental data obtained showed that the birth mode has a marginal effect on the *Bifidobacterial* population, especially with the *B. catenulatum* and *B. longum*, however large population of the cohort showed counts above the median in normal delivery as compared to C-section born children (Figure 2a). *Clostridium spp.* found to be abundant in C-section born 13-14 year old children as is *Akkermancia muciniphila*.

The secretion studies carried out within the same cohort; indicate that the secretor status (fut2 gene) of the individual affects the Bifidobacterial population in the gut, especially of *B. bifidum* and *B. adolecentis* populations. Similar results were obtained for other bacteria like *C. leptum*, suggesting the possibility of *fut2* secretor status of the individual modulating the population of certain important gut microbes. The DGGE fingerprinting of the total bacterial community and qPCR experiments based on the selected bacterial strains revealed that the Indian and Finnish cohort of 13-14 years individuals harbor

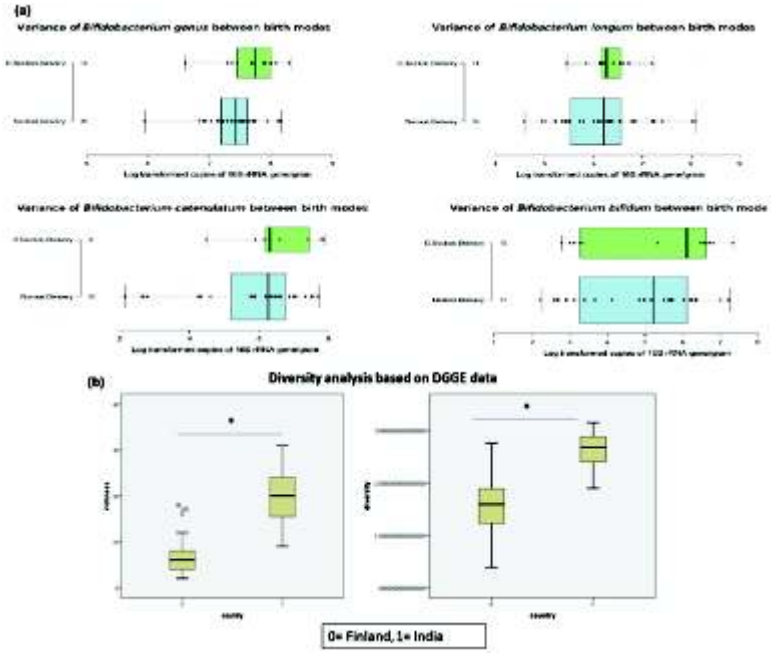

Fig. 2: a) qPCR results. Box plot showing variance in *Bifidobacteria* between individuals with different birth mode. b) the differences in diversity and richness of the bacterial diversity between the two cohorts (Indian and Finnish) based on the DGGE data.

distinct gut bacterial community (Figure 2b). The results obtained exhibit the significant differences in the gut bacterial community between the two distinctly located geographical locations (India and Finland) which also differ in their diet, lifestyle and other environmental conditions.

Gut microbiome of diabetic individuals in Indian population

Dysbiosis in gut microbiota of individuals with diabetes is being increasingly recognized, some studies have demonstrated direct correlations of certain members of gut microbiota with the development of diabetes. In most of the cases, bacteria belonging to Firmicutes are in more abundance in diabetic subjects over the healthy subjects. Surprisingly, bacteria belonging to genus *Lactobacillus* found to be enriched in diabetic individuals along with the decrease in bacteria that produce Short Chain Fatty Acids (SCFAs) such as butyrate in the gut. Till date, there are no reports if the micro-Eukaryotic and Archaeal diversity is different in diabetic and healthy subjects. Eukarya is one of the three domains of life, there are ~70 lineages in this domain most of which are microbial in nature. Human gut is home to many eukaryotes, including helminths, amoebae and flagellates. Microbial eukaryotes in the human gut have been studied primarily from a parasitological point of view. Many diseases are mediated by infections of single cell Eukaryotes (E.g.: *Entamoeba histolytica* causing amoebiasis and *Giardia* intestinalis causing giardiasis). Interestingly, some eukaryotic microbes are considered probiotics, e.g. *Saccharomyces boulardii*. Clearly, Eukaryotes and Archea are important component of the mammalian microbiome and studies characterizing these components from

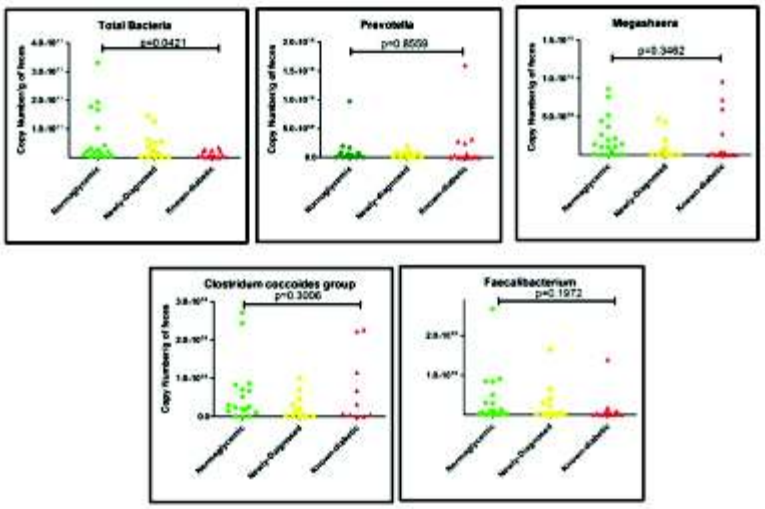

Fig. 3: Copy number of specific group of bacteria in normo-glycemic, newly diagnosed and known diabetic subjects.

human gut are lagging behind bacteria. Thus, characterizing Eukaryotic and Archeal diversity along with Bacterial diversity will shade more light on their role in general well-being and disease development.

We performed qPCR based absolute quantifications of specific groups of bacteria in the human gut from healthy individuals and compared it with the newly diagnosed and known diabetic individuals. We then assessed if these specific group of bacteria has any association with diabetes risk factors such as fasting glucose, high triglycerides and high BMI. In addition, we have also attempted to characterize the micro-eukaryotic and archaebacterial diversity from these subjects using PCR-DGGE fingerprinting approach. We observed that butyrate producing bacteria such as Clostridium coccoides group and *Feacalibacterium* were reduced in known diabetic individuals (Figure 3). We observed inter-individual variation in micro-eukaryotic and archeal diversity. Sequencing of of some of the representative bands from DGGE gel showed presence of organisms belonging to groups: Metazoa, Stramenopiles and Viridiplantae. We noted that *Blastocystis sp* was most dominant microeukaryote found in all the individuals and had no difference in its abundance in healthy and diabetic subjects.

In conclusion, we observe that diabetic subjects display bacterial dysbiosis over archaeal and eukaryotic microbes. Many transient eukaryotic species detected in DGGE profile: Approach used was limited, groups' specific primers may help study eukaryotic diversity with respect to disease. Optimization of archaebacterial DGGE primer is needed to obtain clearer diversity profile.

All these studies taken together emphasize the uniqueness of the microbiome of the Indian population and the need for the detailed investigations.

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

Background

Cellular phenotypes arise due to complex spatio-temporal interplay of biomolecules. Characterizing these biomolecules through genomics and other high throughput techniques has made comprehensive computational modeling of biological systems possible. Such models structured within a mathematical frame work can be powerful tools to predict cellular phenotypes aiding construction of therapeutic designer circuits.

Life has evolved to evolve, and the propensity to evolve is known as "Evolvability". A living cell responds to environmental cues like temperature, pH, stress, disease etc. by changing the expression level of proteins, which are the cellular effectors. The constant change in the effector concentration, leads to cellular plasticity, tightly regulated by transcription factor (TF) activation state. They can either stimulate or repress the transcription of a specific gene, by recognizing short DNA motifs specific to certain TFs.

How evolvability correlates with the treatment strategy and how to drive pathogens into regions of low evolvability where they are eradicated most easily, is of importance to efforts for vaccine and drug engineering.

The protozoan parasite Leishmania (causative agent of leishmanaisis), have evolved evading strategies of host immune response by deregulating the milieu of transcription factors within the host. The outcome of such a deregulation is anti-inflammatory response leading to safe intracellular survival

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of the parasite. Synthetic circuits can be designed to counteract the evolved combat strategy of the parasite to push them to a region of low evolvability. This could be achieved by delineating and comparing the TFs that are deregulated in health and diseased condition within a mathematical frame work.

Aims and Objectives

- To reconstruct transcriptional factor target gene network (TFTG).
- Use knowledge of experimentally characterized transcription factor binding sites to analyse the promoter sequences for identification of potential binding site.
- To map the evolvability of the built-in TFTG network.

Work Achieved

Reconstruction of TFTG Network

The transcription factor target gene (TFTG) network construction of CD14-TNF-EGFR *in silico* signaling network was reconstructed to find out the crosstalk points in this network. Seven TFs involved in the signaling network were considered for TFTG network reconstruction. They are namely – NFkB, Stat1/3, Atf1/2, Irf1/3, Elk1, CREB and AP1. The transcription factor target gene network reconstructed consists of possibly all the genes that are involved in an immune response in leishmaniasis. In all 67 genes were incorporated in the network, that has a bipartite architecture. The network was then put through node selection in by considering each nodes betweeness centrality and edge betweeness (Fig. 1(a). By doing so, the complexity of the network in terms of parameter search was reduced i.e. from 67 genes the numbers of genes were reduced to 8. This TFTG network was numerically simulated considering the following kinetic laws:

- Δ Mass Action Equation - TF binding to Gene
- Hill Hinze Equation - Gene translated to Protein
- Michele Menten Equation - Protein Degradation

Using the above kinetic parameters three network designs were considered

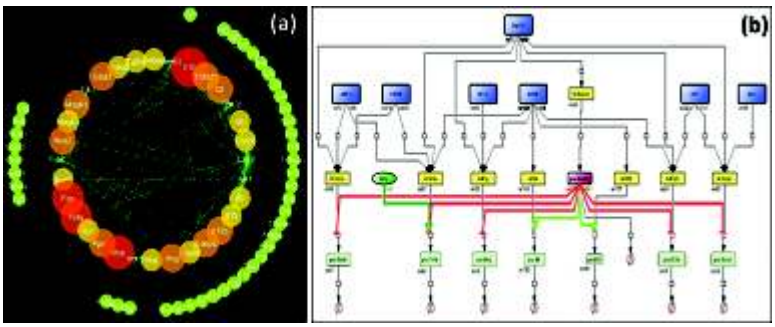
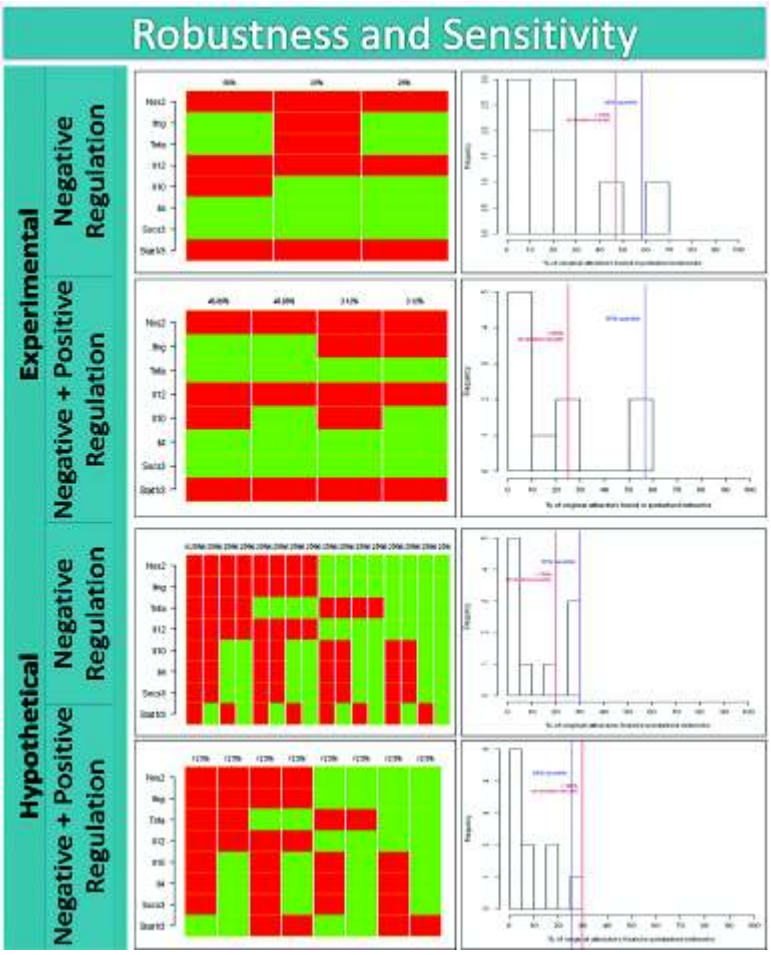


Fig.1: **(a)** Node Selection **(b)** Network Circuitry Showing Negative and Positive Regulation

Fig. 2: Robustness and Sensitivity built for TFTG Network



- Network with no regulatory control
- Network with negative regulatory loop
- Network with negative and positive regulatory loop (Fig. 1 (b)

In numerical simulation the parameters for the reaction were set in such a way that the final concentration of the gene products depicted diseased and a transition from diseased to healthy state.

Robustness, Sensitivity and Evolvability

Evolutionary developments often selected traits enhance robustness of the organism and therefore robustness is ubiquitous in living organisms that have evolved. But, a robust system is often fragile facing performance setback as an inherent trade-off. So, understanding the associated trade-offs is essential for identifying their faults (1) that may help design countermeasures for effective synthetic circuit therapeutics. TFs while acting on their target genes modulate

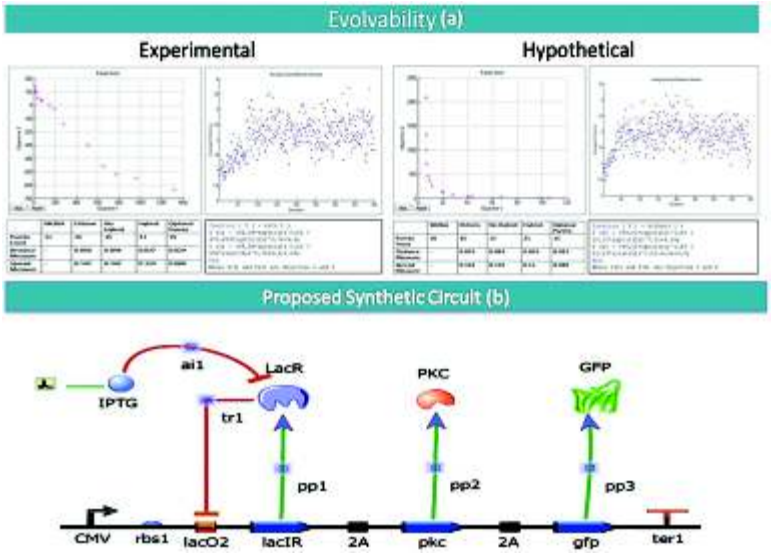


Fig. 3: **(a)** Evolvability of the built-in TFTG network of Leishmania **(b)** Proposed Synthetic Circuit

the fundamental molecular functions of a cell by specifying promoter regulatory elements, modulating gene output, tuning molecular noise, recruiting co-activator/repressor complexes and cooperating with other TFs to regulate a gene, giving rise to complex behavior of transcriptional networks. A framework of transcription regulation would be valuable to synthetic biology efforts, which construct synthetic regulatory elements based on the design principles of a gene regulatory network (2). Such bottom-up circuit construction, enable us to improve our understanding of the natural cell signaling in diseased condition, but also help modify the phenotypic fate at a cellular level, by engineering them to perform new customized tasks.

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Future Research Plans

- The synthetic circuit designed would be constructed in vitro for its application for therapeutic intervention in cutaneous leishmaniasis (Figure 3(b).

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Monoamine Oxidase-A (MAO-A) is an important source of oxidative stress and promotes Cardiac Dysfunction, Apoptosis, and Fibrosis in Diabetic Cardiomyopathy

Background

Diabetic cardiomyopathy characterized by cardiac dysfunction with subsequent heart failure in patients with diabetes mellitus in the absence of coronary atherosclerosis is a major cause of morbidity and mortality in diabetic patients. Oxidative stress has been widely implicated in diabetes and its many complications. The heart is particularly susceptible to oxidative damage, as it possesses lower levels of free radical scavengers in comparison to other organs. In vivo studies demonstrated that diabetes induced cardiac remodeling and dysfunction can be reduced by targeting ROS with oral or systemic antioxidant administration. However, the precise sources of ROS in the myocardium under diabetic condition are unknown. Despite the presence of various antioxidants and detoxifying enzymes, the mitochondria appear to be the major and most powerful source of reactive oxygen species (ROS) because these organelles continuously generate superoxide ($O_2^{\cdot-}$), as a byproduct of electron transport, so it is not unexpected that mitochondria have been shown to be a primary target of damage in diabetes. The mitochondrial apoptotic pathway appears to play an important role in diabetes-induced myocardial cell apoptosis, and among the apoptotic stimuli, ROS and/or reactive nitrogen species (RNS) play a critical role in the mitochondrial cytochrome c release and caspase-3 activation.

In addition to the respiratory chain, monoamine oxidase (MAO), a flavoprotein localized on the outer mitochondrial membrane, is another important mitochondrial source of ROS, in particular of H_2O_2. MAOs are responsible for oxidative deamination of neurotransmitters and dietary amines. During this

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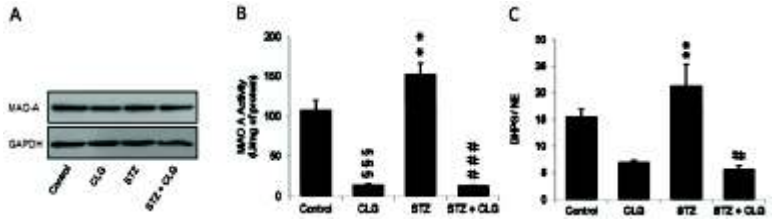

Fig. 1: Diabetes induces myocardial MAO A activity. **(A)** MAO A protein expression **(B)** MAO A activity and **(C)** Quantification of DHPG/NE ratio by HPLC method. All values are given as mean ± SE(n=5/group); ** p< 0.01 vs control group, §§§p< 0.001 vs control group, # p< 0.05, # # #p< 0.001 vs STZ group

process they generate H_2O_2 and reactive aldehydes as by products. Based on substrate specificity and inhibitor sensitivity two isoforms of MAO have been identified MAO A and MAO B. However, MAO A appears to be the predominant isoform in the myocardium of several species. Recent findings demonstrate that pharmacological or genetic inhibition of MAO A prevent maladaptive remodeling and left ventricular dysfunction in mice hearts subjected to pressure overload. Overexpression of MAO A in mouse heart causes oxidative stress mediated mitochondrial damage and cardiomyocyte necrosis leading to ventricular dysfunction. Moreover the important role of MAO as relevant source of ROS in ischemia/reperfusion (I/R) injury was demonstrated both ex vivo and in vivo. Yet the involvement of MAO A in diabetic cardiomyopathy is not defined.

Aims and Objectives

- To investigate whether MAO A can potentially be a relevant source of ROS and contribute to development of diabetic cardiomyopathy.

Work Achieved

Our previous work showed that exposure of H9c2 cardiomyoblast cells to high-glucose (HG) resulted in apoptosis via an increase in the activity of MAO-A, ROS levels, loss of mitochondrial membrane potential and activation of caspase cascade. These changes could be normalized after the treatment with a specific inhibitor of MAO A clorgyline. Further, to validate our *in vitro* findings *in vivo* we induced diabetes in male Wistar rats by single Intraperitoneal (ip) injection of streptozotocin (55mg/kg body weight). MAO A inhibition was carried out by clorgyline (1mg/kg body weight/day, ip) for 2 months. Induction of Diabetes in

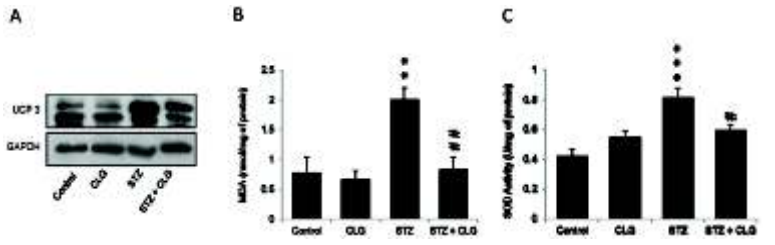

Fig. 2. MAO A inhibition limits diabetes-induced myocardial oxidative stress. **(A)** UCP3 protein expression, **(B)** Lipid peroxidation (tissue malondialdehyde (MDA) concentrations)and **(C)** SOD activity. All values are given as mean ± SE (n=5-9/group); ** p< 0.01, ***p< 0.001 vs control group, #p< 0.05, # #p< 0.01 vs STZ group.

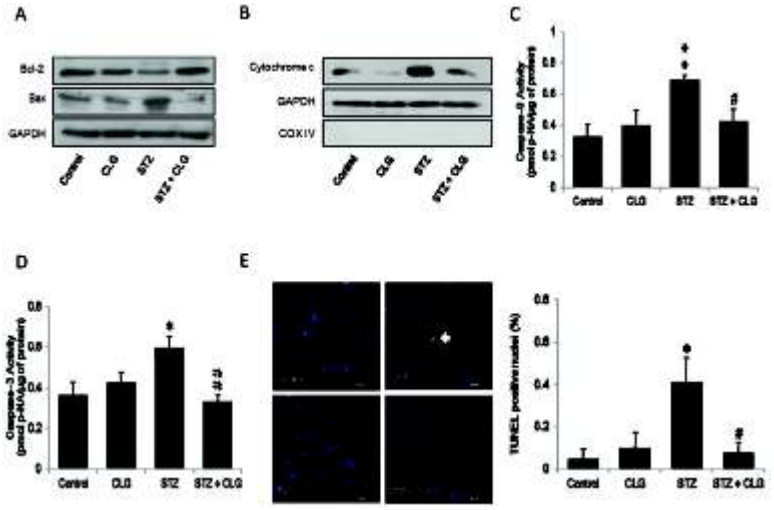

Fig. 3. MAO A inhibition prevents diabetes-induced myocardial apoptosis. **(A)** Bcl-2 and Bax protein expression, **(B)** Analysis of cytosolic cytochrome c levels, **(C)** Caspase-9 activity, **(D)** Caspase-3 activity and (E) Examination of cardiac apoptosis using TUNEL staining; Representative TUNEL section shown on left, arrow indicate TUNEL positive nucleus and quantification (right). All values are given as mean ± SE (n=5/group); *p < 0.05, **p< 0.01 vs control group, #p< 0.05, # #p< 0.01 vs STZ group.

STZ treated animals was confirmed by increase blood sugar levels, decline in serum insulin levels. Diabetic cardiomyopathy was characterized by declined systolic and diastolic performance, increased heart weight to body weight ratio and Biochemical Markers of Myocardial Injury in serum. Diabetes up regulated MAO A activity, elevated markers of oxidative stress such as cardiac lipid peroxidation, superoxide dismutase (SOD) activity and UCP3 protein expression, enhanced apoptotic cell death and increased fibrosis. All these parameters were significantly attenuated by CLG treatment. In addition, treatment with CLG substantially prevented diabetes-induced cardiac contractile dysfunction as evidenced by decrease in QRS, QT and QTc intervals measured by ECG and LV systolic, LV end diastolic pressure measured by microtip pressure transducer. These beneficial effects of CLG were seen despite the persistent hyperglycemic and hyperlipidemic environments in STZ-induced

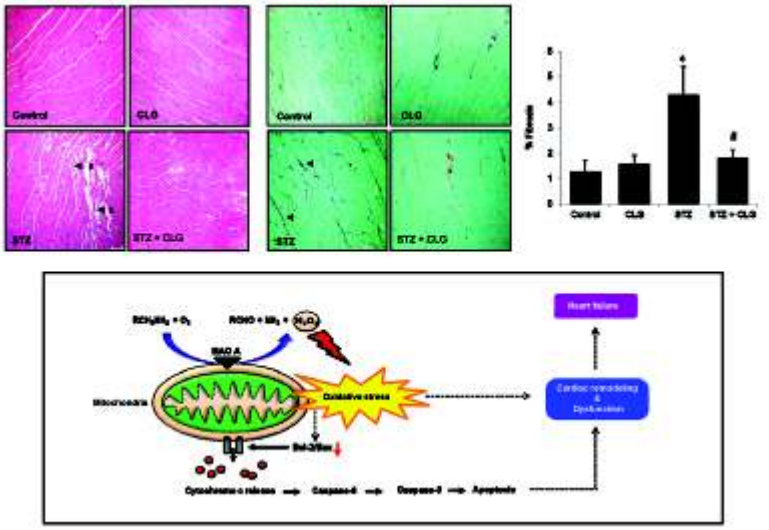

Fig.4: MAO A inhibition attenuates diabetes-induced changes in myocardial histology and cardiac fibrosis **(A)** Representative H&E stained cardiac sections; arrows demonstrate a: separation and b: degeneration of myocardial fibers, **(B)** Cardiac fibrosis. Representative Sirius red-stained sections (left), collagen appears red as indicated by arrows and quantification (right). All values are given as mean ± SE, (n=5/group); *p< 0.05 vs control group, #p< 0.05 vs STZ group.

experimental diabetes. In conclusion, this study provides strong evidence that MAO-A is an important source of oxidative stress in the heart and that MAO-A-derived reactive oxygen species (ROS) contribute to DCM.

Future Research Plans

- Further, efforts are under way to investigate whether reactive aldehyde produced during MAO A catalyzed reaction contributes to mitochondrial dysfunction and to elucidate the cross talk mechanism between MAO A and mitochondrial aldehyde dehydrogenase (ALDH2) in diabetic cardiomyopathy.

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Moving fates around: understanding the role of endocytosis in cell fate transitions

Background

Vesicular transport or trafficking is required for the accurate transport of molecules within a cell. This cellular process requires the simultaneous action of a number of cellular components including proteins that can alter the curvature of the membrane, modify cytoskeletal architecture and allow fusion of membranes to form vesicles. A number of studies have shown that alterations in the process of trafficking can affect the acquisition of pluripotency through reprogramming assays. However, a detailed analysis of the role of trafficking in the maintenance and acquisition of pluripotency remains to be carried out. It is towards this goal that the following aims have been proposed.

Aims and Objectives

- To uncover components of the vesicular transport machinery that play a role in reprogramming and maintenance of pluripotency.
- To define differences in the endocytic proteome between somatic cells and ESCs.

Work Achieved

Knockdown of genes involved in endocytosis affects pluripotency of mouse embryonic stem cells

In order to determine whether genes involved in endocytosis and vesicular transport indeed regulate the pluripotency of stem cells, we used commercially available siRNAs against 112 genes implicated in the process of endocytosis. siRNAs were introduced individually into each well of a 96 well plate in which mESCs were plated. In order to prime mESCs to differentiate, LIF was withdrawn from the media at the time of siRNA transfection (Fig. 1a). A number of controls

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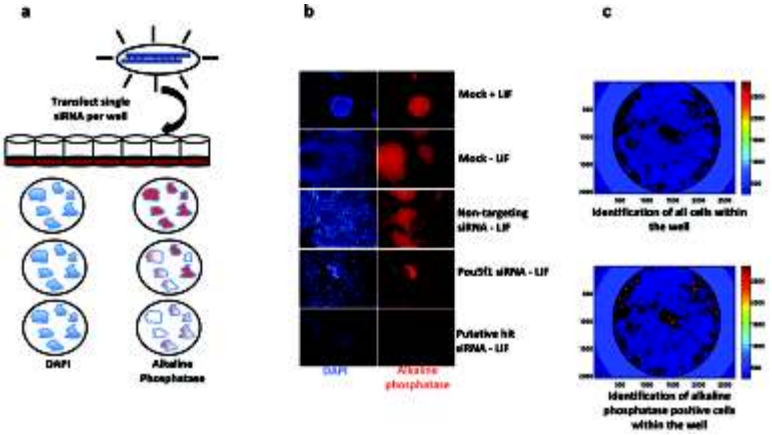

Fig. 1: schematic of siRNA screen to knockdown genes involved in endocytosis in mouse embryonic stem cells: (a) Schematic of siRNA screen. mESCs were plated in 96 well plates. 24 hours post plating, individual siRNAs were introduced into each well at a final concentration of 50nM. At the time of transfection, cells were shifted to ES media minus LIF. Cells were fixed on day 5 and stained for DAPI and alkaline phosphatase (AP) activity. (b) Representative images of mESCs on day 5 treated with different conditions and showing the extent of AP staining. (c) Analysis workflow involving identification of all cells within a well, followed by identification of alkaline phosphatase positive cells within that well.

were included in this screen. These included mock transfected cells that received only the liposome-based transfection reagent. Mock transfected cells were maintained both in ES media containing and deprived of LIF. Consistent with a role for LIF in maintaining pluripotency, cells maintained in the presence of LIF displayed higher alkaline phosphatase (AP) activity, while cells maintained in ES media minus LIF showed less AP activity (Fig. 1b). SiRNA against the pluripotency regulator Oct3/4, showed a further decrease in AP activity, consistent with the ESCs undergoing differentiation. Other controls included siRNAs against Nanog, another major regulator of pluripotency. Non-targeting siRNA controls were also included in the screen. Image analysis was done in an automated manner to avoid any bias or variation in the scoring process. Briefly, image analysis involved acquisition of an image of the complete well. Each well was individually imaged in every experimental plate. This was followed by identification of all cells within each well (N1). Within N1, identification of alkaline phosphatase positive cells was carried out (N2) (Fig 1c). N2/N1 gives us the percentage of alkaline phosphatase positive cells within each well. This value was then normalized to the N2/N1 obtained from mock-transfected cells maintained in ES-LIF conditions. If the normalized value remained close to or equal to 1, then it indicated that the knockdown of that particular gene did not affect differentiation or pluripotency compared to the control. If the value was

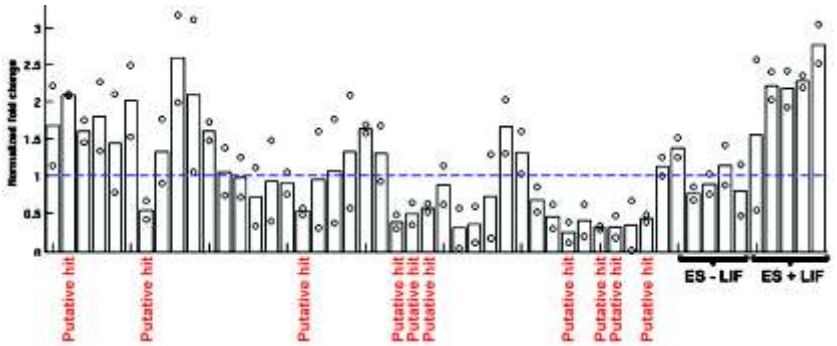

Fig. 2: Knockdown of genes involved in endocytosis and vesicular trafficking impact pluripotency of mouse embryonic stem cells: Graphs showing normalized fold change in Alkaline phosphatase staining compared to staining observed in mESCs grown under ES-LIF conditions. Each screen was repeated twice and values from one-third of the screen are represented. The bar represents the mean value of both screens. The blue dotted line represents the mean value of all the control ES-LIF wells from each plate. Marked in red are genes that showed a significant difference in AP staining compared to controls upon knockdown.

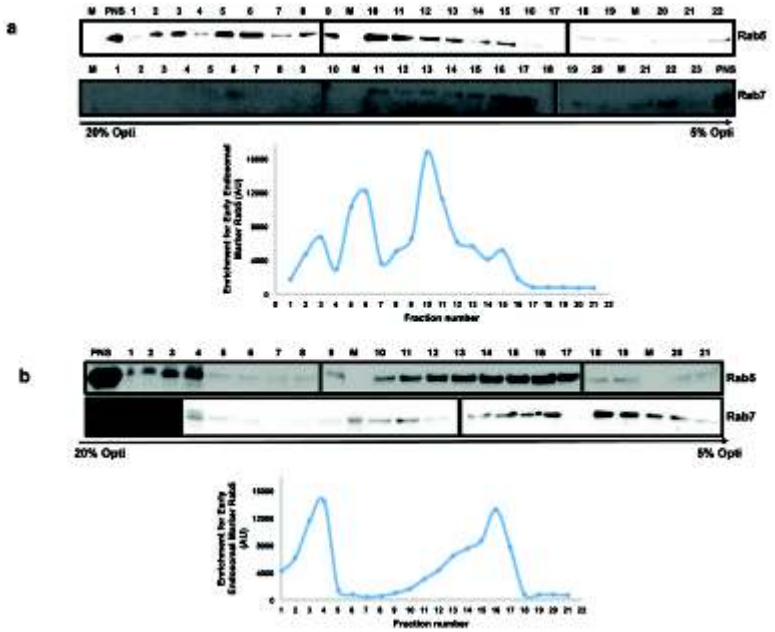

Fig. 3: Isolation of early endosomes from mouse embryonic stem cells and mouse embryonic fibroblasts. Western blotting of fractions isolated from mESCs **(a)** and MEFs **(b)** after density gradient centrifugation. Fractions were probed for the presence of the early endosomal marker Rab5 and late endosomal marker, Rab7. The graph shows a quantitation of the fractions that were enriched for Rab5.

below 1, it indicated that there were less alkaline phosphatase positive cells upon knockdown of a particular gene compared to the control wells, indicating that the particular gene positively regulated pluripotency. Conversely, a value above 1 indicated that knockdown of the gene caused an increase in alkaline phosphatase (AP) activity, suggesting that the gene negatively regulated pluripotency. We obtained a number of hits from this screen (Fig 2), which we are validating at present.

Isolation of early endosomes from mESCs and MEFs

In order to determine the proteome of the early endosome in both mESCs and MEFs, we adopted a previously published protocol to isolate early endosomes . We have been successful in isolating early endosomes from mESCs (Fig 3a) and MEFs (Fig 3b) and are in the process of purifying these fractions to enable detection of the proteins present in these fractions by mass spectrometric analysis.

Future Research Plans

- Validation of targets from the siRNA screen and determination of content of the endosomes by mass spectrometry is under progress. The function of these hits will further be validated during early mouse embryonic development.

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Role of IL-3 in regulation of cartilage biology

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Background

Osteoarthritis (OA) is a degenerative disease of joints, characterized by progressive loss of cartilage and subchondral bone. Genetic predisposition and environmental factors such as gender, obesity, misalignment and injuries increase the risk of developing OA. Cartilage at articular surface helps in smooth mobility of joints with least friction due to the tensile strength of matrix secreted by cartilage cells. Cartilage is composed of a sparse population of chondrocytes distributed in collagen and proteoglycan-rich extracellular matrix. Chondrocytes participate in synthesis as well as degradation of cartilage matrix, and are highly sensitive to pathological changes in the joint microenvironment. Any injury that leads to irreversible physical damage to cartilage induces an inflammatory microenvironment in joints. The inflammatory microenvironment at the affected joints attracts immune cells leading to enhanced degeneration of cartilage matrix and bone. The pro-inflammatory cytokines such as IL-1β and TNF-α are the key players in pathophysiology of OA. These cytokines promote the onset of disease and enhance the degenerative processes by stimulating apoptotic and matrix degrading pathways in affected cartilage. Matrix metalloproteinases (MMPs) triggered by pro-inflammatory cytokines, assist in cartilage destruction during arthritic conditions. Hypertrophic chondrocytes lose their proliferative potential as well as the property of matrix synthesis and hence cannot compensate for the cartilage damage.

IL-3, a cytokine secreted by activated T lymphocytes, stimulates proliferation, differentiation and survival of pluripotent haematopoietic stem cells. Previously,

we have documented that IL-3 irreversibly inhibits *in vitro* osteoclast differentiation induced by receptor activator of NF-B ligand and TNF-α in both mouse and human osteoclast precursors. IL-3 is a potent inhibitor of pathological bone resorption induced by TNF-α and other pro-inflammatory cytokines such as IL-1α, TGF-β1, TGF-β3, IL-6 and prostaglandin E2. Recently, we demonstrated that IL-3 has an anti-inflammatory activity *in vivo* and indirectly protects cartilage and bone damage in inflammatory arthritis and collagen-induced arthritis model of rheumatoid arthritis. However, the role of IL-3 on chondrocyte differentiation and cartilage biology is not yet known. In this study, we investigated the effect of IL-3 on chondrocyte differentiation and function under both *in vitro* and *in vivo* conditions.

Aims and Objectives

- To evaluate the role of IL-3 in regulation of chondrocyte differentiation.
- To investigate the role of IL-3 on cartilage degeneration in mouse model of human OA.

Work Achieved

IL-3 protects cartilage matrix by down-regulating pro-inflammatory cytokines induced expression of MMPs

To evaluate the effect of IL-3 on chondrocytes, we first examined the expression of IL-3Rα on cultured chondrocytes. Both mouse and human chondrocytes showed strong expression of IL-3Rα and chondrocyte specific genes Sox9, Col2a and aggrecan at gene and protein levels. It was observed that IL-3 alone does not alter the proliferation and differentiation of chondrocytes. IL-1β and TNF-α are key players in amplifying the inflammatory conditions and induce cartilage degeneration in OA joints. IL-3 has been reported to have anti-inflammatory activity *in vitro* and *in vivo*, therefore, we further investigated the effect of IL-3 on mouse chondrocytes in the presence of IL-1β. Mouse chondrocytes were incubated with IL-1β (5 ng/ml) and different concentrations

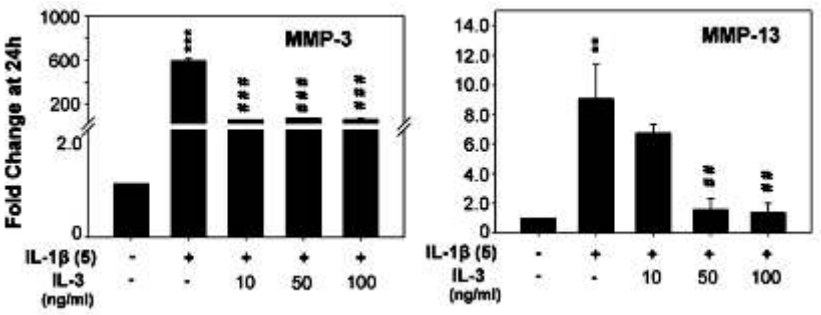


Fig. 1: IL-3 down-regulates pro-inflammatory cytokines-induced expression of matrix degrading enzymes in mouse chnondrocytes. Mouse chondrocytes were treated with IL-1β and different concentrations of IL-3 for 24 hours. The expression of matrix degrading enzymes - MMP-3 and MMP-13 was evaluated by real-time PCR. **p < 0.01 and ***p < 0.001 versus untreated controls. ##p < 0.01 and ###p < 0.001 versus IL-1β. Results are representative of two independent experiments.

of IL-3 for 24 hours. IL-1β inhibited the expression of chondrocyte specific genes including Sox9, Col2a and aggrecan, and up-regulated the expression of matrix degrading enzymes- MMP-3 and MMP-13. We observed that IL-3 significantly up-regulated the expression of Sox9 and Col2a which were down-regulated by IL-1β, and the effect was more evident at 50 and 100 ng/ml of IL-3. Interestingly, IL-3 treatment significantly down-regulated IL-1β-induced expression of matrix degrading enzymes - MMP-3 at all the concentrations and MMP-13 at 50 and 100 ng/ml of IL-3 (Fig. 1). This suggests an anabolic role of IL-3 on cartilage matrix by promoting synthesis of matrix components and by down-regulation of matrix degrading enzymes secreted by chondrocytes.

In human chondrocytes, both IL-1β and TNF-α individually as well as in combination down-regulated the expression of Col2a and aggrecan which was not restored to normal levels upon IL-3 treatment while no change in the expression of Sox9 was observed. In agreement with mouse system, IL-3 significantly down-regulated the expression of MMP-3 induced by IL-1β. However, a slight decrease was observed in the expression of MMP-1 and MMP-13 by IL-3. TNF-α showed a non-significant increase in the expression of all the three MMPs, which was also down-regulated by IL-3. Interestingly, the synergistic effect of IL-1β and TNF-α on the increased expression of MMP-1, MMP-3 and MMP-13 was also significantly down-regulated by IL-3 (Fig. 2). These data suggest the inhibitory role of IL-3 on catabolic processes induced in chondrocytes in the presence of pro-inflammatory cytokines. It shows that under inflammatory conditions, IL-3 prevents cartilage damage by regulating matrix degradation mediated by MMPs along with neomatrix synthesis.

IL-3 prevents cartilage degeneration in mouse model of human OA

To evaluate the *in vivo* role of IL-3 on cartilage damage, we developed a mouse model of human OA by surgical transection of anterior cruciate ligament (ACL) in knee joints. ACL transection alters the joint biomechanics and subsequently leads to the development of OA. We observed OA changes in articular cartilage and subchondral bone microarchitecture at day 8 post-surgery. Cartilage in OA mice was disrupted and discontinuous, hypo-cellular with clonal clusters and

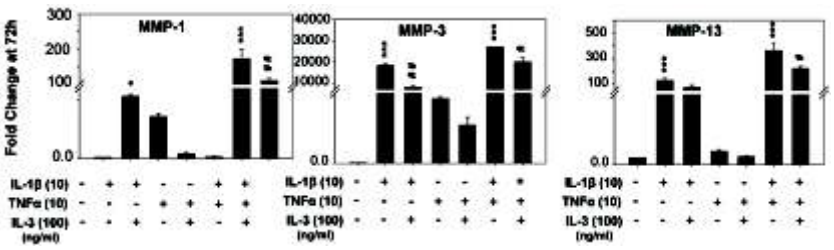
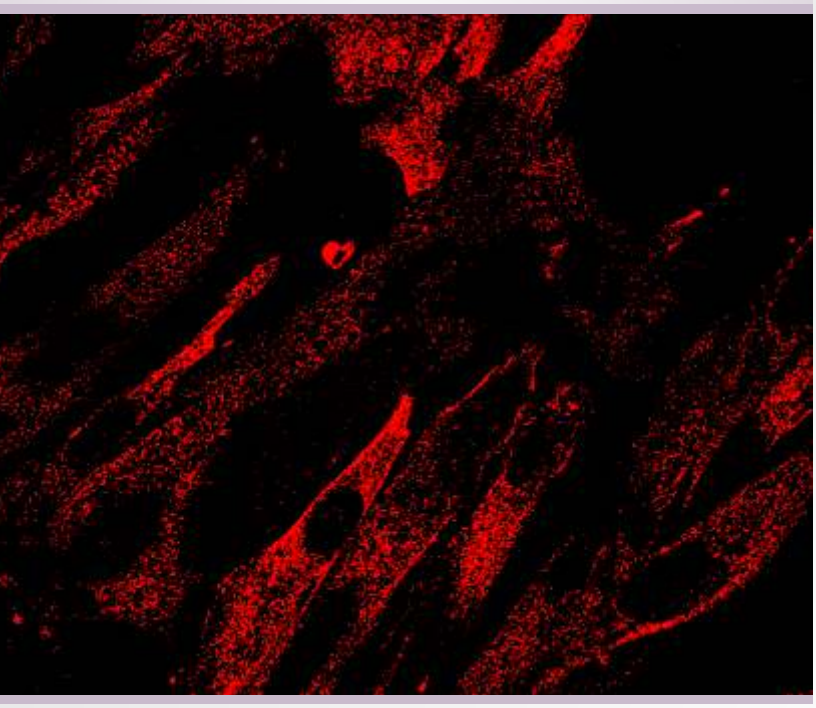


Fig. 2: IL-3 down-regulates pro-inflammatory cytokines-induced expression of matrix degrading enzymes in human chondrocytes. Human chondrocytes were treated with IL-1β and/or TNF-α and different concentrations of IL-3 for 72 hours. The expression of matrix degrading enzymes - MMP-1, MMP-3 and MMP-13 was evaluated by real-time PCR. *p < 0.05 and ***p < 0.001 versus untreated controls. #p < 0.05 and ##p < 0.01 versus IL-1β or TNF-α treated groups. Results are representative of two independent experiments.

with hypertrophic chondrocytes or empty lacunae towards the articulating surface. The superficial zone of resting cells was either eroded or differentiated to hypertrophic stage. The OA mice were then injected with recombinant mouse IL-3 (100 ng/day) intra-articularly for four different time points. Sections of knee joints were evaluated for osteoarthritic changes in joint articulation. In preliminary findings we observed distinct cartilage articulation and normal phenotype of chondrocytes in different zones of cartilage in OA mice treated with IL-3. Also, there was reduced number of clonal clusters and hypertrophic cells near articulating surface of cartilage in IL-3 treated mice. These results suggest that IL-3 has a potential to prevent cartilage damage.

Future Research Plans

- In further studies we will investigate the mechanism(s) of IL-3 action in regulation of MMPs in chondrocytes. The degenerative changes in cartilage percolate down to subchondral bone region as both are interdependent. We will also evaluate the effect of IL-3 on subchondral bone microarchitecture by microcomputed tomography.



Support Units & Other Facilities

Experimental Animal Facility

Dr. Ramanamurthy Boppana

(Facility In-Charge)

The Experimental Animal Facility is a core scientific department of the Institute with an objective to breed, maintain and supply quality laboratory animals for research and development. The facility is registered with the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA) and operates in compliance with the guidelines laid down by the Committee. It is a barrier-maintained facility for the breeding, maintenance of small laboratory animals viz. inbred and mutant mice, rats, rabbits etc. for the ongoing research projects of the Institute. The section also extends complete research and technical support to facilitate animal experimentation in the Institute. The following is the list of various laboratory animals maintained at the facility:

MICE:

- BALB/cJ
- C57BL/6J
- DBA/2J
- DBA/1/J
- 129/SvJ
- FVB/NJ
- SWISS#
- BALB/c*
- NZB
- AKR#
- CF1

Genetically engineered mutant mice (knock-out, transgenic and mutant mice - 38 lines)

RATS:

- WISTAR

RABBITS:

- NEWZEALAND WHITE

The Team

Dr. Rahul M. Bankar
Mr. Md. Shaikh
Mr. A. Inamdar
Mr. P.T. Shelke
Ms. Vaishali Bajare
Mr. Mahavir Rangole
Mr. Rahul B. Kavitake
Mr. Ganesh B. Yadav
Mr. Sanjay Gade
Mr. Harshal G. Gaonkar
Mr. Dilip B. Thorat

* BALB/c with cataract mutation
# Outbred

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

The breeding program for the propagation of the inbred mice is based on the established principles of genetics and breeding and is planned and executed to meet the needs of Scientists of the Institute for the conduct of animal experiments. The breeding program involving mutant mice is structured as per the genetic requirement of the specific strain concerned.

The total number of mice strains, inbred, outbred, and mutant and hybrids, being maintained at the Experimental Animal Facility stands at 51. The foundation/nuclear colonies of mice are housed in Individually Ventilated Caging systems.

Complete scientific support and advice is extended regularly to Scientists and their group members for the conduct of experiments under IAEC approved projects.

The facility conducts training/course work for the research fellows of the Institute in the area of Laboratory Animal Experimentation and Ethics.

Proteomics Facility

Dr. Srikanth Rapole
(Facility In-Charge)

The Team

Mrs. Snigdha Dhali, *Technician*

Mr. Venkatesh Naik, *Technician*

4800 MALDI-TOF/TOF

4000 Q-Trap LC-MS/MS

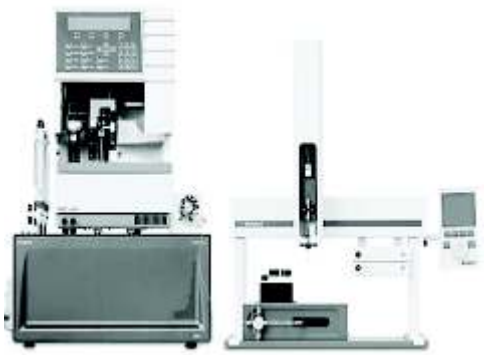
The proteomics facility is a core service facility of the institute with an objective to provide mass spectrometric analysis of biological samples. The following is the list of various instruments available at the facility:

4800 LC-MALDI TOF/TOF system (AB Sciex) is a tandem time-of-flight MS/MS system that is used for high-throughput proteomics research. The system identifies proteins by determining accurate masses of peptides formed by enzymatic digestion. Additionally, the system can more definitely identify and characterize proteins by isolating and fragmenting a molecular ion of interest and measuring the fragment ion masses. The number of samples analyzed is approximately 799 samples including 176 external samples from April-2014 to March-2015.

4000 Q-Trap LC-MS/MS system (AB Sciex) is a hybrid triple quadrupole/linear ion trap mass spectrometer. The system is ideal for proteomic applications including post-translationally modified proteins discovery, protein identification, and biomarker validation. The number of samples analyzed is approximately 152 samples including 10 external samples from April-2014 to March-2015.

Eksigent Tempo Nano MDLC system is a high performance, reliable, nano-scale liquid chromatography for proteomic applications. This Nano-LC system delivers precise and reproducible micro-scale gradients at constant flow-rates, creating ideal conditions for optimized mass analysis and a stable ionization spray. It is connected to 4800 MALDI-TOF/TOF system for proteome analysis.

Eksigent Express Micro LC-Ultra System is an advanced micro-LC technology with its pneumatic pumps, integrated autosampler, ultra-sensitive, full-spectral UV detector, and temperature-controlled column oven. And with Eksigent's intuitive software, users get full system control as well as complete analysis and



EKSIGENT NANO-LC and SPOTTER

EKSIGENT MICRO-LC

AGILENT GC-MS

reporting capabilities. The advantage of Eksigent's state-of-the-art micro-LC is running fast analysis with excellent reproducibility and only a small fraction of the solvent used.

Eksigent EKSpot MALDI Spotter couples Nano MDLC to MALDI mass spectrometer which results in an extremely powerful tool for the analysis of complex peptide/protein samples. This spotter holds 16 AB SCIEX 4700 targets or eight microtiter plate size targets. Each of the targets can hold up to 1,000 spots and it generates up to 8,000 spots on an overnight run.

Shimadzu Prominence UFLC is higher speed and uncompromised separation liquid chromatography instrument. It provides ten times higher speed and three times better separation when compare with normal conventional HPLC. In addition to ultra fast analysis, UFLC is also used for many applications such as conventional HPLC analysis and semi-preparative analysis.

2-D DIGE proteomics set-up including Ettan IPGphor isoelectric focusing unit, Ettan DALT unit, DIGE Typhoon FLA 9000 scanner, DeCyder 2-D DIGE analysis software, and Ettan spot picker. This set-up is used for differential protein expression studies, biomarker discovery, quantitative proteomics etc.

Gas Chromatography Mass Spectrometry (GC-MS) system (Agilent) with new 7890B GC and 5977A MSD provides unmatched sensitivity for ultra-trace analysis, and increased performance. It is highly suitable for volatile and semi-volatile compounds. GC-MS set-up is used for identifying volatile metabolites involving in cancer.

Bioinformatics and High Performance Computing Facility

Dr. Shailza Singh
(Facility In-Charge)

The bioinformatics facility at NCCS provides access to high-performance computing resources and programming expertise. The compute infrastructure serves scientists at NCCS to master the informatics needs of their research in a proficient and cost-effective manner.

Hardware Infrastructure

SGI Altix XE 1300 Cluster

Head Node:

SGI Altix XE 270 Serve.

Dual Quad Core XEON 5620 @ 2.4GHz / 12MB cache,12GB Memory,5 x 2TB SATA Disk @ 7.2K RPM RAID 5

Compute Nodes:

SGI Altix 340 Servers

2 x HEXA Core XEON 5670 @ 2.93GHz / 12MB cache, 24GB Memory, 250GB SATA Disk @ 7.2K RPM, Dual Gigabit Ethernet Card

SGI Cluster Software Stack:

SLES Ver 11

SGI ProPack 7

SGI Foundation Software Ver 2.0

Interconnect:

24-Ports Gigabit Ethernet Switch

GPU Computing HP Proliant SL6500

2x Intel Xeon X5675 @3.06GHz/6 core/12MB L3 Cache

96 GB (8 GB x 12) PC3 – 10600 (DDR3 – 1333) Registered DIMM memory

2 x 1 TB hot Plug SATA Hard Disk @7200 rpm

Integrated Graphics ATI RN50/ES1000 with 64 MB memory

2x NIVIDIA Tesla 2090 6 GB GPU computing module

Specialized Workstations:

HP Elite 8200 CMT PC

Second generation Intel core i7-2600 processor 3.40 GHz, 8M cache, 4 cores/8 threads

Intergrated 4 port SATA 6GBs controller

Intergrated Intel HD graphics

HP Z800 High End Work Station (2 in number)

2x Intel Xeon E5649 6 core @2.53 GHz, 80 watt 12MB cache

5.86GTs QPI, DDR3 1333 MHz, HT Turbo

NVIDIA Quadro FX380 Graphics with 256MB memory

SATA 6 GBs controllers with RAID 0/105 & 10 support

19" LCD wide Display with Windows OS

HP Z820 High End Work Station

2x Intel Xeon E5-2690@2.9GHz, 8 core/20MB L3 cache

8 GTs QPI, DDR3 1600 HT Turbo 2 with vPro support

NVIDIA Quadro 4000 Graphics with 2GB DDR memory

SATA 6 GBs controllers with RAID 0/105 & 10 support

22" LCD wide Display with Windows OS

High End Desktop (2 in number)

HP workstations of Intel Core 2 Duo @3.00GHz with 8 GB of DDR2 memory, 320 GB of SATA storage and 19" LCD wide Display with Linux/Windows OS

Desktop Computers

Desktop computers with Intel core 2 duo processor @1.8Ghz to 2.8GHz with 2 GB to 4 GB of DRR2 memory, 160GB to 320GB of SATA storage with 17" wide LCD display and with Windows XP OS

iMAC: For running specialized software like Biojade

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

APC UPS 10 KVA for supporting the HPCF

Software infrastructure

The Bioinformatics Facility at NCCS has procured several software for scientific research having commercial and/or academic license. These are:

Sequence analysis: BLAST, CLUSTAL-W, MEGA, Eisen

Molecular Modeling: Modeler (DISCOVERY STUDIO 3.0), Protein Families (DISCOVERY STUDIO 3.0), Protein Health (DISCOVERY STUDIO 3.0), Protein Refine (DISCOVERY STUDIO 3.0), Profiles-3D (DISCOVERY STUDIO 3.0)

Molecular Docking: Flexible Docking (DISCOVERY STUDIO 3.0), LibDock (DISCOVERY STUDIO 3.0), Ludi (DISCOVERY STUDIO 3.0), LigPrep (DISCOVERY STUDIO 3.0), LigandFIT (DISCOVERY STUDIO 3.0), LigandScore (DISCOVERY STUDIO 3.0), AUTODOCK, Database of 1.5million Compound Library (DISCOVERY STUDIO 3.0)

Pharmacophore Modeling: Auto Pharmacophore generation, Receptor-ligand pharmacophore egeneration,3D QSAR pharmacophore generation, Steric Refinements with excluded volumes. (DISCOVERYSTUDIO3.0)

Toxicity Prediction: ADMET (DISCOVERY STUDIO 3.0), TOPKAT (DISCOVERY STUDIO 3.0),

QSAR: Create Bayesian Model, Recusrive Partioning Model, Multiple Linear Regression Model, partial least squares model, genetic function approximation model, 3D QSAR model (Discovery Studio 3.0). Intelligent QSAR using molecular fragments of interest and their features, evaluation of descriptors from template scaffold to form relationship with the activity.

Molecular Dynamics: CHARMM, GROMACS, NAMD, MOIL

Molecular Visualization: Rasmol, MolMol, WinCoot, Swiss PDB viewer, MolScript, VMD

ab initio modeling: GAUSSIAN

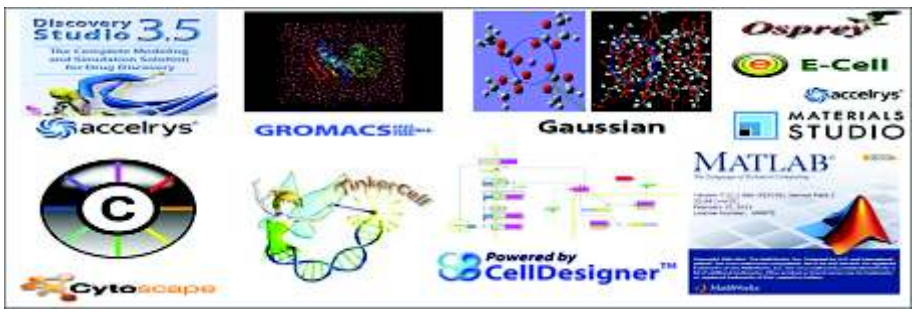
Systems Biology Tools: Virtual Cell, M-cell, Cell Designer, GEPASI, Cytoscape, Osprey, E-Cell, SimBiology

Artificial Intelligence: SVMlight and SNNS

Material Modeling and Simulation: Material Studio 5.5

Graphs and Graphics: Sigma Plot, GNU Plot, Corel Draw and Adobe PhotoShop

Statistical packages: MATLAB and R



Library

The NCCS library has a collection of publications in frontier areas of biotechnology having relevance to NCCS research activities. In the development of its collections, the library's priority is to support NCCS research activities. Therefore, the library collection is expanded in consultation with NCCS scientists. The library's print collections are growing by approximately 600 volumes per year. The library holds approximately thirteen thousand five hundred bound journals, three thousand books and two hundred NCCS Ph.D. theses. It subscribes to twenty scientific journals and twenty eight other periodicals in print form. Additional documentation facilities include local area network for library activities and PubMed database access, a number of CD-ROMS for books, journals & Ph.D. theses. The library is listed in the Union Catalogue of Biomedical Serials in India created by the National Informatics Centre, New Delhi and continues to be a member of the Medical Library Association of India. The Library provides access to 734 online publications of various Publishers such as Springer, John Wiley, Nature Publishing group, Mary & Libert, Oxford, Elsevier & Science Direct (including one Online Book, Methods in Enzymolgoy series) and those available through 'DeLCON', the DBT online journal consortium. In addition to DeLCON, it also subscribes to seven other online journals that are related to NCCS research activities.

The library also maintains other useful information in Hindi and English on the NCCS website and intranet, which includes NCCS research publications, library forms, 'NCCS in the News', Ph.D. theses of NCCS students, a list of NCCS Alumni, as well as links for a free Online Medical database, and Scientific Grants\Funds and fellowships. Furthermore, the library provides in-house services to scan documents for plagiarism using the iThenticate Anti-Plagiarism Software.

The library is equipped with Linux-based SLIM21 with RFID-Interfaced library software for library housekeeping operations, and Web-OPAC for online searching of the library documents. The library has also installed barcode technology for the circulation (issue & return) of library documents.

During the period under review, the library has further expanded its digital archive of NCCS Ph.D. theses, NCCS Publications and Book Chapter archives, and NCCS Annual Reports, which are accessible through the NCCS Intranet.

The Team

Mr. Krupasindhu Behera, *Technical Officer*

Mr. Rameshwar Nema, *Technical Officer*

Computer Section

Dr. M.V. Krishnasastry
(Facility In-Charge)

The computer section has developed the new NCCS Intranet Portal using a 'model-view-controller' architecture which helps the scientists and heads of the various departments to upload seminar notices, office circulars, notices etc. This new portal has been made more user-friendly, to enable any NCCS user to retrieve required information, such as department-wise staff list, telephone lists, inventory of all the staff members, current status of the stock in the stores section, information about the facilities etc., at the click of a mouse.

Acquisition of New Hardware

NCCS has acquired new HP Blade Servers. These systems are configured and installed in the Linux Server to implement and host the following services on VMware virtualize environment:

a) WEB server, DNS server, SMTP Mail server .
b) File sever and Print server
c) Setting up four Linux Proxy server for Internet connectivity.
d) Windows DHCP and Antivirus server

Storage upgradation of NAS server is configured to take a backup of the above configured servers.

Expansion of LAN

A secured NCCS wireless LAN has been installed and configured in many locations inside the campus.

General Assistance

Regular maintenance and updating of the NCCS website and intranet website is done by the Computer Section. Several operating systems and common application softwares were installed / updated on user computers at NCCS. These include MS-OFFICE 2010, Adobe Suite X, Sigma Plot Suite 12.0 and Reference Manager 12.0. In addition, the Paypack salary software was installed and configured for the NCCS salary process, which takes care of TDS, EPF and NPS deductions.

The Team

Mr. Rajesh Solanki, *Technical Officer*
Mr. Shivaji Jadhav, *Technical Officer*
Mrs. Rajashri Patwardhan, *Technical Officer*
Mrs. Kirti Jadhav, *Technical Officer*

Installation of Biometric Time Attendance

NCCS successfully installed and commissioned the Biometric Time Attendance, Access Control, Visitor Management and CCTV (Video) Surveillance System this year. This system takes care of attendance and leave records, etc., of all the staff and students. For visitor management, a kiosk has been installed at the main gate of NCCS in the security cabin, which takes care of issuing gate passes to visitors. Continuous CCTV surveillance recording is done at the NCCS main gate as well as residence gate.

Other Facilities

1) FACS Core Facility

The Team

- **Dr. L. S. Limaye** (Facility In-charge)
- Hemangini Shikhare (Technician)
- Pratibha Khot (Technician)
- Amit Salunkhe (Technician)
- Ashwini Kore (Technician)
- Dnyaneshwar Waghmare (Technician - joined on 11/03/2015)
- Atul Khirwale (operator provided by BD and posted in NCCS under the BD-NCCS Stem Cell COE from May 2014)
- Pooja Shinde (Consultant: June 2014-June 2015)

There are seven instruments in this facility, which are operated on a rotation basis by the seven technicians mentioned above. The usage of these instruments during the period under consideration is summarized below:

Immunophenotyping & Cell Cycle Analysis:

Equipment	Surface / Intracellular staining	DNA Cell cycle	CBA flex	CBA	Total Samples Acquired
FACS Calibur	3196	2677	---	---	5873
FACS Canto II (Old)	5008	---	---	---	5008
FACS Canto II (New)	13948	50	---	136	14134

Sterile Sorting:

EQUIPMENT	SORTING	ACQUISITION **
FACS Aria II SORP	391	1666
FACS Aria III SORP	112	230
FACS Aria III Standard	365	2937

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

BD Pathway 855:

Around six users from NCCS have used this instrument during the period under consideration. Imaging of samples was done for live cell staining (96 well plates) as well as for fixed cells in the form of sections on slides. Images were captured using mercury arc lamp and transmitted light in montage form (e.g. 8X8, 10X10). Macros are done as per the user's request.

Samples from outside NCCS:

Since June 2012, NCCS has been charging a fee for the analysis of samples received from outside NCCS, due to a considerable increase in the work load from such samples. For academic and research institutes, the charges are less and for private institutes/ companies the charges are higher. Institutes like IBB, IISER, and departments of the S.P. Pune University, such as the Biochemistry department, have utilized our facility during the period under consideration. We acquired 223 samples like Surface/ Intracellular staining and DNA cell cycle analysis.

BD-NCCS COE programme:

The BD –NCCS Center of Excellence (COE) was renewed for 3 more years from August 2014 to August 2017. The activities carried out under this programme are given below:

a) National workshop:

A national workshop on "Multicolor Panel Design –Reveal, Elevate & Resolve" under BD-NCCS Centre of Excellence in 'Stem Cell Research' was conducted at NCCS, Pune from August 11-12, 2014. Around 08 students from NCCS were short listed for the program. The program was conducted by BD Application specialist and supervised by Dr. Limaye, Head FACS Central Facility.

b) Calibur training:

The training on Calibur for NCCS students by BD application specialist was carried out throughout the year every month. 33 students from NCCS had got training on Calibur and they started using it independently.

c) Canto-II training and examination:

We had organized training on Canto-II during the period Jan 2015- Feb 2015. 14 students from NCCS had got training. The training was followed by conducting an examination for the trained students on 26-27 March 2015. 12 students appeared for the exam and out of those 11 students successfully

qualified and started using the instrument independently. We had also conducted an examination for the trained students of Jan - Mar 2014 batch on canto II on 29 – 30 April 2014. 19 students had successfully qualified the exam that time.

Participation in the National Science Day:

On the occasion of the national science day on 28th February 2015, the FACS facility operator, Atul Khirwale, displayed the poster, 'NCCS Flow Cytometry Core Facility' and explained the FACS technique to school and college children through active interactions.

2) Imaging facility

The Team

- **Dr. Milind S. Patole** (Facility In-Charge)
- Mrs. Ashwini Atre (Technical Officer)
- Mrs. Trupti Kulkarni (Technician)

The confocal microscopy imaging facility at the NCCS provides state-of-the-art microscopes and software for imaging and data analysis. The facility also offers time-lapse live cell imaging workstations. The facility has three microscopes from three different manufacturers. The most recent microscope is the Leica SP5 II system with a high-end Broadband Confocal Laser Scanning Microscope with 4 cooled spectral PMTs and AOBS technology equipped with a CO2 incubator. The other microscopes are Zeiss LSM 510 and Olympus Fluoview-10, which are used widely for imaging fixed samples. These microscopes also have imaging capability for the full spectrum of dyes. The facility is operated by highly trained technical officers who support imaging and analysis of samples received from the students of NCCS and as well as from other organizations.

3) DNA sequencing facility

The Team

- **Dr. Yogesh Shouche** (Facility In-Charge)
- Dr. Sarang Satoor (Technical Officer)
- Mr. Mandar Rasane (Technician)
- Ms. Ankita Viashampayan (Consultant)

The number of samples run on the sequencers in the central sequencing facility was 4388, during 2014-2015.

4) IVIS Imaging System

The Team

- **Dr. Gopal C. Kundu** (Facility In-Charge)
- Dr. Mahadeo Gorain (Technician)

The In Vivo Imaging System (IVIS) is a central facility of NCCS. This instrument provides the Bioluminescent and Fluorescent Imaging of cells or whole small animal under in-vitro and in-vivo conditions. Recently, more than 20-25 researchers from various laboratories at NCCS and collaborators from other institutes used the IVIS system. This system allows to capture bioluminescence as well as fluorescence based imaging in different strains of mice (NOD/SCID/ C57/Balb-C, etc) as well as tissue culture plates (96 well, 24 well 12 well, etc).

The Xenogen IVIS-Spectrum System is capable of imaging bioluminescence and fluorescence in living animals. The IVIS uses a novel in-vivo biophotonic technique to use real-time imaging for monitoring and recording cellular and genetic activity within a living organism. A light- tight imaging chamber is coupled to a highly sensitive CCD camera system.

IVIS Imaging System

The IVIS Imaging System includes a custom lens with 5-position carousel and adjustable field of view (FOV) of 4-26 cm, more uniform light collection, and improved resolution with single cell sensitivity for in-vitro use. An integrated fluorescence system and 24-position emission filter wheel allow easy switching between fluorescent and bioluminescent spectral imaging, while a laser scanner provides 3D surface topography for single-view diffuse tomographic reconstructions of internal sources. A 25 mm (1.0 inch) square back-thinned CCD, cryogenically cooled to -90°C (without liquid nitrogen), minimizes electronic background, and maximizes sensitivity. This camera system is capable of quantitating single photon signals originating within the tissue of living mice. Up to five or six mice can be imaged simultaneously and an integrated isoflurane gas manifold allows rapid and temporary anesthesia of mice for imaging.

Standard filter sets for IVIS Imaging System

Fluorescence Filters

Set	Name	Excitation (nm)	Emission (nm)
1	GFP	445-490	515-575
2	DsRed	500-555	575-650
3	Cy5.5	615-665	695-770
4	ICG	710-760	810-875

Spectral Imaging Filters

Set	Name	Emission (nm)
5	560 nm	550-570
6	580 nm	570-590
7	600 nm	590-610
8	620 nm	610-630
9	640 nm	630-650
10	660 nm	650-670

Features:

- High-sensitivity in vivo imaging of fluorescence and bioluminescence
- High throughput (6 mice) with 26 cm field of view
- High resolution (up to 60 microns) with 3.9 cm field of view
- Dual 12-position emission filter wheels (24-position total) and 12-position excitation wheel
- A set of four filter pairs for fluorescent imaging come standard with the instrument, in addition to a set of four background filters for subtraction of tissue autofluorescence
- 25 x 25 cm alignment grid on the imaging platform ensures consistent accurate placement of animals for imaging
- Spectral imaging filters that acquire images at different wavelengths (ranging from 560 nm to 660 nm) facilitate 3D diffuse tomographic reconstruction and determination of the depth and location of a bioluminescent reporter
- Heated animal shelf (up to 40° C)
- NIST traceable absolute calibrations
- Class I Laser Product

5) Central Sterilization Facility

The Team

- Dr. Mohan Wani (Facility In-Charge)
- Suresh Basutkar, Technical Officer (Lab)
- Narayan Kadlak, Technician
- Pramod Surve, Technician
- Gayatri Sagare, Asst. Technician
- Kailash Bhandalkar, Helper

The central sterilization facility is an infrastructure service department of the institute. It provides services to all the research laboratories, cell repository, media section and other service and support units through washing, packing and sterilization of glassware and other material required for research. It also supplies high grade distilled water to all the sections of the institute. Furthermore, some of the technical staff is involved in ensuring the safe disposal of radioactive and biohazardous waste material.

Microbial Culture Collection

Yogesh Shouche
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Participants
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Amaraja Joshi, *Scientist*
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Amit Yadav, *Scientist*
Mahesh Chavdar, *Scientist*
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Venkata Raman, *Scientist*
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Vishal Thite, *Technician*
Sonia Thite, *Technician*
Mahesh Sonawane, *Technician*
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Mrinal Mishra, *Technician*
Mandar Rasane, *Technician*
Abhijeet Pansare, *Technician*
Swapnil Kajale, *Technician*
Mitesh Khairnar, *Technicain*
Tushar Ghole, *Technician*

The MCC Team

Background

The Department of Biotechnology established a Culture Collection in June 2008 with a broad charter to preserve, characterize and authenticate microbial resources. These are valuable raw material for the development of biotechnology in India, because of its vast area with varied topology and climate has a rich reservoir of biological diversity which needs to be conserved judiciously and carefully, to prevent enormous economical loss. It is most important to build and enhance human and technological capabilities to isolate, preserve and characterize microorganisms in order to accrue a greater share of the benefits from such microbial resources.

MCC's startup facility operated from the Hindustan Antibiotics Limited premises in Pune until March 2012, after which it moved to an interim laboratory facility of approximately 5000 sq. ft. at the National Centre for Cell Science (NCCS), Pune campus. MCC is currently functioning in the interim laboratory facility at Sai Trinity, Pashan from March 2014.

Aims and Objectives

- The main objectives of this MCC are to act as a national depository, to supply authentic microbial cultures and to provide related services to the scientific community working in research institutions, universities and industries.

Work Achieved

Microbial Prospecting Project

Since its establishment, MCC received ~200000 cultures collected from various ecological niches as part of the microbial prospecting project undertaken by DBT in collaboration with nine participating institutes/univeristies. These safe deposit cultures have been categorized as below on the basis of their screening for different bio-active compounds:

a. 'Normal' Cultures: All the cultures which are isolated by each institute.
b. 'Three star' Cultures: All normal cultures are then screened by Piramal Life Science Limited (PLSL), Mumbai for four different activities. Three star cultures are the ones which show one or more of these activities
c. Re-fermented' Cultures: All the three star cultures undergo second level of screening (fermentation) to check whether they retain the activity. Re-fermented cultures are those three star cultures which retain their activity after second fermentation.
d. Scale-up' Cultures: Re-fermented cultures that have shown potentially novel molecules/compounds during screening at PLSL and have been selected for large scale fermentation by PLSL are designated as 'Scale-up' cultures. These cultures are being sent by PLSL to MCC.

Culture Preservation Status

All cultures received from the nine participants have been preserved in -80 °C freezers In addition, all three star and re-fermented cultures and a significant proportion of the normal cultures have also been preserved in liquid Nitrogen (-196 °C, LN).

Cultures passage status

MCC initiated the passage activity in later half of 2013 as many of the microbial prospecting cultures were preserved in -80 °C freezers almost three years ago. Since then, MCC has finished the 1st passage of all three star and re-fermented cultures. Further, major proportions of the normal cultures have undergone first passage and will be completed for the remaining cultures by 2015.

Cultures dispatched to Academia/Industry

MCC has supplied ~2000 cultures to PLSL for scale up activity and other purposes. MCC is also supplying culture to North Maharashtra University for research project purpose which is funded by DBT, Government of India.

Identification of cultures

So far, a total of 7938 pure 'Three Star' cultures have been received at MCC and all of them are now identified. The DNA sequencing was done using three/ four different universal 16S rRNA gene specific primers. Preliminary classification of these three star sequences was done using Ribosomal Database Project (RDP) using Bayesian naïve classifier. Those that could not be identified by sequencing even after multiple trails, were identified using Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry.

Since September 2013, all normal category cultures are also being processed for 16S rRNA gene sequence based identification and MALDI based identification. From that 5610 have been sequenced and identified and 7803 have been identified by MALDI

Fatty acid methyl ester (FAME) analysis

To build a strong Meta database of the pure 'three star' cultures, MCC processed them for FAME analysis by MIDI. To begin with, some of the cultures that were processed for MALDI-TOF were also processed for FAME analysis. Out of the 504 cultures processed so far, 478 have been analysed by FAME and 14 could not be identified. The remaining are in process. The common aerobic genera identified were *Bacillus*, *Paenibacillus*, *Staphylococcus*, *Pseudomonas*, *Microbacterium*, *Klebsiella*, *Arthrobacter*, *Virgibacillus*, *Shigella*.

Services

Supply of Cultures

MCC began supplying its public access general deposit cultures to researchers in India. Although there have been numerous requests for supply of cultures, many of these could not be processed due to the non-availability of the cultures. So far, MCC has supplied 160 bacterial and 32 fungal cultures in form of stabs and slants, respectively. MCC has started supplying lyophilized culture vials of many high demand cultures to its customers.

General Deposit

MCC is continually receiving cultures for general deposit from several institutions across India and overseas. Authentication of the taxonomic identity of the cultures is done by rRNA gene sequencing (>1200 bp). Upon authentication and preservation, an aliquot of the Deposit is sent to the depositor for confirmation and accessioned in MCC collection. So far, MCC has

authenticated 1424 cultures (1225 bacterial and 199 fungal) in its collection. While all of these have been preserved by two different methods: two cultures stocks in 20% glycerol are stored at -80 °C and two vials in Liquid nitrogen (-196 °C); preservation by lyophilization is in process under which 168 lyophilized ampoules are stored at 4 ° C.

IDA/Patent deposits

In addition to the general deposits, MCC is also receiving deposits for IDA from Indian and overseas institutes. So far, MCC has accessioned 52 bacterial cultures under IDA. On priority, all IDA deposits have been preserved by lyophilization in addition to -80 °C and LN storage. For each culture, 14 lyophilized vials are prepared and two of these are sent to the depositor and the remaining vials are stored at MCC at 4°C.

Identification services

Since October 2012, MCC began offering paid identification services like rRNA gene sequencing, Phylogenetic analysis, MALDI-TOF typing, FAME (fatty acid methyl ester) analysis, G+C mol% (Tm & HPLC), and DNA-DNA Hybridization.

16S/18S rRNA gene sequencing (~ 700 and ~1200 bp) or ITS region sequencing

In addition to the sequencing of deposit cultures for authentication, a total of 1074 cultures for bacteria and fungi together (including 108 for paid service) were sequenced at MCC. For bacterial identification 16S rRNA gene sequence was used whereas for fungi ITS region sequence was used.

Phylogenetic analysis

MCC has received 17 requests for bacterial identification and phylogenetic analysis. Currently MCC is providing phylogenetic analysis based on two methods; Neighbor joining and Maximum parsimony. For phylogenetic analysis, only type strain sequences from databases like RDP and EzTaxon are used. The alignment is done in ClustalX software. Phylogenetic trees are constructed in MEGA software.

MALDI-TOF typing

Matrix-assisted Laser Desorption/Ionization (MALDI-TOF) mass spectrometers are frequently used for the rapid and sensitive analysis of biomolecules. One of the main uses of MALDI-TOF-MS is in the identification of proteins, by peptide mass fingerprinting (PMF).

Since its installation in April 2013, the methods for sample preparation and analysis have been standardized for the Bruker MALDI-TOF MS. So far, only internal MCC cultures have been run on the instrument with very high congruence to rRNA gene sequence identification. MCC has provided MALDI analysis as a part of bacterial identification service for 30 cultures.

FAME Analysis

MCC has start providing FAME analysis as a service in February 2013. Since then, a total of 279 bacterial cultures have been analyzed on the MIDI system. The major anaerobic genera identified were *Bacteroides Camylobacter*, *Tissierella*, *Treponema*, *Peptococcus*, *Clostridium*, *Coprococcus* etc. Among the aerobes, *Sphingomonas*, *Virgibacillus*, *Bacillus*, *Paenibacillus*, *Psuedomonas*, *Rhodococcus*, and *Rhizobium* were identified as common genera. However, FAME profile of four cultures did not match with MIDI aerobic (RTSBA) or anaerobic (SMOORE6) libraries.

DNA-DNA hybridization and GC (mol %)

DNA-DNA hybridization (DDH) and G+C content (mol %) are important molecular characteristics and also plays a key role in polyphasic approach of microbial taxonomy. MCC standardized and started DDH and GC content analysis as a service in June 2013. These services are now being utilized by scientists of MCC and other national institutes for classification and delineation of taxa at species and subspecies level. This study leads to proposal of novel species, reclassification of existing taxa and also to resolve taxonomic conflicts. So far, MCC has analyzed 75 internal MCC cultures. A revised fee structure is proposed for providing these as a service.

ISO Certification

MCC has implemented ISO 9001 in October 2013 for its general and IDA deposit services. After expert opinion from DSMZ, technical specifications to invite ISO consultants were prepared, tenders were invited and one company was given the order for consulting for ISO certification. A set of standard operating procedures (SOP) for various activities performed during processing of cultures for deposit have been devised, they were deliberated upon by the entire MCC staff and revised versions were approved by the ISO consultant. As per the ISO 9001 requirement, the first audit review for certification is due in 2015.

Future Plans

Additional Services to be offered

MCC plans to implement and/or extend certain services in the near future and needs approval of fees for the same. The details are given below.

MALDI-TOF

In addition to identification of cultures by MALDI-TOF, customers also require creation of a MALDI database for which the number of replicates required is large and needs additional consumables along with technical expertise. A revised fee structure for MALDI-TOF typing of single cultures as well as database creation is submitted to the SAC meeting and is approved.

DNA-DNA Hybridization and GC content analysis

MCC is now receiving requests from Indian and overseas customers for DNA-DNA hybridization and GC content analysis. MCC has presented revised fee structure for DNA-DNA hybridization and GC content analysis and is been approved in the SAC meeting.

DNA isolation from cultures

In the past year, there have been many demands especially from colleges and universities about providing DNA preparations to them. Since, there are many universities those cannot perform high quality DNA isolation procedures in their laboratories, MCC is now providing this as service to the customers.

Anaerobic microbes

The facility for the cultivation of anaerobic microbes is ready and we are receiving anaerobes for deposit in all the three categories. Currently more than 10 strains of anaerobes are deposited.

Hazard group 3 microbes

For the establishment of Biological Safety Level 3 (BSL-3) laboratory to handle Hazard Group 3 organisms specifications have been drawn by an expert committee chaired by Dr. D. T. Mourya, Director National Institute of Virology, Pune. The global tender for the construction of this facility has been released.

Supply of cultures to Industry

As part of DBT's initiative to share the microbial prospecting cultures for screening of additional bioactive compounds, DBT has sanctioned two projects and MCC is supplying them these cultures.

MCC Publications

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2. Dastager SG, Mawlankar R, Mual P, Verma A, Krishnamurthi S, Joseph N, Shouche Y. *Bacillus encimensis* isolated from marine sediment sample. Int J Syst Evol Microbiol. 2015 Feb 12. pii: ijs.0.000114. doi: 10.1099/ijs.0.000114. [Epub ahead of print] PubMed PMID: 25678682.
3. Dastager SG, Mawlankar R, Srinivasan K, Tang SK, Lee JC, Ramana VV, Shouche YS. *Fictibacillus enclensis* sp. nov., isolated from marine sediment. Antonie Van Leeuwenhoek. 2014 Mar;105(3):461-9. doi: 10.1007/s10482-013-0097-9. Epub 2013 Dec 17. PubMed PMID: 24343101.

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19. Thorat MN, Mawlankar R, Sonalkar VV, Venkata Ramana V, Joseph N, Shouche YS, Dastager SG. *Deinococcus enclensis* sp. nov., isolated from a marine sediment sample. Antonie Van Leeuwenhoek. 2015 Jan;107(1):141-8. doi: 10.1007/s10482-014-0311-4. Epub 2014 Oct 25. PubMed PMID: 25344421.

Awards / Honours / Memberships (MCC Scientists)

- Dr. Kamlesh Jangid was elected as the secretary of Bergey's international society for microbial systematics a part of the Bergey's Manual Trust.
- Dr. Om Prakash received Young Achiever Award for the "Significant contribution in Agricultural Microbiology" from Institute of Agricultural Science, Banaras Hindu University, Varanasi, India.
- Dr. Avinash Sharma was selected as Young Fellow of the Maharashtra Academy of Science.

Conferences / Workshops (MCC Scientists)

- Dr. Avinash Sharma visited DSMZ for training purpose regarding IDA and Database management during June 2014.
- Dr. Avinash Sharma was invited by European Culture Collection Organization (ECCO) for delivering a talk on status on microbial culture collection in India.
- Dr. Dhiraj Dhotre attended WFCC, WDCM and UNESCO sponsored 15 days' workshop on "Training Course of Microbial Resources Information Management and Utilization for Developing Countries" in Beijing, China during August 2014.
- Dr. Prashant Singh attended POLAR ecology conference 2014 in Czech Republic during September 2014 which was funded through DST grant.

Talks by MCC Scientists

- Dr. D. R. Ranade

1. Invited as the Guest of Honour for the National Conference on 'Recent Advances in Biodegradation of Human wastes' held at Defence Research Laboratory, Min of Defence, Tezpur (Assam) during 16-17 December 2014.
2. He also delivered the invited talk during the Conference entitled 'Anaerobic Biological Treatment of Human Waste with Fuel Gas Production'.
3. Delivered an invited talk on 'Future Directions of Research in Biotechnology for Energy' at ONGC Energy Centre, New Delhi on 26th September, 2014.
4. Delivered one talk each in Marathi and English on as part of National Science Day- 2015 celebrations (28th Feb 2015) on the topic 'Bacteria for Bioenergy' at MCC.

- Dr. Kamlesh Jangid

1. Conventional & Recent Trends in Microbial Identification. Winter School in Microbial Technology for Waste Water Management. West Bengal University of Technology, Kolkata. 24 Jan 2015.
2. The Microbial Diversity Assessment Club for Learning Systematics in the Classroom. 2nd Meeting of the Bergey's International Society for Microbial Systematics, Edinburgh, UK. 09 April 2014

- Dr. Om Prakash

1. Invited lecture delivered on "Role of Microbial Taxonomy in Determination of Biosafety Classes of Plant Growth Promoting (PGP) Bacteria "by Dr. Om

Prakash Sharma in the National Workshop on "Advances in PGPR Research" on October 7-8, 2014 at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi organised by Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi and Asian PGPR Society, Hyderabad.

- Dr. Dhiraj Dhotre

1. Invited lecture delivered on "Advances in Microbial genomics" at Garware college, pune as a part of AMI event
2. Invited as resource person and chaired the valedictory function of the workshop on "Techniques in molecular biology" at Yashwantrao mohite college, pune
3. Invited as a guest lecturer at Modern college, ganeshkhind, pune to teach bioinformatics and molecular phylogeny. (5 lectures)
4. Invited as a guest lecturer at Department of Bioinformatics, Pune to teach genomics, metagenomics and transcriptomics (6 lectures).
5. Delivered a talk about "Microbial Culture collection, NCCS, pune" in "Training Course of Microbial Resources Information Management and Utilization for Developing Countries" at Institute of Microbiology, Chinese Academy of Sciences, Beijing, China during the period 2nd September 2014 to 15th septemeber 2014. Sponsored by WFCC, UNESCO, WDCM, IMCAS and CODATA.

- Dr. Avinash Sharma

1. Invited talk on Microbial Cuture Collection, India in 33rd Annual Meeting of the European Culture Collections Organisation, Valencia, Spain (11- 13th June 2014).
2. Invited Talk at Institute of Biology II, Microbiology, Albert-Ludwigs-University, Freiburg, Germany on Extremophiles; diversity and taxonomy (24th June 2014)

- Dr. Amit Yadav

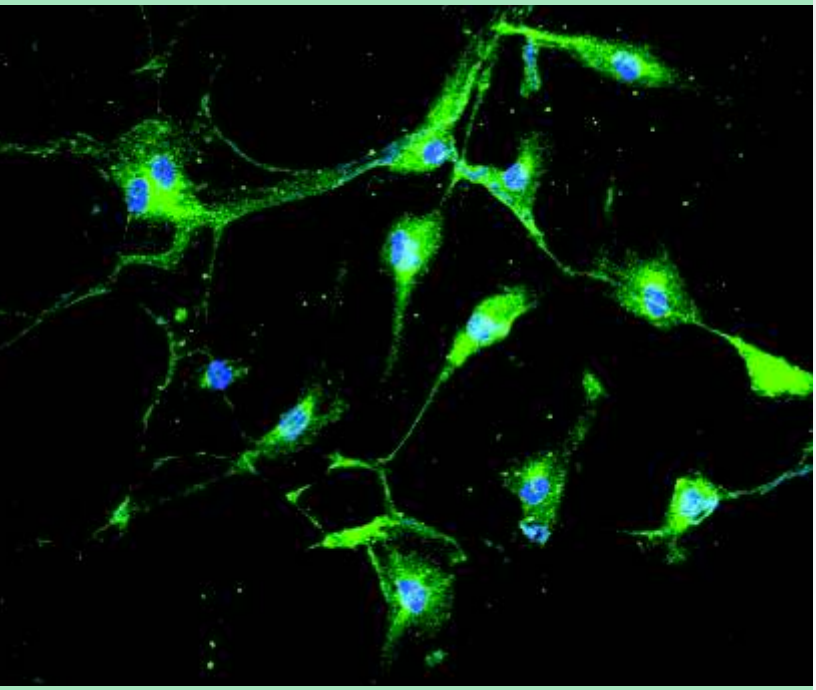
1. Invited talk on "DNA Sequencing" at Desai College, Pune on November 20, 2014

Events Organized by MCC

- A five day workshop on 'Microbial Identification and Preservation' was organized from 5th to 9th January 2015 at its own facility. Forteen participants including Ph.D students, scientists from government institutes and industries, faculty members from colleges and universities across the India attended this workshop.
- On the occasion of Science day students from schools were invited to visit MCC and were exposed to basic microbiology. Dr. Ranade delivered a talk on "Bioenergy and biofuels" in English and Marathi.





Other Information

Publications / Book Chapters / Patents

Publications*

(*Publications of scientists from MCC & DST-INSPIRE Faculty are listed in their respective reports)

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2. Bajaj MS, Ghode SS, Kulkarni RS, Limaye LS, Kale VP. Simvastatin improves hematopoietic stem cell engraftment by preventing irradiation-induced marrow adipogenesis and radio-protecting the niche cells. Haematologica. 2015 Apr 17. pii: haematol.2015.124750. [Epub ahead of print] (In press)
3. Bandopadhyay M, Kundu GC et al: Osteopontin as a therapeutic target for cancer. Expert Opin Ther Targets. 2014 Aug; 18(8): 883-95. doi: 10.1517/14728222. 2014.925447.
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Book Chapters / Invited Reviews / Editorials

- Butti R., Ghosh P., Kumar TVS., NNV Radharani, Nimma R. and Kundu G. C. (2015) 'Role of Osteopontin in Tumor Microenvironment: A New Paradigm in Cancer Therapy'. In *Multi-Targeted Approach to Treatment of Cancer*. (Springer International Publishing Switzerland). DOI 10.1007/978-3-319-12253-3-6.
- Ghosh SK, Lalsare K, Saha B (2015). 'Toll-like receptor signaling in macrophages' (Ch.08). In *Leishmania: Current Biology and Control*. Subrata Adak and Rapk Datta, eds. (UK: Caister Academic Press and Horizon Press).
- Lausi A, Polentarutti M, Onesti S, Plaisier JR, Busetto E, Bais G, Barba L, Casetta A, Campi G, Lamba D, Pifferi A, Mande SC, Sarma DD, Sharma SM, Paolucci G. Status of the crystallography beamlines at Elettra. Eur Phys J Plus. 2015; 130: 43.
- Mande SC. Early developments in crystallography. Resonance 2014; 1077-1086.
- Mandlik V, Bejugam PR and Shailza Singh S (2014) 'Application of ANN in Drug Discovery'. In *Artificial Neural Network for Drug Delivery, Drug Design and Drug Disposition*. (Elsevier Inc.).
- Phulera S, Akif Mohd, Sardesai AA and Mande SC. Redox proteins of *Mycobacterium tuberculosis*. J. IISc. 2014; 94:127-137.
- Roy G., Shetty D., Yadav A. and Kundu G. C. (2015). 'Nanomedicine: Therapeutic applications and Limitations' In *Handbook of Research on Diverse Applications of Nanotechnology in Biomedicine, Chemistry and Engineering* (pp. 64-89)'. (Hershey, PA: IGI Global). Doi:10.4018/978-1-4666-6363-32.
- Saha B (2015). Antigens [v3.0]. In *Encyclopedia of Life Science*. (UK: John Wiley and Sons, Ltd.)

Patents (filed / sealed)

Dr. Vaijayanti P. Kale / Dr. Lalita Limaye

Method for preservation of human hematopoietic stem or progenitor cells (filed by DBT)

(Co-inventor: Prof. Avadhesha Surolia, IISc, Bengaluru)

Country: USA	Patent No. 8859282	Date granted: 24th Nov, 2014
Country: Singapore	Patent No. 167894	Date granted 8th Aug, 2014

Dr. Samit Chattopadhyay

- New reagent for selective detection of cysteine and histidine in pure aqueous environment and in human blood plasma.

  (Co-inventors: Amitava Das, NCL, Pune; Upendar Reddy Gandra; Hridesh Agarwalla)

  India: Application number: 1697/DEL/2014; Provisional Filing date: 6/25/2014.

- Novel uracil and 5-fluorouracil conjugated ruthenium (II) polypyridyl complexes as cellular and DNA damaging agents.

  (Co-inventors: Amitava Das, NCL, Pune; Vadde Ramu; Nandaraj Taye)

  India: Application number: 2864/DEL/2014; Provisional Filing date:10/8/2014

Dr. Manoj Kumar Bhat

Method of preparing dialyzed extract of fenugreek seeds which induces hypoglycemia, mediated, in part, via stimulation of insulin signaling pathway.

(Co-inventor: Dr. Vijayakumar MV)

Country: USA Patent No. 8865237 Date granted: 21/10/2014

Dr. Sharmila Bapat

- A Tumor Deconstruction Platform for the Analysis of Intra-Tumor Heterogeneity

  (Co-inventor: Rutika R. Naik)

  Indian Patent Application No. 173/MUM/2014; Date of filing: 20 Jan, 2014

- Identification, quantification, monitoring and analysis of intra-tumor heterogeneity

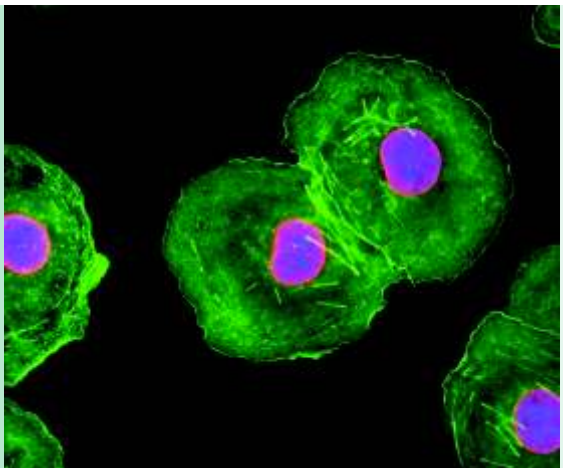
  (Co-inventor: Rutika R. Naik)

  PCT Application Number: PCT/IB2015/050358; Date of filing: 17 Jan, 2015

- A Monoclonal Antibody Targeting the Tumor Regenerative Hierarchy

  (Co-inventor: Rajkumar S Kalra, Avinash M. Mali, Pradeep B. Parab)

  Indian application No. 2980/MUM/2014; Date of filing: 18 Sep, 2014



Awards/ Honours/ Memberships/ Extramural Funding

Awards / Honours / Memberships

Faculty

Sharmila Bapat

- Elected Fellow of the Indian Academy of Sciences, Bangalore
- Awarded Fulbright Fellowship (2015 – 2016)

Gopal Kundu

- Elected as Associate Editor, J. of Cancer Metastasis and Treatment (2015-)
- Elected as Associate Editor, Molecular Cancer (2015-)

Girdhari Lal

- Life member, The Indian Science Congress Association, India (since 2015).
- Active Member, Society of Leukocyte Biology, USA (since 2015).

Nibedita Lenka

- Chairperson, Institutional Ethical Committee, OCT Therapies & Research Pvt. Ltd. Mumbai.

Lalita Limaye

- International Society of Experimental Haematology
- Indian society of cell biology
- Biotechnology society of India
- Indian women scientists association
- Indian association of Microbiologists of India

Srikanth Rapole

- Executive council member, Proteomics Society, India (PSI)
- Life member, Indian society for mass spectrometry (ISMAS)
- Member, American society for mass spectrometry (ASMS)

Arvind Sahu

- Elected member, Guha Research Conference, India (2014)

Shailza Singh

- DST Young Scientist Award
- International Travel Award by CSIR and DBT
- Life Member-Indian Biophysical Society (IBS)
- Life Member -Biotechnology Society of India (BSI)
- Life Member-Society of Biological Chemists, India (SBC)
- Life Member-Association of Microbiologists of India (AMI)
- Life Member-Association for DNA Fingerprinting and Diagnostics

Deepa Subramanyam

- Member of the Indian Society for Developmental Biology.

Mohan Wani

- Academic Editor, PLOS ONE journal, 2014-15.
- Member, Stem Cell Task Force, DBT, 2014-17.
- Adjunct Professor, Shivaji University, Kolhapur, 2014-15.

Anjali Shiras

- **Prime Minister's delegate** at the Indo-Japan S & T Joint Committee Meeting held at Tokyo, Japan; 27th-29th October, 2014.
- Membership of the International Society for Stem Cell Research (ISSCR); Illinois, USA
- Member of Society for Translational Research: New Delhi, India

Yogesh Shouche

- **'ABP Majha Sanman Puraskar - 2014'**, presented by the Hon'ble Chief Minister of Maharashtra, Shri. Prithviraj Chavan & the Union Surface Transport Minister, Shri Nitin Gadkari.

Madhav Gadgil (Emeritus faculty):

- **'Tyler Prize for Environmental Achievement - 2015'** - Awarded in recognition of his achievements in ecological research and the promotion of environmental stewardship.

Students' Awards & Honours

Aman Sharma: 1st Prize for 'Early stage lung cancer detection'
Award for Propagation of Innovative Ideas in Biotechnology at UNCONVENTIONIL PITCH PUNE, sponsored by Villgro Foundation, Chennai, for promoting Social Innovation. (March, 2015)

Debasish Paul:
The 'Prof. P. R. Sudhakaran Best Poster Award'; 'Cell biology and cell signaling section' - 83rd Annual Meeting of the Society of Biological Chemists of India, Bhubneswar, Odissa. (18-21 December, 2014)

Ramesh Butti: 1st Prize in poster presentation 1st International Conference on Translational Research, at KIIT University, Bhubaneshwar.
(5-7 February, 2015)

Tushar More: Best poster award; 6th Annual Meeting of the Proteomics Society, India & International Conference on "Proteomics from Discovery to Function", IIT, Bombay. (7-9 December, 2014)

Avneesh Kumar Gautam: ICS trainee award, at the 25th International Complement Workshop, Rio de Janeriro, Brazil. (14-18 September, 2014)

Ajitanuj Rattan: ICS trainee award, at the 25th International Complement Workshop, Rio de Janeriro, Brazil. (14-18 September, 2014)

Dinisha Kamble: 1st Prize in poster presentation; International Symposium on Current Advances in Radiobiology, Stem Cells and Cancer Research, JNU, New Delhi. (19-21 February 2015)

Mangesh Suryavanshi: Second prize for poster presentation at 'Probiotics - From Bench to Community', held at New Delhi, India (7 - 8 March, 2015).

Prachi Umbarkar: Best 'oral presentation' award at the 'Indo-Canadian Symposium on Heart Failure: Progress and Prospects', at RGCB, Trivandrum (13-14 March 2015)

Pranav Pande: First prize for poster presentation at 'Probiotics - From Bench to Community', held at New Delhi, India (7 - 8 March, 2015).

Late Sachin Rathod: ISNO 2014 President's Award for the Best Students in Neuro Oncology, (Basic Sciences) at ISNO Meeting; Lucknow; 2014

Extramural Funding

Sharmila Bapat

- Molecular Pathways regulating Ovarian Cancer Cell Plasticity and Stem Cell Properties 2012 - 2016 (DBT, India - AISRF, Australia)
- Prostate and ovarian cancer detection from innovative use of antibody libraries, human lectins, exosomes and simple affordable assay platforms - PROVATECT 2015 - 2018 (DBT, India - TEKES, Finland)

Manoj K. Bhat

- Relationship between obesity and cancer, its ramifications in cancer progression and chemotherapy. 2013-2016 [Project under Department of Science and Technology-Science and Engineering Research Board (DST-SERB); DST Grant No. SR/SO/HS-0136/2012, India]

Samit Chattopadhyay

- Samit Chattopadhyay, Regulation of T cell Development and Differentiation by nuclear protein SMAR1: Its implication in immune responses (BT/PR14746/BRB/10/899/2010), 2012-2016, Department of Biotechnology, New Delhi. India
- Samit Chattopadhyay, Regulation of CD44 splicing by tumor suppressor SMAR1: Implications in cancer metastasis (BT/PR3624/MED/30/662/2011-2016). 2012- 2016, Department of Biotechnology, New Delhi. India
- Samit Chattopadhyay, Role of Nuclear Matrix protein SMAR1 as regulator of suppressor T cell in inflammatory Boweel Disease (IBD), 2015- 2018, Department of Biotechnology, New Delhi. India

Radha Chauhan

- Structural and functional studies on Nup93 subcomplex of the nuclear pore complex. 2012-2017. (DST-SERB Ramanujan Fellowship)
- Reconstitution and structural studies on Nup93oNup205 complex of the nuclear pore complex. 2013 - 2016. (DST-SERB EMR funded)
- Reconstitution and structural studies on Nup93oNup62oNup54oNup58 quaternary complex 2015-2018. (DBT-basic sciences EMR funded)

Jomon Joseph

- Regulation of RNA metabolism by Dishevelled, a critical player of Wnt signalling. 2011-2014. (DBT, India)
- Exploring the functional connection between Par polarity proteins and Nup358 in cell polarity. 2012-2015. (DBT, India)

Vaijayanti Kale

- The role, and mechanism, of Free Radical Scavengers and / or Cell Death Cascade regulators in mitigating the Diabetes Mellitus-induced Endothelial Progenitor Cell (EPC) Dysfunction 2012 -2015 DBT, India
- A study to determine the safety and efficacy of extra-cellular matrix (ECM) embedded Endothelial Progenitor Cells (EPCs) in the treatment of impaired wound healing. 2011-2015. BRNS, India

Janesh Kumar

- Molecular Mechanisms for Regulation of Ionotropic Glutamate Receptors by their Auxiliary Subunits. 2014 - 2019. (Wellcome Trust/DBT India Alliance, India)

Gopal Kundu

- Peptide nanoparticle mediated drug/siRNA delivery to tumor vasculature that suppresses tumor growth and angiogenesis in breast and prostate cancers" (2011-2014) (Department of Biotechnology, Government of India).
- Therapeutic application of targeted shRNA libraries in treatment of breast and prostate cancers" (2012-2015) (Department of Biotechnology, Government of India).

Girdhari Lal

- Cellular and molecular mechanism of CD4 T cell and endothelial cell interaction to control inflammation and autoimmunity (2012-2015) (Department of Biotechnology, Government of India; BT/PR4610/MED/30/720/2012)
- Role of chemokine and its receptors in the pathogenesis and regulation of autoimmunity (2011-2016) (Department of Biotechnology, Government of India; BT/RLF/Re-entry/41/2010)
- CCR6 as therapeutic target to control inflammation and autoimmunity. (2011-2014) (Department of Biotechnology, Government of India; BT/03/IYBA/2010)

Nibedita Lenka

- Nibedita Lenka. Indo-Australia Biotechnology Fund (Round 6) (2012-2015).
- Nibedita Lenka. Department of Biotechnology, India (2014-2016).

Lalita Limaye

- "Generation of Mesenchymal stem cells from human umbilical cord tissues, their characterization and differentiation to neural cells." 2012-2015, BRNS, Mumbai
- "Evaluation of the effect of apoptotic inhibitors on ex vivo expansion and cryopreservation of Hematopoietic stem/progenitors cells in a co-culture system with cord derived Mesenchymal stem cells." 2013- 2016 , DRDO LSRB, New Delhi.
- "Studying the Effect of oral feeding of nutraceuticals belonging to the class of polyunsaturated fatty acids on hematopoiesis and Thrombopoiesis of mice." 2013-2015,DBT,New Delhi

Shekhar Mande

- SysTB: A network program for resolving the intracellular dynamics of host-pathogen interactions in TB infection. PI in a project involving 11 PI's and 4 co-PI's. 2012-17 (DBT)
- Elucidation of gene regulatory networks in Mycobacterium tuberculosis from the available high-throughput data and prediction of transcription regulation. 2014- 16. (DST-RFBR)

Debashis Mitra

- Identification of novel cellular targets and new lead molecules to inhibit HIV-1 infection. (2012-2015) Tata Innovation Fellowship grant, Department of Biotechnology, India

Srikanth Rapole

- An attractive and promising strategy for early cancer diagnosis through the assembly of the human cancer volatome. 2013-2016 (DBT New INDIGO)
- Metabolomic profiling for identification of novel potential biomarkers in breast cancer using mass spectrometry and bioinformatics. 2013-2016 (DBT RGYI Grant)
- Identification and characterization of novel potential biomarkers for breast cancer using gel based (2-D DIGE) and LC based (iTRAQ-LC-MS/MS) proteomic approaches and bioinformatics tools. 2013-2016 (DBT Basic Science)

Bhaskar Saha

- TLR2- dependent engagement of the host cell kinases and phosphatases that dictate disease progression or resolution. 30.01.2015-29.01.2017. (DBT, India)

Arvind Sahu

- Studies of species specificity in poxviral complement regulators. 2011-2014 (Department of Biotechnology, India).
- Soluble mediators of the immune system against *Aspergillus fumigatus*. 2014-2016 (Department of Science and Technology and Indo-French Centre for the Promotion of Advanced Research).
- Fine mapping of functional sites in Kaposica, the complement regulator of Kaposi's sarcoma-associated herpesvirus (HHV-8), 2015-2018 (Department of Biotechnology, India).

Vasudevan Seshadri

- Role of protein disulfide isomerase in glucose stimulated insulin biosynthesis. June 2011- Dec2014, DBT

Anjali Shiras

- Identification of Bio-markers for Diagnosis and Prognostication by Nex-Gen Sequencing of Oligodendroglial tumors. 2012-15 (DBT, India)
- Novel strategy for Reprogramming of somatic cells to induced pluripotent stem (iPS) cells by a single Non-coding RNA - Ginir for applications in regenerative medicine; 2014-16 (DST-UKIERI, India)

Yogesh Shouche

- Establishment of Microbial Culture Collection and Biological Research Centre. 2009-2015 (DBT)
- Maharashtra Gene Bank 2014-2019 (RGSTC)
- The origins and process of microbata development in different geographic areas: creating new nutritional tools for microbiota modulation 2012-2015 (DBT)
- Tracking the shift in gut microbiome from healthy to diabetic state: an omic approach. 2015-2016 (Unilever)
- Pune Microbiome Study - Molecular analysis of human microbiome. 2013-2015 (DBT)

Shailza Singh

- Systems and Synthetic Biology for Leishmania, funded by Department of Science and Technology (2013-2016)
- Systems Biology of L.major: Therapeutic Implications, funded by Department of Biotechnology (2012-2015)

- Drug Target Identification in *L.major* and *S.mansoni* through Biochemical Network Modeling, funded by Department of Biotechnology (2012-2015)

Sandhya Sitaswad

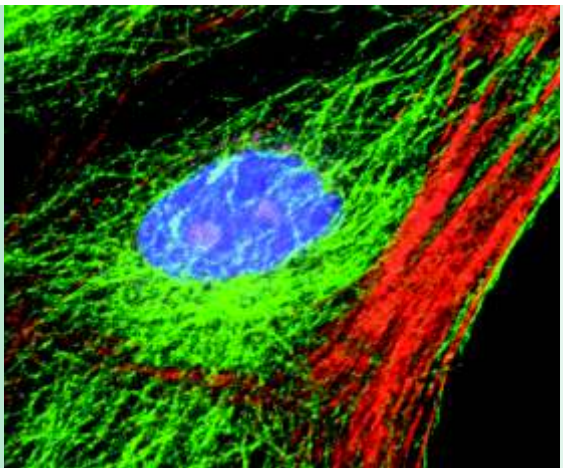
- "Investigation of anti-angiogenic efficacy/ potencyand molecular mechanism of the novel anti-cancer compound AECHL-1". (2013- 2016) Department of Science &Technology, Government of India

Deepa Subramanyam

- 'The role of endocytosis and vesicular trafficking in regulation of stem cell functions and cell fate decisions during early development.' 2013- 2018 (Wellcome-Trust DBT India Alliance Intermediate Fellowship).

Mohan Wani

- "Studies on understanding the role of IL-3 in regulation of human osteoclasts and osteoblasts differentiation", 2013-2015, (DBT).



Conferences / Seminars / Symposia / Workshops / Meetings

Participation by Faculty

Sharmila Bapat

- Participated in the symposium "Science on the Swan" on invitation from Curtin University at Perth, Australia, 21st - 23rd April, 2015
- 20 years of Cancer Stem Cells: Translating achievements to drug development and evaluation. Invited Talk at the BioPune Seminar Series, NCL Ventures Pune, India, 30th June, 2014.
- Cellular Heterogeneity - Not just noise in tumors - Plenary talk - Josephine Nefkens Institute Lecture, Erasmus MC, Netherlands, 24th September, 2014.
- Ovarian Cancer Biology, Invited Talk at the University of Turku, Turku, Finland 29th September, 2014
- Tumor Deconstruction identifies Definitive End-points in Drug Screening at the EMBO meeting on "Cancer stem cells 20 years later: Achievements, controversies, emerging concepts and technologies" at Catanzaro, Italy from 3-6 Oct., 2014
- Tumor Deconstruction identifies Definitive End-points in Drug Screening. Invited Talk during Indian Science Congress at University of Mumbai, Mumbai on 7th January, 2015.
- Recent concepts in Cancer Biology - Cancer Stem Cells. Invited Talk during ISSRF Annual Meeting at Nehru Science Centre, Mumbai from 14th – 17th February, 2015.
- Ovarian Cancer Stem Cells: Implications for effective treatment. Special Talk during IACR Annual Meeting at Jaipur from 19th - 21st February, 2015
- Ovarian Cancer Stem Cells: Implications for effective treatment. Invited Talk during Cancer Stem Cell Workshop organized by Curtin University at Perth, Australia on 24th April, 2015
- Ovarian Cancer Biology, Invited Talk at the Queensland University of Technology, Brisbane, Australia, 28th April, 2015
- Heterogeneity -Not just noise in tumors. Invited Talk at the Cancer Talks Series, Translational Research Institute, Brisbane, Australia, 29th April, 2015

Manoj K. Bhat

- Cancer and therapies; Invited talk; Department of Biochemistry, Shivaji University, Kolhapur, India, December 3rd, 2014.
- Membrane cholesterol: Potential target to enhance therapeutic effect of cancer chemotherapy; Invited talk; National Conference on Recent Trends in Drug Development organized by Department of Chemistry, Savitribai Phule Pune University, Pune, India, March 12th-14th, 2015.
- Potential Implications of bystander effect in cancer chemotherapy: The role of tumor microenvironment; Invited talk; Molecular Immunology Forum, Bhubaneswar, India, January 16th-18th, 2015.

Samit Chattopadhyay

- Control of regulatory T cell fate during Inflammatory Bowel Disease (IBD) Indian Science Congress, January 3rd to 7th, 2015, Invited Speaker.
- Switching regulatory T cell phenotype during Inflammatory Bowel Disease (IBD), TranscriptionAssemblyMeeting, IISER, Pune, 17-19th March, 2015. Invited Speaker
- Regulation of alternative splicing by SMAR1 through Sam68 deacetylation: Implications in cancer metastasis, Chromatin Asia, JNCASR, Bangalore, 15-18th January, 2015, Invited Speaker and chairing a session.
- Regulation of CD44 alternative splicing: An important clue to understand cancer and cancer stem cell behavior. Invited talk at National Institute of Biomedical Genetics, NIBMG, Kalyani, West Bengal, July 14th, 2014.
- Alternative splicing of CD44 controls cancer cell metastasis, 15th May, 2014, All India Institute of Medical Science (AIIMS), New Delhi, Invited talk.
- Control of regulatory T cell fate during Inflammatory Bowel Disease (IBD), Invited Speaker. Indian Science Congress, Mumbai, January 3rd to 7th, 2015.
- Switching regulatory T cell phenotype during Inflammatory Bowel Disease (IBD), Invited Speaker, Transcription Assembly Meeting, IISER, Pune, 17-19th March, 2015.
- Regulation of alternative splicing by SMAR1 through Sam68 deacetylation: Implications in cancer metastasis, Invited Speaker and session chair. Chromatin Asia, JNCASR, Bangalore, 15-18th January, 2015.
- Regulation of CD44 alternative splicing: An important clue to understand cancer and cancer stem cell behavior, Invited speaker, National Institute of Biomedical Genetics, NIBMG, Kalyani, West Bengal, July 14th, 2014.
- Alternative splicing of CD44 controls cancer cell metastasis, Invited talk, All India Institute of Medical Science (AIIMS), New Delhi. 15th May, 2014.
- Cancers and Causes, A lecture in Hindi at Government High School, Khajuraho, 5th December, 2014, Invited Speaker.

Radha Chauhan

- Recent Advances in crystallography, IIT Mumbai, 17th November, 2014.
- Frontiers in Structural Biology: New Advances in X-ray Diffraction and Cryo-electron Microscopy. Indian National Science Academy/Regional Centre for Biotechnology (INSA/RCB). 15-17th December 2014.
- Structural basis of mammalian Nup62 functions within nuclear pore complex and beyond. Invited talk. IIT Mumbai, Recent Advances in crystallography, 17th November, 2014.

Jomon Joseph

- Non-traditional role of a nucleoporin in cell polarity (Jomon Joseph), 10th Indo-Australian Workshop on Biotechnology, 11-13, April, 2014, School of Life Sciences, Manipal University, Karnataka, India.
- Novel role for a nucleoporin in miRNA pathway (Manas Kumar Sahoo, Deepak Khuperkar, Swati Gaikwad, Maitreyi Ashok, Mary Helen, Vasudevan Seshadri and Jomon Joseph). Cold Spring Laboratory Meeting on "Regulatory & Non-Coding RNAs", 26 – 30, August, 2014, Cold Spring Harbor, USA.
- Non-traditional role of a nucleoporin in cell polarity; Invited Talk; School of Life Sciences, Manipal University, Karnataka, India, April 2014.
- Novel role of a nucleoporin in miRNA pathway; Invited Talk; National Institute of Child Health and Human Development, NIH, Bethesda, USA, September 2014.
- Novel role of a nucleoporin in miRNA pathway; Invited Talk; Department of Surgery, Texas A&M Health Science Center, Temple, Texas, USA, September 2014.
- Novel function for a nucleoporin in miRNA pathway; Invited Talk; Department of Zoology, Savitribai Phule Pune University, Pune, India, March 2015.

Janesh Kumar

- Structural mechanism of glutamate receptor activation and desensitization, Indo-US conference on "Recent advances in Structural Biology and Drug Discovery (RASBDD-2014) at IIT Roorkee, 9-11, October, 2014, Roorkee, India

Gopal Kundu

- Indo-Australian Workshop, Manipal University, 11-13th April, 2014
- 6th Mayo Clinic International Angiogenesis Conference, Section VI (Session Chair): Regulation of Microenvironment in Disease, Rochester, MN, USA, 22-24th August, 2014

- Hallmarks of Cancer Asia, Cell Press Conference, Beijing, China 9-11th November, 2014
- Indo-US Nanotechnology Workshop, AIIMS, New Delhi, 17-19th December, 2014
- Molecular Immunology Forum 2015, KIIT School of Biotechnology, 16-18th January, 2015
- AVISHKAR-2015, MAFSU, Nagpur, 21-22nd January, 2015
- Therapeutic implication of osteopontin in breast and other cancers, Sastra University, 4th April, 2014
- Nanoparticle mediated drug delivery in breast cancer: Osteopontin as important theragnostic target, INST, Mohali, 18th November, 2014
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, iNSGTD Conf, Burdwan University, 21st November, 2014
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, ICDBT Conference, IASST, Guwahati, 3rd December, 2014
- Therapeutic and diagnostic significance of osteopontin, a chemokine like protein in breast and other cancers, NCCS-Curtin University Meeting, NCCS, Pune, 11-12th December, 2014
- Osteopontin regulated signaling network: implications in breast tumor growth and angiogenesis, International Conference on Cell Signaling, IICB, Kolkata, 15th December, 2014
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, Shivaji University, Kolhapur, 22nd December, 2014
- Therapeutic and diagnostic significance of osteopontin, a chemokine like protein in cancer, SVKM, Mumbai, 23rd December, 2014
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, ICGEB, New Delhi, 29th December, 2014
- Identification of anticancer formulations from plant derived extracts using invitro and invivo melanoma and breast cancer models, NIF, Ahmedabad, 20th January, 2015
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, 1st International Conference on Translational Research, KIIT University Bhubaneswar, 5-7th February, 2015
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, Carcinogenesis Conference, ACTREC, Mumbai, 10-12th February, 2015

- Nanoparticle mediated drug delivery in breast cancer: Osteopontin as important theragnostic target, ISNACT 2015, IIT Mumbai, 19th February, 2015
- Therapeutic targeting of osteopontin: implication of breast tumor microenvironment, 34th Annual Convention of Indian Association for Cancer Research, Jaipur, 20th February, 2015
- Chitosan nanoparticle-mediated drug delivery in breast and other cancers: Osteopontin as potential therapeutic target. Nanomedicines and Biopharmaceutical R & D Symposium, South China University of Technology, Guanzhou, China, 9-10th March, 2015
- CD133+ cells control melanoma growth and metastasis through differentially modulating EMT regulators in response to TGF beta, 18th Transcriptional Assembly Meeting, IISER, Pune, 12- 14th March, 2015
- Osteopontin, a chemokine like protein acts a novel theragnostic target covering all hallmarks of cancers, National Conference on NTCDR, IASST, Guwahati, 27-28th March, 2015

Girdhari Lal

- Lal G (2015) Chemokine receptor CCR6 intrinsic signaling controls the differentiation of Th17 and Treg in autoimmune colitis. Symposium on Systems Immunology in Infectious Diseases at Pasture Institute, Lille, France on February 6th, 2015. (Invited talk).
- Lal G (2014) Chemokine receptor signaling in autoimmunity, Department of Internal medicine I, University of Ulm, Germany on November 3rd, 2014. (Invited talk)
- Kulkarni N, Singh AK, Sethi A, Shaikh S, Joshi S and Lal G (2014) CCR6 intrinsic signaling in CD4 T cells promotes the differentiation of inflammatory Th17 cells. Immune Homeostasis and inflammatory disease: A herrenhausen symposium organized by Nature Medicine and Volkswagen Foundation, on 6-8th November at Hanover, Germany.

Nibedita Lenka

- N. Lenka. S. Krishnan, S. Sinha, D. Rangasamy. Identification of Epigenetic Modulators governing Embryonic Stem Cells Maintenance and Differentiation by Insertional Mutagenesis Approach. International Society for Stem Cell Research and Stem Cell Society, Singapore sponsored Regional Forum on Global Control on Stem Cells, Nov. 5-7, 2014. Singapore.

- Nibedita Lenka. Current Regulatory Requirements for Members of Institutional Ethics Committee, workshop organized by Clinical Development Services Agency (CDSA), New Delhi, India, Feb. 11-12, 2015, NCCS, Pune, India.
- N. Lenka. Stem Cells –The Biology and Implications. Department of Biotechnology, Jaypee University of Information and Technology, Himachal Pradesh, November, 2014 (Invited Speaker).
- N. Lenka. Loss of Function Mutagenesis and Exploration of Cell Fate Modulation using Embryonic Stem Cell Model. XXXVIII All India Cell Biology Conference and International Symposium on Cellular Response to Drugs, CDRI, Lucknow, UP, Dec. 2014. (Invited Speaker).

Lalita Limaye

- Invited talk on "Stem Cell Technology-An overview" in National Seminar on " Science for National development 2015" on 14th and 15th March 2015 in Applied Physics Deptt., VNIT, Nagpur.

Shekhar Mande

- Structural studies on redox enzymes of *M. tuberculosis*; Invited talk at the International Conference on Cellular and Molecular Mechanisms of Disease Processes, University of Kashmir, Srinagar, April 2014
- History of Crystallography; 43(B) National Seminar on Crystallography, Sardar Patel University, Vallabh Vidya Nagar, September 2014
- Early Indian Contributions to Crystallography; 80th Annual Meeting of the Indian Academy of Sciences at IIT-Madras, Chennai, November 2014
- History of Crystallography; 43(C) National Seminary on Crystallography, CSIR- Central Drug Research Institute, Lucknow, November 2014
- Structural understanding of redox reactions in M. tuberculosis; Invited talk at Frontiers of structural biology: new advances in X-ray diffraction and cryo-electron microscopy, Indian National Science Academy, December 2014
- Structural studies on *M. tuberculosis* redox proteins; Invited talk in International workshop drug development and neglected tropical infectious diseases, Department of Biophysics and Crystallography, Madras University, Chennai, February 2015
- Networks in Biology; Invited talk; Jamia Milia Islamia; April 2014
- Interdisciplinary Sciences in Modern Biology; INSPIRE Camp at IISER, Pune, May 2014

- Interdisciplinary Sciences in Modern Biology; INSPIRE Camp at Manipal University, Manipal, May 2014
- The power of molecular structure in understanding nature; INSPIRE Camp at KIIT University, Bhubaneshwar, August, 2014
- History of Crystallography and Early Indian Contributions; Invited talk; University of Hyderabad, Hyderabad, September 2014
- Networks in Biology; Invited talk at Institute of Advanced Study in Science and Technology, Guwahati, October 2014
- Structure-function and mechanisms; Invited talk at the Academic Staff College, University of Hyderabad, Hyderabad, November 2014
- Early historical developments in X-ray diffraction and Indian contributions; Invited talk at the Department of Chemistry, SP Pune University, Pune, December 2014
- Role of molecular structure in understanding nature; INSPIRE camp at Shivaji College, Nagpur, January 2015

Debashis Mitra

- Anti-HIV Drug Discovery: Past, Present and Future. Amrita Pharmaceutical Conference (APC 2014), 29th-30th August 2014; Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, Kochi, Kerala
- Cellular stress proteins in HIV pathogenesis and their potential as antiviral targets; Infectious Diseases- Current Trends, January 05, 2015. CSIR-Indian Institute of Chemical Biology, Kolkata
- HIV-1 Tat protein: viral transcriptional activator to regulator of cellular gene expression Molecules to Systems, 29th-31st Jan, 2015. Department of Biological Sciences.Presidency University, Kolkata
- The fight against HIV/AIDS: Novel molecules and strategies targeting the virus. Lupin visiting fellowship Lecture, 28th May 2014. Institute of Chemical Technology, Mumbai

Milind Patole

- Ebolavirus Genomics and Vaccine; Invited talk; Hyatt Regency Pune; 5th Annual Vaccine World Summit 2015 Meeting; Pune, India, March 2015.

B. Ramanamurthy

- Delivered 2 lectures during the Workshop under the Quality Improvement Programme (QIP), UoP on 'Guidelines on Use of Animals in Teaching and Research as per CPCSEA and UGC'. 25th February 2014; Department of Zoology, S. P. Pune University.

Srikanth Rapole

- National seminar on clinical proteomics for veterinary and allied sciences organized by
- Rajiv Gandhi Institute of Veterinary Education and Research, July 18-19, 2014 in Puducherry.
- Indo-US workshop on proteomics for translational research organized by IIT-Bombay, December 6, 2014 in Mumbai.
- International proteomics conference on proteomics from discovery to function organized by Indian Institute of Technology, Bombay from 7-9 December, 2014 in Mumbai.
- Identification of novel potential biomarkers for breast cancer using proteomic approaches and bioinformatics. Invited talk at National seminar on clinical proteomics for veterinary and allied sciences organized by Rajiv Gandhi Institute of Veterinary Education and Research, July 18-19, 2014 in Puducherry.
- Basic aspects of mass spectrometric techniques used in biological sciences. Invited talk at workshop on proteomic technologies for biosciences organized by KBP College, August 25-26, 2014 in Mumbai.
- Identification of novel potential biomarkers for breast cancer using integrated proteomic and metabolomic approaches. Invited talk at Indo-US workshop on proteomics for translational research organized by IIT-Bombay, December 6, 2014 in Mumbai.
- Metabolomic and lipidomic profiling towards novel theragnostic markers for breast cancer. International proteomics conference on proteomics from discovery to function organized by Indian Institute of Technology, Bombay from 7-9 December, 2014.
- Mass spectrometry basics, quantitative mass spectrometry, and iTRAQ proteomic approach. Invited talk at proteomics workshops organized by Indian Institute of Technology, Bombay from 10-11 December, 2014.

Arvind Sahu

- Viral teachings on human complement regulation', Guha Research Conference, (GRC), 6th-10th December 2014, Khajuraho, India.
- Structural basis of cofactor activity of Kaposica, the complement regulator of Kaposi's sarcoma-associated herpesvirus', 23rd Molecular Immunology Forum, 16th-18th January, 2015, Bhubaneswar, India.
- 25th International Complement Workshop, 14th-18th September, 2014, Rio de Janeiro, Brazil.

Manas Santra

- Talk at Tejpur University: 'Genome-wide screen identified APC/C complex as an important regulator of tumor suppressor FBXO31'; 21st April, 2014.
- Talk at the 83rd annual meeting of Society of Biological Chemists (India): 'Protein phosphatase 1 regulatory subunit p90 suppresses tumor growth through inactivation of MAPK pathway'; 21st December, 2014
- Talk at Vidyasagar University: 'Mammalian tissue culture: a tool for understanding the modern biology and therapeutics'; 2nd February, 2015.

Padma Shastry

- Conference - Indian Society for Neuro-oncology, Lucknow 11-13 April, 2014: "Role of Par-4 in glioma development"
- International Conference on Cell Culture & Cell Based Assays 'CellTech India 2015', 2-3 March, 2015. Bengaluru.
- Development & characterization of glioma stem cell lines and application of these cell lines as a tool for screening anti-cancer activity' International Conference on Cell Culture & Cell Based Assays 'CellTech India 2015', 2nd-3rd March 2015, Bengaluru.
- Targeting tumor suppressor-Prostate Apoptosis Response 4 (Par-4) for cancer therapy: Challenging the Limitations'. Recent trends in cell and molecular, and translational neuro-oncology research in India. Dept. of Biochemistry, Karpagam University, Tamil Nadu. 13th Feb 2015.
- Expert Lecture – DBT sponsored program for DBT star college- Modern college, Ganeshkhind, Pune- Exploring the potential of Tumor suppressor Prostate Apoptosis response-4 (Par-4) for cancer therapy. 19th Jan 2015
- DBT sponsored workshop for in house and college teachers of Mumbai on Biosafety and related issues. The workshop is to be organized on 11th April, 2015. Research Grant Writing with emphasis on Biosafety issues.

Anjali Shiras

- Tumor suppressive microRNA let-7a in Glioma Progression (Anjali Shiras, Sachin Rathod, Sandhya Rani) International Society of Neuro-oncology, 11th-13th April, 2014, Lucknow, India
- MicroRNA-34a suppresses proliferation of Glioma Stem Cells by targeting Wnt Signalling pathway (Anjali Shiras, Sandhya Rani, Sachin Rathod), 1st International Conference on Translational Research: From Basic Science to Clinical Application; 5th-7th February, 2015, Bhubaneshwar, Odisha, India
- Induced Pluripotent Stem Cells (iPSC): Opportunities and Challenges in Banking Activities at National Centre for Cell Science; Pune: Tokyo; JCM Meeting: Japan- Oct-2015

- Tumor Suppressive miRNAs in Glioblastoma with highlight on miR-145 family; Prognostic role in Glioma Progression: Recent trends in cell and molecular and translational Neuro-oncology research in India; Karpagam University; Coimbatore, India Feb 2015

Workshops

- Techniques in Handling of Stem Cell Culture, Anjali Shiras, Workshop on Cell Biology and Regenerative Medicine 4th -6th Aug. 2014, Nairobi, Kenya
- Provocative Questions Cancer Workshop: 4th- 6th Nov 2014. Rajiv Gandhi Centre for Biotechnology (RGCB) Trivandrum, India

Yogesh Shouche

- How to describe new species. National workshop on teaching and learning biology: problem solving approach Department of Microbiology, University of Pune 7-9 August 2014, Pune India
- What mother does for baby: Microbes contribution to animal world. National Symposium on Academic and Research Excellence in Animal Sciences 26-27 September 2014, S.N. Arts, D.J.M. Commerce, B.N.S. Science College, Sangamner, India
- Me & Us understanding human microbiome. Recent Trends and Future Prospects in Multidisciplinary Approaches in Microbiology, 4-5 October 2014, Rajaram College Kolhapur, India
- In Silico Comparative analysis of 16S rRNA gene sequences. Short course on metagenomics, October 6-15, 2014. Anand Agricultural University, Anand, India
- Metagenomics and human microbiome. October 13, 2014, Sardar Patel University, Anand, India
- Human gut microbiome in Indian perspective and need for indigenous probiotics. 2nd Probiotic Association of India Conference, New Delhi November 3-4, 2014
- Metagenomics: Basics and applications. Human Microbiome in Indian perspective. International course on Genomics, Metagenomics and Metabolic Engineering, 8-18 December 2014, IIT Kharagpur, India
- Our other genome: Human Microbiome. 10th National Research Students Meet in Life Sciences, December 18-19, 2014, ACTREC Navi Mumbai, India
- Genes Genomes and Metagenomics: Revolution in Microbiology. XII Conference of Society of Cytologists and Geneticists and National Symposium On Challenges for Biologists in 21st Century, December 22-24 2014, Shivaji University Kolhapur, India
- Human microbiome and its biotechnological relevance. India Genetic Congress, March 4-6, 2015, SRM University, Chennai, India

- Microbial Resource Centres and Need of Cryopreservation of Microorganisms for Their Sustainable use. International Conference on 'Low Temperature Science & Biotechnological advances 27-29 March 2015, New Delhi, India

Shailza Singh

- Invited talk on "Systems pharmacology strategies and discovery avenues in Bioactive Natural Products: (Re)-defining Lessons from the Past and Future" at GITAM University, Department of Biochemistry and Bioinformatics, Vizag, 26th-27th March 2015.
- Invited Talk on "Engineering signal transduction pathways through synthetic modular systems: Dissecting the puzzle in infectious disease" at 50th Indian Biophysical Society Conference, Jamia Millia Islamia, New Delhi, 14th-17th Feb 2015.
- Key Note Talk on "Systems engineering and the integration of mechanistic explanation in infectious disease through synthetic biology::A golden braid" at Pre-Science Congress, Aurangabad, 30th-31st December 2014
- Invited Talk on "Elucidating the interactome in *Leishmania*: Molecular insights with ligand pharmacophoric sites through combinatorial QSAR and Molecular Dynamics simulation" in a State-level Seminar titled "Recent Advances in Computational Medicinal Chemistry" on 14th November 2014 at Smt.Kashibai Navale College of Pharmacy, Singhad Technical Education Society, SP University of Pune, Pune.
- Invited Talk on "Biological Network Inference and Rational Drug Design for Antileishmanial Therapy: A Multi-Centric View" in the 17th Biomedical Informatics and Drug Discovery Workshop at Mahatma Gandhi Institute of Medical Sciences, Wardha, 1st-2nd December 2014.
- Invited Talk on "Modeling the Regulatory Networks in *Leishmania major* : An outlook based on Molecular Simulations" , International Conference on Emerging Trends in Drug Discovery, 23-28 July 2014, AICADD, Amrita Viswa Vidyapeetham University, Amritanagar, Ettimadai, Coimbatore Session Chair: Technical Research Paper Presentations.

Sandhya Sitaswad

- Attended "Indo-Canadian Symposium on Heart Failure: Progress and Prospects", March 11-14, 2015, RGCB, Trivandrum, India.
- Attended "International Symposium on, "Current Advances in Radiobiology, Stem Cells and Cancer Research", February 19-21, 2015, JNU, New Delhi, India.

- Attended National Conference on "Natural Therapeutics for Cancer, Diabetes and Renal Diseases (NTCDR)" March 27th & 28th, 2015, GITAM University, Visakhapatnam, India.

Deepa Subramanyam

- 160th Wellcome Trust Advanced Course on 'Genetic Engineering of Mammalian Stem Cells'; 16-28 February, 2-15, Hinxton, UK.

Mohan Wani

- "Regulation of bone regeneration from human mesenchymal stem cells by interleukin-3". Indo-Australian Conference on Epithelial Development, Function and Disease – New Frontiers and Therapies at Manipal University, Manipal, April 12, 2014.
- "Stem cell applications in Dentistry" at Sinhgad Dental College & Hospital, Pune, April 21, 2014.
- "Interleukin-3 in stem cell migration: Implications in bone regeneration". Guha Research Conference (GRC) at Khajuraho, Mandhya Pradesh, December 8, 2014.
- "Interleukin-3 prevents pathological bone loss by regulating RANK and TNF receptor signaling". Cell Signaling & Network (CeSiN)" meeting at CSIR-Indian Institute of Chemical Biology, Kolkata, December 13, 2014.
- "Role of IL-3 in regulation of migration and osteogenic differentiation of human mesenchymal stem cells". National Symposium on "Emerging Trends in Biochemical Sciences" at Department of Biochemistry, M.S. University of Baroda, Baroda, December 31, 2014.
- "Role of mesenchymal stem cells in amelioration of collagen-induced arthritis in mice". Molecular Immunology Forum, at Institute of Life Sciences, Bhubaneshwar, Januray 17, 2015
- "Osteoimmunology: Crosstalk between bone and immune systems". Prof M. C. Nath Memorial Oration, Department of Biochemistry, RSTM Nagpur University, Nagpur, January 10, 2015.
- "Laboratory Animal Models in Biomedical Research". International Course on Laboratory Animal Sciences" at Institute of Genomics and Integrative Biology, New Delhi, January 19, 2015.
- "Dual role of interleukin-3 in regulation of bone homeostasis". International Conference on Translational Research: From Basic Science to Clinical Application, at KIIT University, Bhubaneswar, February 5, 2015.
- "Use of in-vitro techniques in drug discovery" at Department of Pharmacology, Marathwada Mitra Mandal's College of Pharmacy, Pune, February 13, 2015.
- "Novel role IL-3 in maintenance of bone homeostasis" at Sinhgad College of Engineering, Pune, February 23, 2015.

Students' Participation in Workshops, Conferences, etc.

- Akshada Gajbhiye presented a poster entitled 'Identification of urinary proteomic biomarkers in breast cancer using complementary gel-based and gel-free quantitative proteomic approaches' at proteomics conference on proteomics from discovery to function and 6th annual meeting of Proteomics Society, India (PSI), 7-9 December, 2014 organized by Indian Institute of Technology, Bombay.

- Tushar More presented a poster entitled 'Metabolomic and lipidomic profiling using mass spectrometry based approaches towards detection of early disease markers for breast cancer' at proteomics conference on proteomics from discovery to function and 6th annual meeting of Proteomics Society, India (PSI), 7-9 December, 2014 organized by Indian Institute of Technology, Bombay.

- Venkatesh Chanukuppa presented a poster entitled 'Identification of new targets and biomarkers for hematological malignancies using secretome proteomic analysis' at proteomics conference on proteomics from discovery to function and 6th annual meeting of Proteomics Society, India (PSI), 7-9 December, 2014 organized by Indian Institute of Technology, Bombay.

- Khushman Taunk presented a poster entitled 'Volatile organic compounds as potential biomarkers for breast cancer diagnosis: A non-invasive metabolomics approach' at proteomics conference on proteomics from discovery to function and 6th annual meeting of Proteomics Society, India (PSI), 7-9 December, 2014 organized by Indian Institute of Technology, Bombay.

- Aftab Alam, International Conference on Stem Cells and Cancer (ICSCC-2014), Co-ordinated regulation of Calnexin gene by SMAR1 and GATA2 transcription factor and immune surveillance in cancers, 8-10 November, 2014, New Delhi.
- Naoshad Mohammad; Methyl-β-cyclodextrin potentiates doxorubicin mediated apoptosis in breast and hepatocellular carcinoma cells through p53 activation and Fas (Naoshad Mohammad, Shivendra Vikram Singh, Dipti Athavale, Parmanand Malvi, Balkrishna Chaube and Manoj Kumar Bhat), International Symposium on Molecular Pathways to therapeutics:

Paradigms and challenge in Oncology, 11th-13th, February 2015, ACTREC-TMC, Navi Mumbai, India.

- Shivendra Vikram Singh; Metformin induced lactic acidosis renders cancer cells resistant to chemotherapy (Shivendra Vikram Singh, Balkrishna Chaube, Naoshad Mohammad, Parmanand Malvi and Manoj Kumar Bhat), International Symposium on Current Advances in Radiobiology, Stem Cells and Cancer Research, 19th-21st, February, 2015, School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi, India.

- Snahlata Singh; Role of resistin on colon cancer cells (Snahlata Singh and Manoj Kumar Bhat), 5th International Conference on Stem Cells and Cancer (ICSCC-2014): Proliferation, Differentiation and Apoptosis, 8th-10th, November, 2014, Jawaharlal Nehru University (JNU), New Delhi, India.

- Dipti Athavale; Glucose dependent alterations in PCSK9 in hepatocellular carcinoma cells (Dipti Athavale, Vimal Pandey and Manoj Kumar Bhat), International Symposium on Molecular Pathways to therapeutics: Paradigms and challenge in Oncology, 11th-13th, February, 2015, ACTREC-TMC, Navi Mumbai, India.

- Abdul Kalique; National workshop on Scientific/Research paper writing at Department of Chemistry, Savitribai Phule Pune University, Pune, organized by National Academy of Science (NASI), Allahabad, India. (16-17th Dec 2014). Attended Wellcome Trust/ DBT India Alliance Science Communication Workshop, Hyderabad on 20th-21st March, 2015.

- Poonam Pandey; National workshop on Scientific/Research paper writing at Department of Chemistry, Savitribai Phule Pune University, Pune, organized by National Academy of Science (NASI), Allahabad, India. (16-17th Dec 2014).

- Rucha Sarwade; 2nd AIST International Imaging workshop, Tsukuba, Japan, Dec 9-14, 2014

- Ajitanuj Rattan: Poster presentation, 'Critical role of complement in controlling pandemic influenza A(H1N1) 2009 infection' (Ajitanuj Rattan, Shailesh D Pawar, Jayati Mullick & Arvind Sahu), 25th International Complement Workshop, 14th-18th September , 2014, Rio de Janeiro, Brazil.

- Avneesh Kumar Gautam: Oral presentation, 'Bridging of MG2 and CUB domains of C3b by Kaposica is crucial for its cofactor activity' (Avneesh Kumar Gautam, Yogesh Panse, Malik Johid Reza, Jayati Mullick & Arvind Sahu), 25th International Complement Workshop, 14th-18th September, 2014, Rio de Janeiro, Brazil.

- Kamble SC, Budhe SR, Gardi NL, Deshpande TU, Bapat SA. Validation of in silico classification of High Grade Serous Ovarian Adenocarcinoma to identify putative markers; NCCS Retreat, 25th September, 2014, Khandala, India

- Varankar SS, Pansare KJ, Gardi N, Deshpande TU, Bapat SA. Transcriptional mechanisms governing epithelial and mesenchymal properties in ovarian cancer; EMBO Workshop on Wnt Signalling: Stem Cells, Development, Disease; 6th- 9th October, 2014, Broome, Australia

- Kumar B, Uppuladinne MVN, Jani V, Sonavane U, Joshi RR, Bapat SA. Auto-regulation of SNAI2 mediates its activity during epithelial to mesenchymal transition; Symposium - Accelerating Biology 2015: Catalyzing Evolution Organized by C-DAC, 20th- 22nd January, 2015, Pune, India

- Swastik Phulera; Frontiers of Structural Biology: New Advances in X-ray Diffraction and Cryo-Electron Microscopy, Dec 15-17, New Delhi

- Ashwani Kumar; Studies on *Mycobacterium tuberculosis* FHL complex and regulators during latency; 43rd A National seminar on crystallography held in IISER, Mohali on 28th March -30th March 2014 (Oral presentation)

- Ashwani Kumar; Structural and functional study of FHL complex and its regulators in *Mycobacterium tuberculosis* during latency; 43rd C National seminar on crystallography held in CSIR - CDRI, Lucknow on 12th November - 14th November 2014 (Oral and poster presentation).

- Gaurang Mahajan; IISER Pune-ICTS Program on Advances in Mathematical Biology at IISER, Pune (December 5-14, 2014).

- Vipul Nilkanth; IISER Pune-ICTS Program on Advances in Mathematical Biology at IISER, Pune (December 5-14, 2014).

- Rutuja Patwardhan, Nibedita Lenka. Delineation of Early Neural Development using Embryonic Stem Cells Model. 28th Annual Meeting of Society for Neurochemistry, India (SNCI), Workshop & National Conference, Sept. 3-12, 2014, Sri Ramachandra University, Chennai, India.

- Shruthi Krishnan, Danny Rangasamy, Nibedita Lenka. Unravelling the role of USP31 in cell fate decisions. 5th International Conference on Stem cells and Cancer: Proliferation, Differentiation and Apoptosis, Nov. 8-10, 2014, New Delhi, India.

- Shruthi Krishnan, Danny Rangasamy, Nibedita Lenka. Functional genomics- "Eruptions of new functions by disruptions" an approach to identify cell fate decisions. Genome Architecture and Cell Fate regulation, Dec. 1-4, 2014, Hyderabad, India.

- Varun Haran M., Nibedita Lenka. Wnt Signalling in modulating Mesoderm Induction and Cardiomyogenesis. Genome Architecture and Cell Fate regulation, Dec. 1-4, 2014, Hyderabad, India.

- Pravin Dewangan: Structural and functional studies on Nup62 of the nuclear pore complex. (Pravin dewangan, Ankita Chouksey & Radha Chauhan), National Seminar on crystallography 43C, 12-14 November 2014, Central Drug Research Institute, Lucknow. India

- Parshuram Sonawane: Cryo-EM studies of Nup62 and Nup93 sub-complexes of Nuclear pore complex. (Dr. Parshuram Sonawane & Dr. Radha Chauhan). Frontiers of Structural Biology: New Advances in X-ray Diffraction and Cryo-electron Microscopy. 15-17th December, 2014, Indian National Science Academy/Regional Centre for Biotechnology (INSA/RCB), New Delhi India.

- Shilpi, and Lal G. (2014) Role of gamma-delta T cells in transplantation tolerance. NCCS retreat, Lonawala on September 25th. (Poster).

- Kulkarni N, Sethi A, Singh AK, Shaikh AK, Sharma M, Boppana R and Lal G. (2014) Chemokine receptor CCR6 signaling affects the differentiation and function of CD4 T cells. NCCS retreat, Lonawala on September 25th. (Best poster award).

- Sonor S and Lal G (2014) IFN-γ disrupts the blood-brain barrier (BBB) during inflammation and autoimmunity. Microscopy workshop at National Centre for Biological Science (NCBS), Bangalore on 20th September. (Poster)

- Sophia Fernandes: "India Stem Cell Workshop" organized by Life Technologies held on 29th Oct, 2014 at their Head Office at Whitefield, Bangalore.

- Prajakta Shinde: "The Multicolor Workshop" in University of Hyderabad from 2nd -4th July, 2014 organised by BD.

- Kedar Limbkar: 'Mass spectrometry based metabolomics'The workshop was conducted by Institute of Bioinformatics (IOB), Bangalore. It was held on 16th and 17th April, 2014

- Jeetendra Kumar : Poster entitled "Revealing the potential of Human umbilical cord blood derived Dendritic Cells as an allogenic source for cancer immunotherapy."

- Jeetendra Kumar, Vaijayanti Kale and Lalita Limaye* was selected for presentation at DC 2014 International Symposium at TOURS , France 14th - 18th September 2014.(Student could not attend as visa not granted.)

- Dhiraj Kumar: Science Communication Workshop: held on 28-29 November 2014 in Hyderabad, India. Conducted by Wellcome Trust/DBT India Alliance.

- Dhiraj Kumar: 'Functional Characterization of Cancer Stem-Like subpopulation in Melanoma Model' (Dhiraj Kumar, Santosh Kumar, Mahedeo Gorain and Gopal C. Kundu), 1st International Conference on Translational research (ICTR-2015): 5th-7th February, 2015, KIIT University Bhubaneswar, India (2015).

- Dhiraj Kumar: 'CD133+ stem like subpopulation enhances melanoma growth, angiogenesis and metastasis' (Dhiraj Kumar, Santosh Kumar, Mahedeo Gorain and Gopal C. Kundu), Keystone Symposia meeting on Stem *Cells and Cancer*: 2nd-7th February, 2014, Fairmont Banff Springs, Canada.

- Pompom Ghosh: 5th International Conference on Translational Cancer Research, from February 6th-9th, 2014 at Vigyan Bhawan,in New Delhi, India

jointly organized by MD Anderson, Texas, USA and Delhi State Cancer Insitute, India.

- Pompom Ghosh: International Symposium on Genomic Alterations: Therapeutics and Diagnostics (NSGTD) and Annual Meeting of Society of Biotechnologists (India) from November 21-23'2014 at Department of Zoology ,The University of Burdwan, India.

- Ramesh Butti: Poster presentation; 'Role of osteopontin in regulation of mesenchymal to mesenchymal transition leading to cancer associated fibroblast-mediated breast cancer growth' (Ramesh Butti and Gopal C Kundu). 1st International Conference on Translational Research (ICTR-2015): 5th-7th February, 2015, KIIT University, Bhubaneswar, India.

- Debasis Paul attended 83rd annual meeting of Society of Biological Chemists (India) for presenting poster and secured best poster presentation award.

- Srinadh choppara attended 83rd annual meeting of Society of Biological Chemists (India) for presenting poster.

- Rajesh Kumar Manne delivered the oral presentation in the 34th Annual Convention of Indian Association for Cancer Research from 19th-21st February 2015 at Jaipur.

- Parul Dutta presented a poster in the 34th Annual Convention of Indian Association for Cancer Research from 19th- 21st February 2015 at Jaipur.

- Sachin Rathod: Role of miR34a as tumor suppressive microRNA in Glioblastoma (Sachin Rathod, Sadhya Boya Rani and Shiras Anjali), International Society of Neuro-oncology (ISNOCON), 11th-13th April; 2014; Lucknow, India

- Suchismita Panda: Linc RNA Ginir promotes cellular transformation, tumorigenicity & metastasis in mouse fibroblasts (Suchismita Panda, Navjot Kaur, Varsha Shepal and Anjali Shiras), 10th National Research Scholars Meet (NRSM) in Life Sciences 18th- 19th Dec 2015; ACTREC, Mumbai, India

- Mangesh Suryavanshi and Pranav Pande attended "Probiotics - From Bench to Community" on 7th and 8th March 2015 at The Grand Hotel, New Delhi, India.

- Sunil Banskar attended Bat Ecology and Conservation Training workshop (29th-31st Jan 2015)' organized by Mhadei Research Center (Field station) Belgaum, Karnataka, India.

- Pruthvi Raj Bejugam amd Shailza Singh (2014) Exploring Synthetic Biology in Leishmania through Autoregulatory RNA Elements: A Mechanistic Perspective, FEBS-EMBO 2014, 30th August-4th September, Paris. (Poster presentation)

- Mr. Suhas Maske attended "Workshop on MicroRNA" at Krishna Institute of Medical Sciences Deemed University (KIMSDU), Karad, August 5-6, 2014.

- Mr. Suhas Maske attended "Workshop on Fundamentals of Mass Spectrometry-Based Proteomics" at Institute of Bioinformatics, Bangalore, March 11-14, 2015.

Events & Meetings

1) **Guha Research Conference**

6-10 December, 2014, at Khajuraho,

[Conveners: Dr. Samit Chattopadyay, Dr. Mohan Wani & Dr. Debashis Mitra from NCCS]

2) **Mini-symposium on Recent Advances in Proteomics -** 18th March, 2015 (Proteomics Day)

Organized by NCCS in association with the Proteomics Society, India

[Convener: Dr. Srikanth Rapole]

Dr. Geetanjali Sachdeva
NIRRH, Mumbai

Dr. Surekha Zingde
(President, Proteomics Society, India)

Dr. Harsha Gowda
IOB, Bengaluru

Dr. P. Babu
C-CAMP, Bengaluru

Speakers & Organizers

Dr. Mahesh Kulkarni
CSIR-NCL, Pune

3) Scientific Retreat of NCCS at Lonavala (25-26 Sep, 2014)
[Organizers: Dr. Anjali Shiras & Dr. Janesh Kumar]

Presentations by Faculty

Students' Poster Presentations

Faculty Discussions

Team-Building Activities

Students who won the 'Best Poster' awards

Balkrishna Choube

Debasish Paul

Neeraja Kulkarni

Jinumary Mathai & Sonal Patel

4) 'Reaching the unreached to offer better eyesight- so that all may see'
Talk by Prof. D. Balasubramanian (former President of the IAS)
[organized by NCCS for the Science Academies' Summer Research Fellows under training at research institutions in Pune]
19 June, 2014

5) Workshop on: Multi Sectorial Interventions in the context of Maternal and Child Health - "All Children Thriving"
[organized at NCCS on behalf of the DBT, BIRAC & the Bill and Melinda Gates Foundation]
13th November, 2014

6) 10th Indo-Australian Workshop on Biotechnology:
Epithelial Development, Function & Disease - New Frontiers & Therapies
[co-organized with Manipal University, Manipal;
co-organizer from NCCS: Dr. S. A. Bapat]
11-13 April, 2014

7) 1st International Conference on Translational Research:
From Basic Science to Clinical Application
[co-organized with KIIT University, Bhubaneshwar;
co-organizer from NCCS: Dr. G.C. Kundu]
5th -7th Feb, 2015

8) Visit by a team of senior scientists from the J. Craig Venter Institute, USA
9-11 March, 2015

Discussions with the NCCS faculty

Discussions with colleagues from different
research organizations & private companies

9) Gender sensitization on the occasion of International Womens' Day
March, 2015

'Gender Equity in Science: Status,
Goals & Strategies'
Prof. Rohini Godbole (IISc, Bengaluru)

Discussions

'Research in Science and Technology:
A non-traditional view'
Dr. Vineeta Bal (NII, New Delhi)

10) Visit by the CNRS Indo-French Delegation

4th February, 2015

The delegation was headed by Dr. Catherine Jessus (Director, Institute of Biological Sciences, France)

11) 'Awareness Program on 'Current Regulatory Requirements for Members of Institutional Ethics Committees'

[organized at NCCS by the Clinical Development Services Agency (CDSA) - THSTI]

11th & 12th February, 2015

Students Awarded with Ph. D. Degrees

(01.04.2014 – 31.03.2015)

No.	Research Scholar	Thesis Title	Month & Year of Award	Research Guide
1	Subhshri Sahu	Understanding the biology and function of extra-pancreatic insulin producing cells.	April 2014	Dr. Anandwardhan A. Hardikar
2	Rosalin Mishra	Studies on role of semaphorin 3A in regulation of breast tumor growth and angiogenesis.	April 2014	Dr. Gopal C. Kundu
3	Vanita P. Kamat	Studies on MAP kinase activated proteinkinases in uninfected and *Leishmania* infected macrophages	May 2014	Dr. Bhaskar Saha
4	Miotrayee Maiti	Characterisation of CD40 signalosome in uninfected and *Leishmania* infected macrophages.	May 2014	Dr. Bhaskar Saha
5	Shakti Singh Charan	Study of midgut bacteria of *Aedes* mosquitoes with respect to their role in the infectivity of dengue virus.	June 2014	Dr. Yogesh S. Shouche
6	Sijo Chemmannur	Regulation of T cell differentiation by MAR binding protein SMAR1	June, 2014	Dr. Samit Chattopadhyay
7	Sandhya B. Rani	Elucidation of the role of miRNAs in self-renewal and tumorigenicity of brain tumor stem cells derived from neuroepithelial tumors of central nervous system (CNS).	July 2014	Dr. Anjali Shiras
8	Surya Prakash Pandey	Analysis of the regulation of TLR expression	September 2014	Dr. Bhaskar Saha
9	Himanshu Singh Chandel	Studies on TLR-CD40 cross-talk in *Leishmania* infection	November 2014	Dr. Bhaskar Saha
10	Tabish Hasan Khan	Role of Phosphatases in regulation of anti-leishmanial immune response in macrophages by tuning CD40 signaling	2014	Dr. Somenath Roy & Dr. Bhaskar Saha

Silver Jubilee Orations

'Vaccine Science and Vaccine Development' 14th July, 2014

Padma Bhushan Dr. M. K. Bhan M.B.B.S., M.D., D.Sc., FNA, F.ASc, F.A.M.S.

National Science Professor, IIT, New Delhi (& former Secretary, DBT)

'What Is Ayurvedic Biology?' 19th August, 2014

Dr. M. S. Valiathan *Ch.M, FRCS, FRCS (C), FRCP, D.Sc (h.c)*

National Research Professor, Manipal University, Manipal

Foundation Day Lecture

'Science & Nation Building: Lessons from History' 26th Aug, 2014

Prof. Sugata Bose

Gardiner Professor of Oceanic History and Affairs, Harvard University, Cambridge, USA

Talks by Other Invitees

Dr. Judith Clements
Distinguished Professor and Program Leader, Cancer Program,
Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia
'Role of Kallikrein related peptidases in the tumor microenvironment'
16th Apr, 2014

Dr. Vasanta Subramanian
Dept. of Biology & Biochemistry, Univ. of Bath, UK
'Angiogenin and Neurodegenerative disease- making the connection from Structure to function and Induced Pluripotent stem cell models'
22nd Apr, 2014

Prof. K. N. Balaji
Dept, of Microbiology and Cell Biology, IISc, Bangalore
'Mycobacterial infection-dependent microRNAs fine-tune immune responses'
23rd April, 2014

Dr. Ajay Mathuru
Neural Circuitry and Behavior Lab, Institute of Molecular and Cellular Biology (BMSI), Singapore
'The scent of fear: From molecules to behavior'
24th Apr, 2014

Dr. Vidisha Tripathi
'Mammalian long noncoding RNAs: Regulators of gene expression'
13th May, 2014

Dr. Arun Shukla
Biological Sciences and Bioengineering, IIT Kanpur
'Structural basis of β-arrestin dependent regulation and signaling of G Protein-Coupled Receptors'
9th June, 2014

Dr. Aravind Penmatsa
Oregon Health & Science University, Portland, USA
'Structure of the Dopamine transporter unravels mechanism of neurotransmitter transport inhibition'
27th June 2014

Prof. Arun Dharmarajan
Curtin Health Innovation Research Institute (CHIRI), Curtin University, Australia
'Targeting Cancer Stem Cells Via Wnt/β-Catenin Antagonist, Secreted Frizzled Related Protein-4'
4th July, 2014

Dr. Paras Anand
'Cross-Talk between TLRs and NLRs during Host Defense and Inflammasome Activation'
15th July, 2014

Dr. Amitabha Mukhopadhyay, FNASc, FNA, FASc
National Institute of Immunology, New Delhi
'Hemoglobin endocytosis in *Leishmania*: An approach from bench to potential application'
16th July, 2014

Dr. Sankar Maiti
IISER-Kolkata
'Gluδ2 receptor interacting protein nPIST is a novel actin nucleator'
17th July, 2014

Dr. Suchitra Gopinath
Stanford University Medical Center, USA
'Life-altering decisions: To renew, commit, or change fate'
24th July, 2014

Dr. Oishee Chakrabarti
Saha Inst. of Nuclear Physics, Kolkata
'Specificity in the promiscuity of E3 ligases'
14th August, 2014

Dr. Suvendra Bhattacharyya
CSIR-Indian Institute of Chemical Biology, Kolkata
'Inter-cellular transfer of miRNAs in controlling growth and proliferation of cancer cells'
21st August, 2014

Dr. Dasaradhi Palakodeti
inStem, Bangalore
'*Plenaria*, small RNAs and regeneration'
16th September, 2014

Dr. Rashna Bhandari
CDFD, Hyderabad
'Inositol pyrophosphates in eukaryotic cell physiology'
18th September, 2014

Dr. Danny Rangasamy
Australian National University, Canberra, Australia
'Exploring the dark side of the genome: Retrotransposons and cancer'
30th September, 2014

Dr. Manikandan Subramanian
Columbia University Medical Center, USA
'Burying the Dead: Mechanisms, Defects, and Diseases'
5th November, 2014

Dr. M. Gopinath
Institute of Medical biology, Singapore
'A molecular switch from a single mRNA controls skin homeostasis in wound healing and cancer'
10th November. 2014

Prof. Amitabha Chattopadhyay, FNA, FNASc, FASc, FRSC
Centre for Cellular & Molecular Biology, Hyderabad
'GPCR-Cholesterol Interaction: Novel Insights in Health and Disease'
14th Nov, 2014

Prof. Kazuhiko Igarashi, MD. PhD.
Dept. of Biochemistry, Tohoku University School of Medicine, Japan
'Synthesis of s-adenosylmethionine in nuclei for epigenetic regulation'
23rd January, 2015

Dr. Stephen Price
University College London, UK
'Neuronal Nucleus Formation in the Developing Central Nervous System'
11th February, 2015

Ms. Rohini Mukherjee
Chief Policy Officer, Policy and Advocacy cell, Naandi (Public Charitable Trust)
'Large-scale child nutrition survey'.
19th March, 2015

Dr. Ashutosh Tewari, MBBS, MS, M.Ch., FRCS (Hon.) Glasgow
Professor & System Chair Urology, Mount Sinai Hospital, New York, USA
'Current Insights in Prostate Cancer Biology: Lessons for India'
(organized in association with the Institute for Prostate Cancer)
27th March, 2015

Public Outreach

1) Talks

- Public talk by Dr. Karen Nelson (President, J. Craig Venter Institute, USA)
  '*The Zoo in You: Human Microbiome in Health & Disease*' (11th March, 2015)


Organized by NCCS in collaboration with the Association of Microbiologists of India (AMI)
Co-sponsored by the Wellcome Trust/DBT India Alliance, India and the National Cancer Institute, USA.

- Lecture series on X-ray crystallography (December, 2014)
  A series of ten talks were organized by NCCS at colleges & departments of the S.P. Pune University, to commemorate the International Year of Crystallography (IYCr 2014). The talks, aimed at increasing awareness about the science of crystallography, were given by faculty from different research institutions in Pune, including NCCS, IISER-Pune, CSIR-NCL and DIAT.

- '20 Years of Cancer Stem Cells: Translating Achievements to Drug Development & Evaluation' (30th June, 2014)
  Public talk given by Dr. Sharmila Bapat at the BioPune Seminar Series organized by the Venture Center, NCL Innovation Park, Pune.

2) National Science Day : 28 Feb, 2015
(Talks & displays open to all)

(a) Popular science talks

'*Observations on Scientific Temper*'
- Prof. Sunil Mukhi (IISER-Pune)

'*Reaching out to the stars : A journey through the Universe*'
- Prof. Yashwant Gupta
(Dean, GMRT Observatory, NCRA-TIFR)

b) Displays






3) Radio Talks

- 'Stem Cell Therapy: Importance of well-designed clinical trials'
  Talk by Dr. Vaijayanti Kale, broadcast by Akashwani Pune Kendra in July, 2014

4) News paper & Magazine articles

- मूळपेशी उपचारांसाठी
  Article written by Dr. Vaijayanti Kale on stem cell therapy, published in the August 2014 issue of the periodical printed by the Marathi Vidnyaan Parishad.

- 'आईचा वारसा'
  Article written by Dr. Yogesh Shouche, published in the Marathi news paper, Loksatta, on 11th May, 2014. This article mainly discusses the role of the transfer of the maternal microbiome to the baby vis-à-vis the importance of the human microbiome.

Teaching Activities

Scientist	Subject / Talk title /Symposium	Class /Discipline / Dept.	School / College / Institution
Rahul Bankar	a) Biology & Husbandry of Laboratory Animals b) Injection & Blood Collection in Rodents c) Laboratory Animal Handling and Identification of Male & Female. d) Basic Experimentation Techniques in Laboratory Animals	S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	NCCS, Pune NCCS, Pune NCCS, Pune NCCS, Pune
Sharmila Bapat	a) Microarray Technology & Analysis of Gene Expression Data b) Understanding Cancer Stem Cells c) Molecular Classification and Personalized Therapy for Cancer Patients d) Molecular Classification of high-grade serous ovarian adenocarcinoma	M.Sc. Bioinformatics Science Academies' Education Program -Lecture Workshops S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	S.P. Pune University Jai Hind College, Mumbai NCCS, Pune NCCS, Pune
Manoj K. Bhat	a) Cancer and therapies b) Membrane cholesterol: Potential target to enhance therapeutic effect of cancer chemotherapy (National Conference on Recent Trends in Drug Development) c) Cancer therapies	M.Sc. Biochemistry Dept. of Chemistry S.P. Pune University Ph.D. course work	Shivaji University, Kolhapur S.P. Pune University NCCS, Pune
Samit Chattopadhyay	a) Cancer and causes b) Cancer Biology c) Immunobiology	Std. XI S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	Government High School, Khajuraho NCCS, Pune NCCS, Pune
Jomon Joseph	a) Microscopic techniques b) Intracellular transport	S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	NCCS, Pune NCCS, Pune
Vaijayanti Kale	a) Ethical Issues involved in stem cell research and therapy (National seminar on ethical & legal challenges in biotechnology) b) Stem Cell Biology c) Bioethics	LLM, Dept. of Law S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	S.P. Pune University NCCS, Pune NCCS, Pune
M. V. Krishnasastry	a) Fluorescence microscopy and its applications b) Biotechnology	B.Sc. Biotechnology S.P. Pune University Ph.D. course work	Modern college, Ganesh Khind, Pune NCCS, Pune

Scientist	Subject / Talk title /Symposium	Class /Discipline / Dept.	School / College / Institution
Janesh Kumar	a) Over view of Signaling at the Neuromuscular Junction	Neurobiology	National Chemical Laboratory, Pune (Ph.D. prog.)
	b) Synaptic receptors and their regulation	Neurobiology	National Chemical Laboratory, Pune (Ph.D. prog.)
	c) Structural aspects of membrane proteins	M.Sc. Biotechnology	S.P. Pune University
	d) Structure of RNA polymerase and its role in transcription	M.Sc. Biotechnology	S.P. Pune University
	e) Structural biology of membrane proteins	S.P. Pune University Ph.D. course work	NCCS, Pune
	f) Examples of membrane protein structures	S.P. Pune University Ph.D. course work	NCCS, Pune
Gopal Kundu	a) Cancer Awareness in India (DST INSPIRE Program)	Std. XI	KIIT University, Bhubaneswar
	b) Cancer Awareness in India (DST INSPIRE Program)	Std. XI	Amity University, Jaipur.
	c) Application of Nanomedicine in Cancer: Current Strategies and Future Prospects	S.P. Pune University Ph.D. course work	NCCS, Pune
	d) Tumor Immunology and Targeted Therapy, Tumor Immunology	S.P. Pune University Ph.D. course work	NCCS, Pune
Girdhari Lal	a) Genomics and Proteomics: Global genomics and proteomics analysis	M.Sc. Biotechnology	S.P. Pune University
	b) Transplantation Immunology	S.P. Pune University Ph.D. course work	NCCS, Pune
	c) Tumor Immunology	S.P. Pune University Ph.D. course work	NCCS, Pune
Nibedita Lenka	Pluripotent Cell Technologies & Reproduction	M.Sc. Biotechnology	S.P. Pune University
Lalita Limaye	a) Animal tissue culture	M.Sc. Biotechnology	S.P. Pune University
	b) Cell separation FACS	M.Sc. Biotechnology	S.P. Pune University
	c) Hematopietic stem cells	M.Sc. Biotechnology	S.P. Pune University
	d) Flow cytometry	S.P. Pune University Ph.D. course work	NCCS, Pune
Shekhar Mande	a) Crystallography	M. Sc. Biotechnology	S.P. Pune University
	b) Crystallography	M. Sc. Bioinformatics	Centre for Bioinformatics, S.P. Pune University
	c) Mycobacterium tuberculosis	M. Sc. Biotechnology	S.P. Pune University
	d) Structural Biology	S.P. Pune University Ph.D. course work	NCCS, Pune
	e) Communication skills	S.P. Pune University Ph.D. course work	NCCS, Pune
Debashis Mitra	a) Immunology	M.Sc. Biotechnology	S.P. Pune University
	b) Virology	M.Sc. Biotechnology	S.P. Pune University
	c) Immunology	S.P. Pune University Ph.D. course work	NCCS, Pune
B. Ramanamurthy	Laboratory Animal Experimentation and Ethics	S.P. Pune University Ph.D. course work	NCCS, Pune

Scientist	Subject / Talk title /Symposium	Class /Discipline / Dept.	School / College / Institution
Manas Santra	a) Cancer Biology b) Molecular Biology c) Proteomics d) Tools and techniques e) Molecular Biology	S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work M. Sc. Biotechnology	NCCS, Pune NCCS, Pune NCCS, Pune NCCS, Pune S.P. Pune University
Anjali Shiras	a) Basics of Cell biology b) Cancer Stem cells c) Neural Stem Cells Differentiation d) Understanding Stem Cells & Cancer Stem Cells e) Induced Pluri-potent Stem Cells (iPSC) f) Stem Cells Basics g) Cancer Biology: Hallmarks of Cancer	Std. IX & X M.Sc. Biotechnology M.Sc. Biotechnology MBBS S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work S.P. Pune University Ph.D. course work	Moving Academy, Pune S.P. Pune University S.P. Pune University Karpagam School of Medical Sciences & Research, Coimbatore NCCS, Pune NCCS, Pune NCCS, Pune
Yogesh Shouche	a) Microbial Genetics b) Advanced Techniques in Microbial Taxonomy c) DNA Fingerprinting RFLP, Next Generation Sequencing	M.Sc. Biotechnology M.Sc. Microbiology M.Sc. Biotechnology	S.P. Pune University S.P. Pune University S.P. Pune University
Shailza Singh	Computer Applications and Bioinformatics	S.P. Pune University Ph.D. course work	NCCS, Pune
Srikanth Rapole	a) Advanced techniques in biological chemistry and molecular biology b) Proteomics c) Proteomics	M. Sc. Biotechnology M. Sc. Biotechnology S.P. Pune University Ph.D. course work	S.P. Pune University S.P. Pune University NCCS, Pune
Deepa Subramanyam	a) Stem Cells b) Stem Cells	MSc. Biotechnology S.P. Pune University Ph.D. course work	S.P. Pune University NCCS, Pune
Mohan Wani	a) Stem cell applications in dentistry b) Use of in-vitro techniques in drug discovery c) Novel role IL-3 in maintenance of bone homeostasis d) Stem cell biology and regenerative medicine e) Autoimmunity	BDS M.Pharm. B.E. (Biotech) M.Sc. (Biotech) & M.Sc.(Zoology) S.P. Pune University Ph.D. course work	Sinhagad Dental College & Hospital, Pune Marathwada Mitra Mandal's College of Pharmacy, Pune Sinhgad College of Engineering, Pune. Shivaji University, Kolhapur NCCS, Pune

Other Happenings at NCCS

1) Hands-on Training

Dr. Anjali Shiras and her group hosted Dr. Farisai Chidzwondo from the University of Zimbabwe from 4th - 25th January 2015, under the Regenerative Medicine programme of the African Academy of Sciences, and imparted hands-on training in cell culture and molecular biology.

2) NCCS and the Symbiosis International University (SIU) signed a Memorandum of Understanding

(for inter-institutional cooperation in education, training and research in biotechnology)

4th December, 2014

3) Fire Safety & Firefighting Demonstration (10th July, 2014)

The PMC officer, Mr. Pathrudkar Gajanan, made a presentation and organized a demonstration on fire safety and firefighting at NCCS. Co-ordinator: Mr. S. H. Basutkar (Technical Officer).

4) Swachh Bharat Abhiyaan (2nd Oct, 2014)

5) Sports 2015

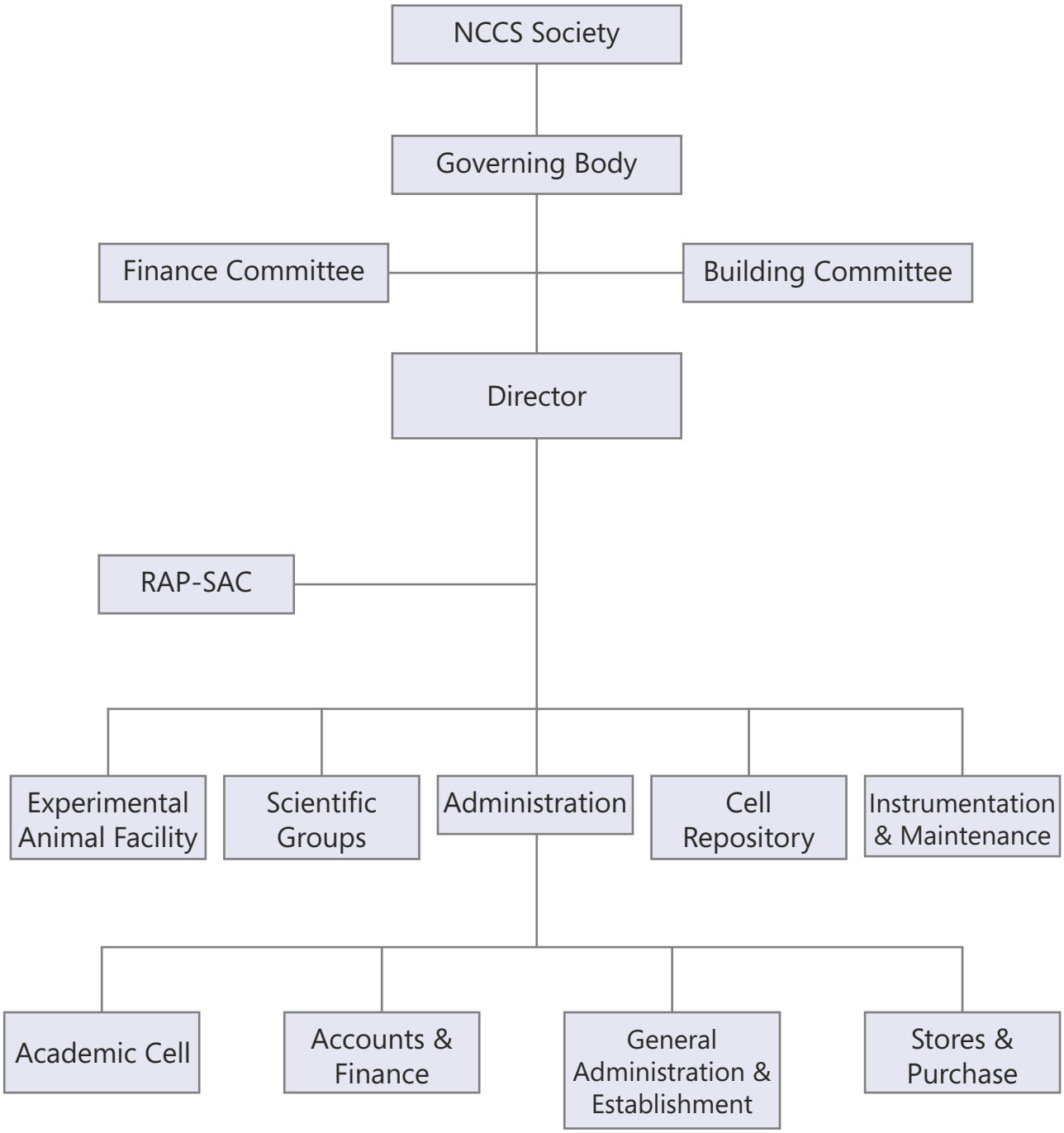



6) ATM on campus

Prof. W. N. Gade, Vice Chancellor of the Savitribai Phule Pune University, inaugurated a Bank of India ATM installed on the NCCS campus.

10th July, 2014

NCCS Organization



The NCCS Teams

Academic Cell

Accounts & Finance

General Administration & Establishment

Instrumentation and Maintenance

Stores & Purchase

NCCS Committees

NCCS Society Members

1. **Dr. Harsh Vardhan** — President
   Honourable Minister of
   Science & Technology & Earth Sciences,
   Anusandhan Bhawan,
   2, Rafi Ahmed Kidwai Marg,
   New Delhi - 110 001

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

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

4. **Dr. Bipin Nair** — Member
   Professor and Dean,
   School of Biotechnology,
   Amrita Vishwa Vidyaopeetham,
   Amritapuri Clappana P.O.,
   Kollam, Kerala - 690 525

5. **Prof. W.N. Gade** — Member
   Vice Chancellor,
   S.P. Pune University,
   Pune - 411 007

6. Mr. J.B. Mohapatra — Member
   Financial Adviser
   Department of Biotechnology,
   Ministry of Science & Technology,
   Block No.2, 7th floor, CGO Complex,
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7. Dr. Arvind Duggal — Member
   Adviser,
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   Lodi Road, New Delhi - 110 003

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

9. Prof. J. K. Pal — Member
   Head,
   Department of Biotechnology,
   S.P. Pune University,
   Ganeshkhind, Pune - 411 007

10. Prof. Rajendra Prasad — Member
    School of Life Sciences,
    Jawaharlal Nehru University,
    New Mehrauli Road,
    New Delhi - 110 067

11. Dr. Yogesh Shouche — Member
    Scientist 'G'
    National Centre for Cell Science,
    Ganeshkhind, Pune - 411 007

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

Governing Body Members

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   Ministry of Science & Technology,
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   CGO Complex, Lodi Road,
   New Delhi - 110 003

2. **Dr. Vishwa Mohan Katoch** — Member
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   Dept. of Health Research and
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   Council of Medical Research,
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    Ganeshkhind, Pune - 411 007

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

Finance Committee members

1. **Mr. J.B. Mohapatra** — Chairman
   Financial Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodi Road,
   New Delhi - 110 003

2. **Dr. Srikanth Tripathy** — Member
   Director,
   National JALMA Institute of Leprosy
   and other Mycobacterial Diseases,
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   Dr. M. Miyazaki Marg,
   Tajganj, Agra - 282 001

3. **Dr. M.V. Deshpande** — Member
   Emeritus Scientist and Professor AcSIR,
   Biochemical Sciences Division ,
   CSIR-National Chemical Laboratory,
   Pune - 411008

4. **Prof J. Pal** — Member
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   Pune - 411 007

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   CGO Complex, Lodi Road,
   New Delhi - 110 003

6. **Dr. S.C. Mande,** — Member Secretary
   Director,
   NCCS,
   Pune - 411 007

Building Committee members

1. Dr. Dinakar Salunke — Chairman
   Executive Director,
   Regional Centre for Biotechnology,
   180, Phase - I, Udyog Vihar,
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2. Shri. Arvind Duggal — Member
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   Department of Biotechnology,
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   Consultant,
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   University of Hyderabad Campus,
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   :

6. Executive Engineer — Member
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7. Shri. N. V. Nagarathnam — Member
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   Pune - 411 007

9. Shri. A. C. Pendhari — Convener
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   Ganeshkhind,
   Pune - 411 007

Scientific Advisory Committee Members

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

2. **Dr. Mahendra S. Rao** — Member
   Vice President for Regenerative Medicine,
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3. **Dr. Vamsi K. Mootha** — Member
   Massachusetts General Hospital,
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   6th Floor, Boston MA 02114, USA.

4. **Dr. Jaya Sivaswami Tyagi** — Member
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   Education in Cancer (ACTREC),
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Pune - 411021, India

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Adviser
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11 Lodi Road, CGO Complex
7-8th floor, II Block
New Delhi 110 003, India

Administration

The NCCS Administration consists of the following sections: General Administration & Establishment, Civil Maintenance, Accounts & Finance, and Stores & Purchase. The centre also has an Instrumentation & Maintenance unit. All these sections provide support services to the main scientific activities of the centre.

The NCCS staff strength (as on 31st March, 2015):

Scientists	:	33
Administrative Staff	:	41
Technical Staff	:	73
Total	:	147

Reservation Policy

NCCS follows the Government of India orders on reservation matters. For direct recruitments, respective rosters are followed, with reservation as follows: 15% for SC, 7.5% for ST and 27% for OBC, on an All India Basis by Open Competition. Liaison officers have been nominated to ensure compliance with the reservation orders issued in favour of SC/ST/OBC. NCCS also follows the Government of India reservation policy for physically handicapped candidates.

Right to Information Act 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. V.S. Shinde, Officer 'B' (Administration) as the CPIO and Dr. Jomon Joseph, Scientist 'E', as the First Appellate Authority.

Dr. Atul Fulzele [(MBBS), IPS, Head of branch, CBI and ACB, Pune & Mumbai], speaking about vigilance at NCCS, on 31Oct, 2014.

Vigilance Matters

Vigilance reports are sent regularly to the nodal ministry, i.e. the Department of Biotechnology, Government of India, New Delhi.

The Vigilance Awareness Week was observed at the National Centre for Cell Science (NCCS), Pune, between 27th October and 1st November, 2014. On the 27th of October, 2014, the acting Director, Dr. Debashis Mitra, administered a pledge in Hindi and English, to the staff and students of NCCS. Dr. Atul Fulzele (MBBS), IPS, Head of branch, CBI and ACB, Pune & Mumbai, was invited as the Chief Guest at a programme organized for the NCCS staff and students, on the 31st of October, in celebration of the Vigilance Week. Dr. Fulzele spoke on the topic of vigilance on this occasion.

Security

NCCS has engaged a private security agency for providing security services on a contractual basis. All important places in the complex are manned by security

personnel throughout the entire day. As on date, there has been no security-related problem at the Centre.

Disciplinary Matters

NCCS follows CCS (Conduct) rules 1964, CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the Centre.

Implementation of Official Language

The Director, NCCS, strongly supports the use of the Official Language in official work, and other related activities carried out at the Centre. NCCS has constituted the Official Language Implementation Committee to implement the Government of India orders to use the Official Language in day-to-day official work. Meetings of this committee are held quarterly, where various ways to implement the Official Language at NCCS are discussed.

Dr. Sunil Keshav Devdhar speaking at the Hindi Day function on 18 Sep, 2014.

Towards ensuring implementation of the Official Language, most of the official documents that are used regularly have been made bilingual (Hindi + English). Also, almost all the official correspondence carried out within the country is in the bilingual format.

The 'Hindi week' in 2014 was celebrated with much enthusiasm by holding 'Hindi essay writing' and 'Hindi Handwriting' competitions. The winners were awarded with cash prizes and certificates. Dr. Sunil Keshav Devdhar, Programme Officer, All India Radio, Pune, was invited as the Chief Guest for the Hindi Day Function held on 18th September, 2014. On this day, the second issue of 'Meemansa' (Hindi Patrika) was released at the hands of Dr. Devdhar, Dr. Dr. G. C. Mishra (former Director of NCCS) and Dr. Shekhar C. Mande (Director, NCCS). This magazine includes articles, photographs and other material written and submitted by the staff and students of NCCS. Four selected Hindi articles from the 1st issue of Meemansa were also included in DBT's in-house Hindi Magazine, 'Jaivashree'.

Committees

The Centre has constituted the following committees as required under various statutes and guidelines for the smooth functioning of the institute:

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

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

Photographs courtesy : Jeetendra Kumar, Jyoti Rao, Mahavir Rangole, Mandar Rasane, Meenakshi Jadhav, Phalguni Rath, Ragini Adhav, Sunil Kachare.

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