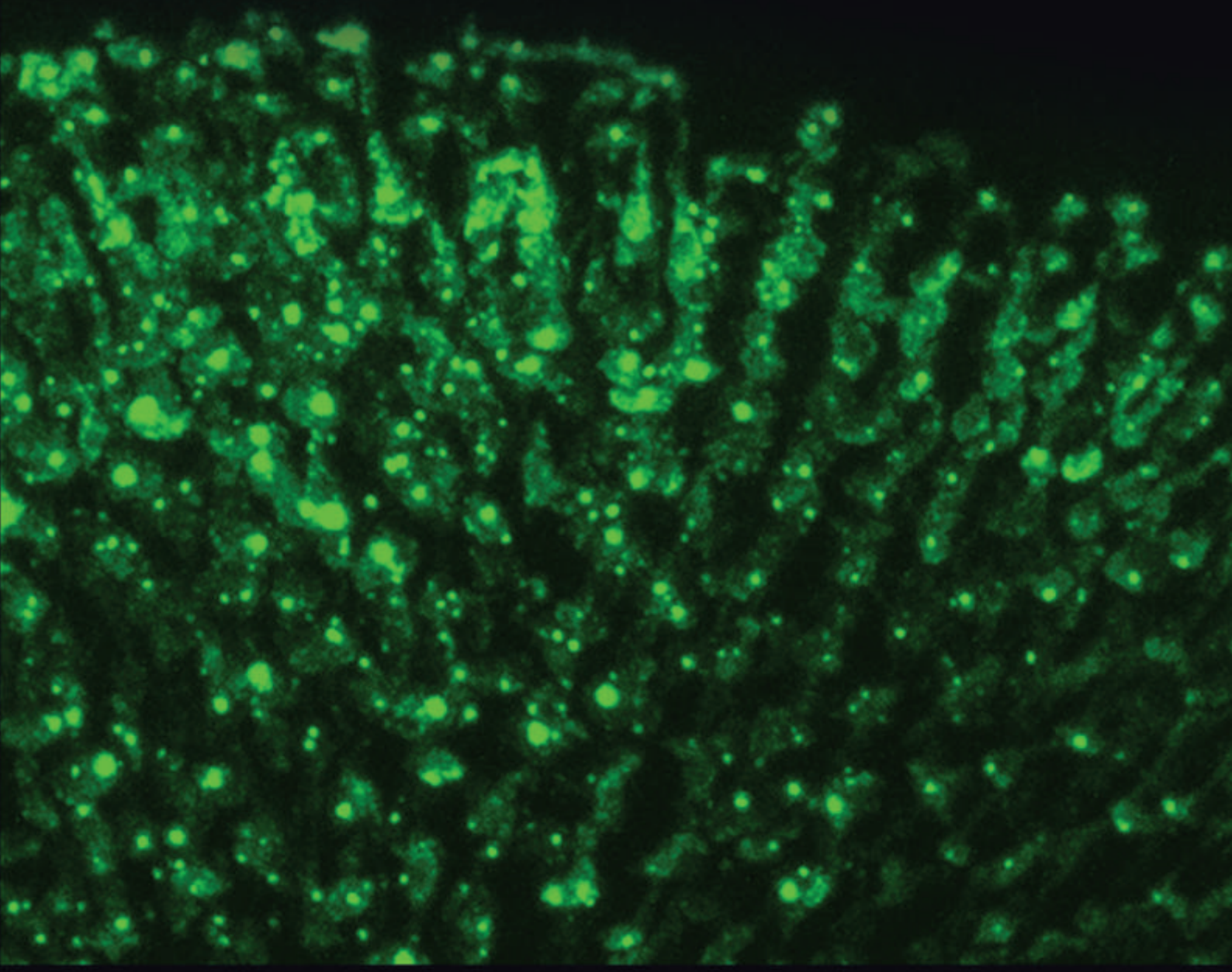
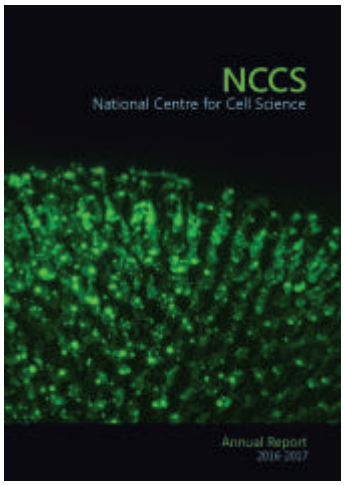
NCCS

National Centre for Cell Science



Annual Report

2016-2017



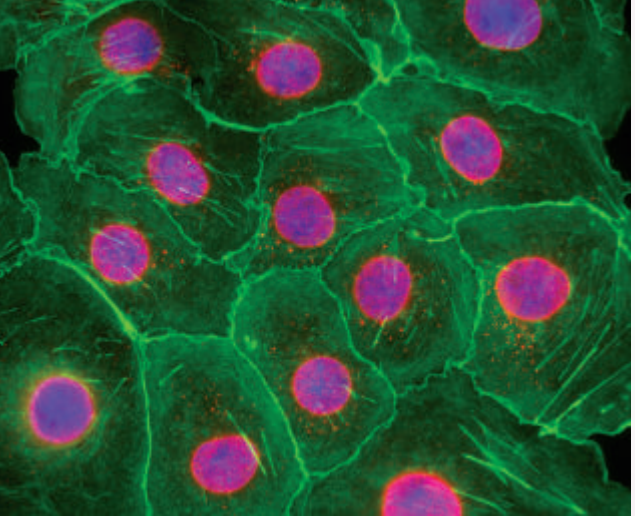
Cover page image

Drosophila laminar neurons in the eye expressing Orb2GFP.

(Image courtesy of Amitabha Majumdar)

National Centre for Cell Science

Annual Report 2016 - 2017



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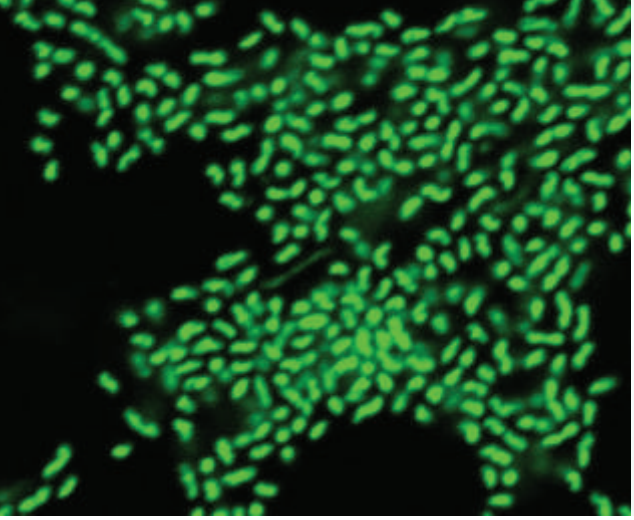


Mandate of NCCS

- 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.

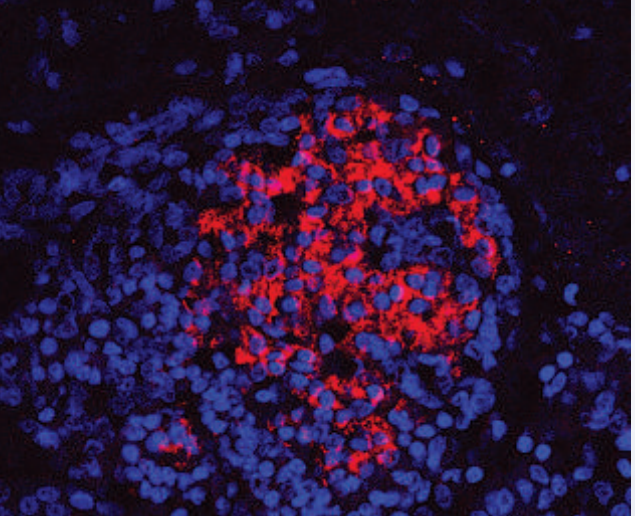
- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

- 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.



Summary of NCCS Activities for the Unacquainted

NCCS carries out research in cell biology, which involves the study of cells, the 'basic unit of life'. The bodies of all animals, including humans, are composed of trillions of different types of microscopic cells. These cells, in turn, are composed of a variety of molecules, including DNA, RNA, proteins, and several others, which determine the properties and biological activities of the cell. Cellular activities are also influenced by other determinants, including interactions of the cells with the environment and molecules outside the cell, with each other, and with microorganisms that they encounter. All these molecules, interactions and other factors that influence the functioning of cells, collectively determine the functioning of the animal as a whole. Consequently, to gain essential insights into how the body functions under conditions of health and disease, it is necessary to study the nuances of how cellular activities operate at the molecular level and decipher all the determinants involved. We carry out such studies at NCCS to address challenging questions about human health, especially those related to cancer, diabetes, infectious diseases, functioning of the immune system, regeneration of bone and other tissues, gut microorganisms in health and disease, stem cell biology, etc. Through achieving the proximal goal of understanding the basic biology of cells, we aspire to eventually contribute towards improvements in methods for diagnosis, and treatment regimens / therapeutics for management of diseases. Our studies hold special relevance for this purpose, since they are mainly focused on the Indian population. While engaging in basic research, we also explore possibilities for translating our promising breakthroughs into tangible benefits for the people through collaborations with clinicians. Transfer of medically useful technologies like 'large scale expansion of human skin culture for the treatment of burns, vitiligo and non-healing ulcers' & 'bone marrow cryopreservation' to Government medical colleges and hospitals exemplify our success on this front. The details of the research carried out at NCCS over the past year are described in the research reports of the individual scientists in the annual report that follows. Some of the key findings are also summarized in the 'From the Director's Desk' section.

NCCS also has service-oriented components which play a big role in facilitating high quality research not only at NCCS, but also at other organizations. One of the aims of NCCS is to function as a national cell repository for animal cell lines, which are essential to study the biology of cells. Cell lines are different types of cells obtained from animals, including humans, which are grown and maintained under laboratory conditions. This cell repository provides cell lines to cell biologists from academic and research institutions across the country. Therefore, a significant proportion of cell lines-based research in India is dependent on the cell repository at NCCS, and is also supported by the training and guidance provided by NCCS to develop the skills required to handle cell lines.

The Microbial Culture Collection (MCC) of NCCS plays a big role in preserving the nation's microbial biodiversity, by serving as a national depository for microorganisms. It has successfully undertaken the enormous task of obtaining several different microorganisms from a variety of environments across India, preserving them in the laboratory in the form of 'cultures', and characterizing them to identify them and to explore their potential for application in biotechnology. The MCC is the largest individual collection of microorganisms in the world and is instrumental in India being internationally ranked as the country with the fourth largest collection of microbial cultures. It also facilitates high-quality research in microbiology in universities, colleges, other research institutions, and industries all over the country, by supplying authentic microbial cultures

and providing related services, such as identifying microorganisms using cutting-edge techniques. Further, MCC has been recognized by the World Intellectual Property Organization (WIPO) in Switzerland, as an International Depository Authority (IDA) for the deposit of microorganisms to fulfill the requirements of the patent procedure in 55 countries.

From the Director's Desk

I am happy to present the Annual Report of the National Centre for Cell Science (NCCS), Pune, for 2016-17. It has been a challenging year in many respects, but taking these challenges in our stride, we have striven hard to improve our science, impart training to the younger generation, provide quality services, and increase our visibility in the society. I am happy to state that the public outreach of NCCS has been improving, with many of our faculty members and students reaching out to different sections of the society to articulate our work. Furthermore, the national cell repository of NCCS, which has been instrumental in supporting research in cell biology all over India, has continued to fulfil its commitment to service by providing six thousand cell lines to four hundred organizations in the country during this year. The repository extended its services further to facilitate human resource development by conducting two national workshops, providing hands-on training in cell culture techniques to researchers from across the country.

This year was an especially important one for many of our graduate students and research scholars, with 33 of them having received the Ph.D. degree, 21 students having submitted their theses to the University, and 22 of our research scholars having registered as Ph.D. students with the University. During this year, 32 new Research Fellows joined us, and we had a total of 125 research scholars registered for a Ph.D., as on 31st March, 2017. Moreover, 38 project trainees & 16 summer trainees also received training at NCCS under the mentorship of our faculty.

Cancer is a major cause for concern, being one of the leading causes of death worldwide. Several factors can result in the development of cancer. At the molecular level, cancer arises mainly due to the inactivation of malignancy-suppressive genes and/or the activation of malignancy-promoting genes. Dr. Manas Santra had earlier reported that levels of the tumor suppressor, FBXO31, are normally low in unstressed cells, and increase substantially following genotoxic stress through a mechanism that remained to be determined at that point. A few years ago, he and his team at NCCS reported that FBXO31 functions as a dedicated DNA damage response checkpoint protein, which can induce growth arrest in the G1 or G2/M phase of the cell cycle through two independent pathways that differ with respect to substrates and p53 dependence. In p53-positive cells, FBXO31 interacted with and directed the degradation of MDM2 following genotoxic stress. This resulted in increased levels of p53, promoting growth arrest and senescence through the transcriptional activation of p21. In p53-deficient cells, following genotoxic stress, FBXO31 interacted with and mediated the degradation of cyclin D1, an important regulator of the G1/S transition, resulting in G1 arrest. Recently, he and his team reported that the oncogenic microRNAs, miR-93 and miR-106a, repress FBXO31, resulting in the upregulation of Slug, which was found to be involved in epithelial-mesenchymal transition and cell invasion. They found that FBXO31 targets and ubiquitylates Slug for proteasomal degradation. Further, they observed that this mechanism was, however, repressed in breast tumors, where miR-93 and miR-106a were observed to be overexpressed. The studies carried out by this group further unravelled an interesting mechanism whereby Slug drives the expression of miR-93 and miR-106a, thus establishing a positive feedback loop that maintains an invasive phenotype. Collectively, these findings have established for the first time, the presence of interplay between microRNAs and the ubiquitination machinery, which together regulate cancer cell invasion. All these findings have strong relevance to designing improved strategies for controlling malignancy.

Another disease that is a cause for concern, especially due to the lack of a cure, is the Acquired Immune Deficiency Syndrome (AIDS) caused by the Human Immuno-deficiency Virus (HIV). To establish itself successfully within the host, HIV-1 exploits a

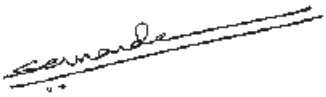
multitude of cellular host factors, and even mimics some of their functions, in order to evade the host's immune system. For self-protection, the host, in turn, employs numerous competitive strategies to inhibit invasion by the virus, including the use of restriction factors, such as the APOBEC3 family of proteins. Thus, the virus and host are engaged in an ongoing, evolutionary 'arms-race' that influences host-virus interactions. Dr. Debashis Mitra and his group at NCCS, who study such interactions in HIV-1 infection, identified novel interactions between the host's cyclin F and the viral protein, Vif, during infection in T cells. They carried out differential gene expression analysis to identify novel gene modulations associated with cell cycle deregulation in CD4+ T cells during HIV-1 infection and observed down-regulation of the cyclin F (CCNF) gene. This led them to speculate that cyclin F could have a possible role in viral pathogenesis. Further investigations revealed that cyclin F, a known F-box protein, has the ability to physically interact with and negatively regulate the expression of the viral infectivity factor, Vif, by inducing its ubiquitination and proteasomal degradation. Vif plays an important role in maintaining viral infectivity by mediating ubiquitination and proteasomal degradation of the APOBEC3G (A3G) protein of the host, which provides innate immunity to the host against HIV. Interestingly, upon further analysis, this group found that cyclin F-mediated Vif degradation could restore A3G expression, resulting in less infectious viral particles being released in the presence of cyclin F and A3G. Therefore, down-regulation of cyclin F in CD4+ T cells during HIV-1 infection is likely to be virus-mediated, and may be a strategy evolved by the virus to retain its virulence. Consequently, deciphering the exact mechanism involved could help identify novel targets for therapeutic interventions.

The research carried out at NCCS also aims to understand the biology of disease caused by other viruses, such as poxviruses, which are among the most successful pathogens. It is believed that one of the key reasons for their success is their ability to subvert both, innate as well as adaptive immune barriers of the host. The most notable member of this family is the variola virus, the causative agent of smallpox, which was successful eradicated in 1977 through mass vaccination with another related virus, the vaccinia virus. The vaccine-derived vaccinia virus is now found to circulate and cause sporadic outbreaks in dairy cattle in India and Brazil. Therefore, studies to understand the biology of the vaccinia virus assume special importance. Earlier studies have shown that the vaccinia virus is susceptible to neutralization by the classical complement pathway (CP) of the host's immune system. However, to counteract this, the virus encodes a soluble complement regulator, the vaccinia virus complement control protein (VCP). Dr. Sahu and his group at NCCS examined whether this protein selectively targets bovine CP, to investigate if it might be one of the unknown factors responsible for host tropism of the vaccinia virus. Their findings showed that it does exhibit selectivity in inhibiting the bovine CP, and that this is primarily determined by three amino acids, E108, E120 and E144. These residues were found to interact with bovine serine protease factor I and inactivate bovine C4b, the non-catalytic subunit of the CP C3-convertase. The variola virus also encodes a complement regulator, smallpox inhibitor of complement enzymes (SPICE), which similarly helps it to subvert the host's complement system. It is noteworthy that, in contrast to the negatively charged glutamic acid residues of VCP, they found that SPICE contains positively charged residues at these positions, which skews its specificity towards human complement. They therefore concluded that these three variant residues in VCP are an interesting example of adaptive mutations that likely enhance the fitness of the virus in cattle, and could be important for determining their host tropism.

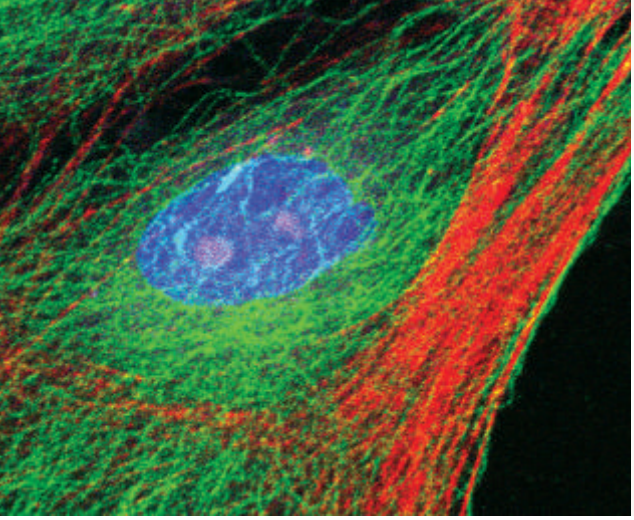
We are the first institution in the country to carry out structural studies on one of the largest protein complexes present in eukaryotic cells, the nuclear pore complex (NPC). Dr. Radha Chauhan, one of our newer faculty members, initiated research here in this challenging field to gain an insight into the complexity of these macromolecular assemblies. NPCs serve as the major route for communication between the nucleus and cytoplasm, mediating the exchange of cellular components. While small molecules diffuse through NPCs, larger molecules are recognised and transported through the pores by specific components called nucleoporins. The central transport channel of vertebrate NPC is made up of the nucleoporins, Nup62, Nup54 and Nup58. The coiled-coil domains in the α-helical regions of these nucleoporins are thought to be crucial for several protein-protein interactions in the NPC sub-complexes. Dr. Chauhan and her group recently reported a hitherto unknown behaviour of Nup62. Based on the crystal structure of the coiled-coil domain of the rat Nup62 (362-425) fragment and supporting biochemical data, they concluded that this fragment displays dynamic behavior in solution, causing Nup62 to exist in two possible states: homodimeric and homotrimeric. Using a comparative structural analysis of the rat Nup62(362-425) homotrimeric structure with available heterotrimeric structures [rat Nup62(362-425)-Nup54(346-407) and *Xenopus*

Nup62(358-485)-Nup54(315-450)-Nup58(283-406) complexes, they demonstrated the structural basis behind the formation of parallel triple helix bundles by Nup62 with different nucleoporin partners. Based on these observations, they showed that the coiled-coil domain of Nup62 is sufficient to interact with other coiled-coil proteins, such as rat Exo70, which is involved in exocytosis, thus demonstrating the role of this motif in providing plasticity for diverse interactions. Summarizing their findings, they proposed a chain replacement mechanism that yields diverse protein assemblies with Nup62. This is an important step forward towards explaining the complexity of the NPC assembly in higher organisms.

In the coming years, we aspire to recruit new faculty members to provide promising young minds with opportunities to establish rewarding careers. We would like to bring on board diverse expertise to complement and enhance our core strengths, and help us realize our vision of expansion into newer and challenging research areas.



Shekhar C. Mande
Director



Human Resource Development

The beneficiaries of the NCCS academic programmes during the year 2016-17 are as follows: Thirty two Research Fellows joined NCCS, and twenty two research scholars registered for a Ph.D. with the University during this year, taking the total number of registered Ph.D. students to 125, as on 31st March, 2017. Twenty one students submitted their theses to the University for evaluation, and thirty three students were awarded the Ph.D. degree during the said year.

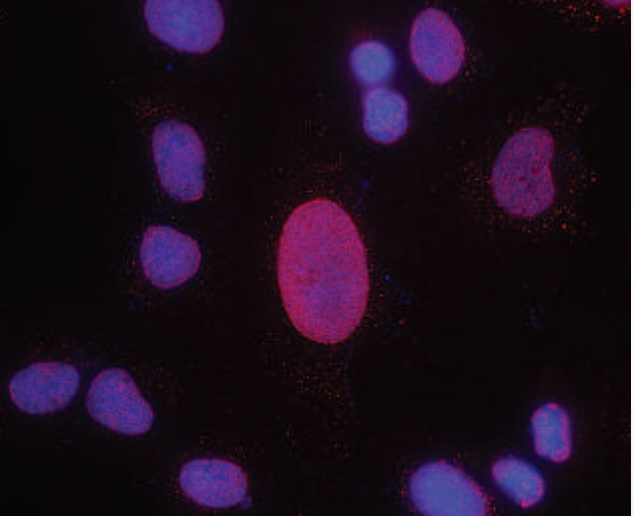
NCCS also conducts training programmes for students every year, as given below:

(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 2016-17 is as follows:

Project trainees:	38
Summer trainees:	16



Cell Repository

Scientists

Dr. Milind Patole

Dr. Punam Nagvenkar

Dr. Rahul Patil

Technical Officers

Ms. Medha V. Gode

Ms. Nivedita A. Bhave

Mr. Dharmendra V. Bulbule

Ms. Anjali M. Patekar

Technicians

Mr. Nitin S. Sonawane

Mr. Bhimashankar G. Utage

Mr. Vikas Mallav

Consultant

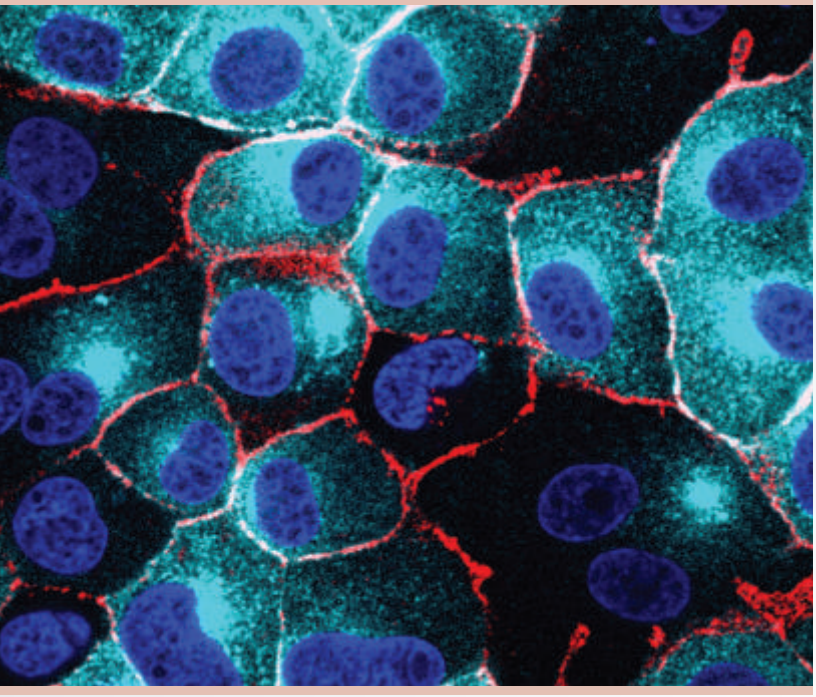
Mr. Sadashiv D. Pawar

NCCS serves as a national cell bank for animal cell lines. The repository manages the expansion, cryopreservation and distribution of cell lines to researchers in academia and government as well as private research institutions in India. In the year 2016-17, six thousand cell lines were supplied to four hundred organizations across the country.

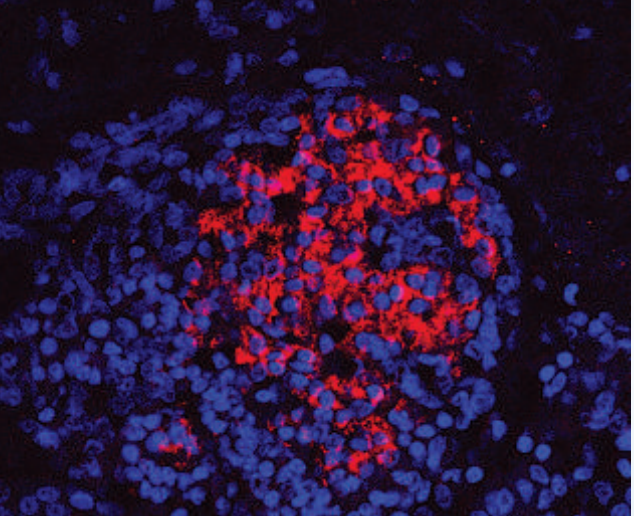
The repository organized two national hands-on training workshops on 'Basic Cell Culture Technology' from 16-19 May, 2016 and 26-28 September, 2016. The workshops included modules for important cell culture techniques related to cell line maintenance, expansion, cryopreservation and revival. Early career researchers, including doctoral students, young faculty and technical staff from academic and other institutions from all over the country were selected to participate in these workshops.

Personnel from the repository participated in the curtain raiser for the 2nd India International Science Festival (IISF) organized at NCCS on 13th November, 2016. This event was open to the general public. For their benefit, cell cultures were kept on display, and their importance was explained to the visitors.

Personnel from the repository also joined in to celebrate the National Science Day on 28th February, 2017. Various types of cell lines were shown to the visitors and the significance of their use in research was explained to them.



Research Reports



Index of Research Reports

Sharmila Bapat

sabapat@nccs.res.in

Phenotypic Plasticity in Ovarian Cancer

Background

Two-way transitions between epithelial and mesenchymal cell states are associated with specific cell fate decisions during development as well as tumor progression. The phenomenon of epithelial to mesenchymal transition (EMT) has been extensively investigated in cancer metastasis, but the reverse phenomenon of mesenchymal to epithelila transition (MET) remains ambiguous. Recent work describing the existence of different phenotypic states and their association with chemotherapeutic responses prompted our investigation of these cellular states in ovarian cancer.

Aims and Objectives

- Profiling of Phenotypic Spectrum in a panel of high-grade serous ovarian cancer (HGSC) cell lines and exploring a functional relevance with cancer metastases.
- Modulation of cellular plasticity by specific transcriptional networks and micro-environmental factors.

Work Achieved

Expression of epithelial and mesenchymal markers (E-cadherin and CK18; FAP and vimentin respectively) was profiled across a panel of eight HGSC cell lines. This associated OVCAR3, OVCA432 cells with epithelial and A4, OVMZ6 with mesenchymal phenotypes, whereas the remaining cell lines appeared to share some markers of both phenotypes (Figs. 1a, 1b). Assessment of invasive properties demonstrated an inability of OVCA432 and OVCAR3 cells to penetrate through a matrigel driven growth factor gradient while the others exhibited differential capabilities of invasion (Fig. 1c). Epithelial properties of these cells were further assessed as a measure of resistance across an intact cell

Participants

Dr. Namrata Erande, *DST Fast track fellow*
Dr. Neha Thakre, *RA*
Swapnil Kamble, *SRF*
Gaurav Soman, *SRF*
Sagar Varankar, *SRF*
Ancy Abraham, *JRF*
Madhuri More, *JRF*
Snehal Gulhane, *JRF*
Avinash Mali, *Technician*

Collaborators

Dr. Judith Clements, *QUT-TRI, Australia*
Dr. Urpo Lamminmaki, Dr. Olli Carpen,
Dr. Kim Pietersson, *University of Turku, Finland*

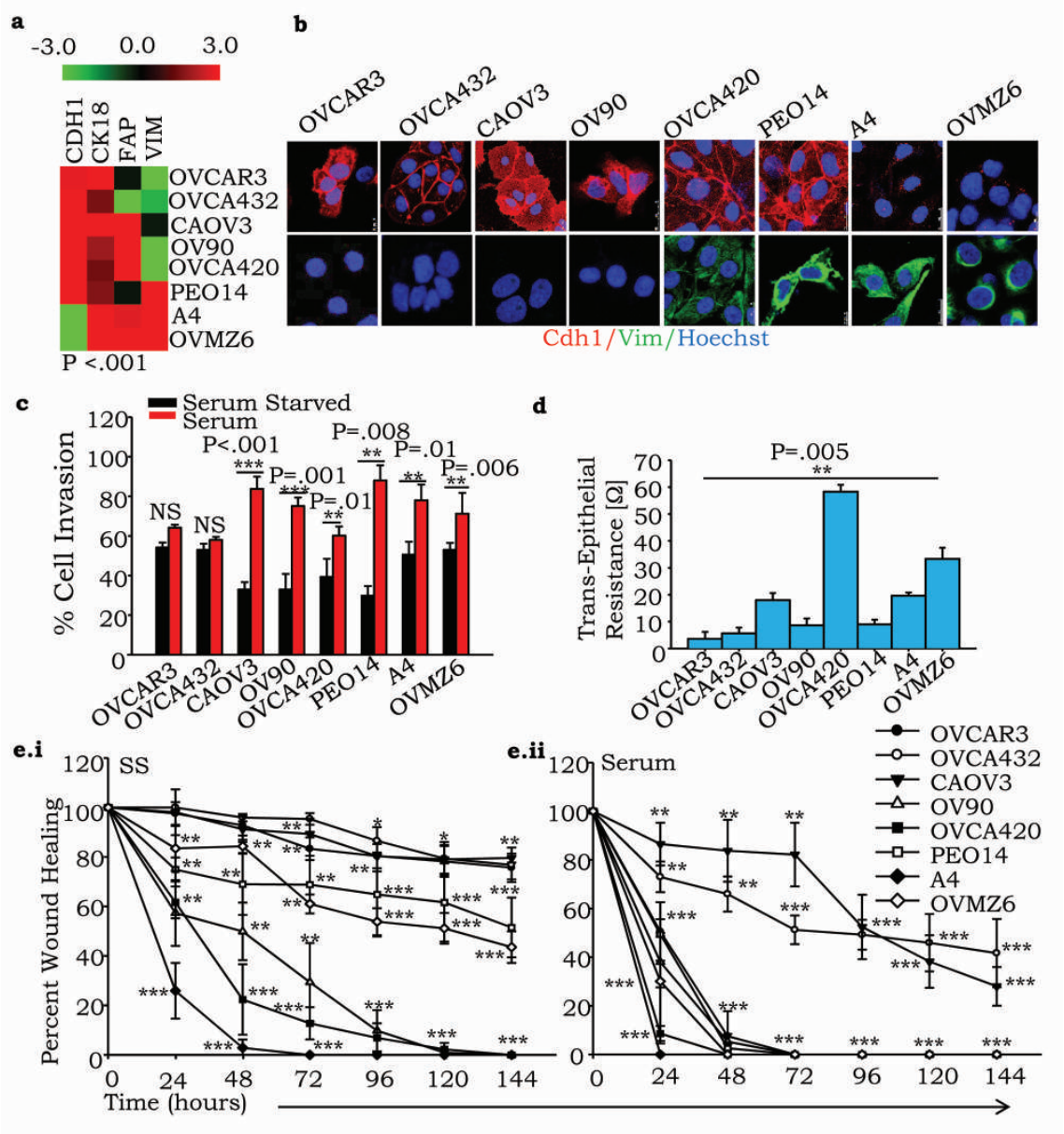


Fig. 1: Phenotypic spectrum of HGSC cell lines. **a.** Expression profiles of epithelial (CDH1, CK18) and mesenchymal (FAP, vimentin] markers in a panel of eight HGSC cell lines (n=3). GAPDH was used as an endogenous control **b.** Immunostained images depicting the expression and localization of E-cadherin and vimentin in a panel of eight HGSC cell lines (n=3). Nuclei were stained with Hoechst 33342. **c.** Percent invasion capacity of HGSC cell lines as measured by trans-well invasion assay (n=3). **d.** Representative graph depicting the trans-epithelial resistance of HGSC cell lines (n=3). e. Representative graph depicting the percent wound closure capacity of HGSC cell lines in a two - dimensional wound healing assay, in e.i - absence and e.ii – prescence of serum (n=3).

monolayer. Cell lines that generated a high trans-epithelial resistance (TER) were indicative of strong cell-cell contacts (Fig. 1d). The inability of OVCAR3 and OVCA432 to grow as groups of tightly packed cells rather than dispersed cells that readily form confluent monolayers, led to a lower TER for the former although they likely have strong cell–cell contacts. Two-dimensional wound healing assays indicated A4 cells to possess fastest migration capability, while OVCAR3 mediated healing through rapid proliferation of cells at the wound edge; CAOV3 and OVCA432 cells exhibited a complete lack of migration till the end of assay at 144 hours (Fig. 1e). Interestingly, wound healing capacity of most cell lines reduces upon serum deprivation, implying influence of growth factors on an alteraed phenotype and associated functionality. Together, these data indicate the existence of the following discrete phenotypic states –

1. Epithelial - represented by OVCAR3 and OVCA432 cells,
2. Intermediate epithelial - CAOV3 and OV90,
3. Dual positive (may be considered as a E-M hybrid cell type) - PEO14 and OVCA420,

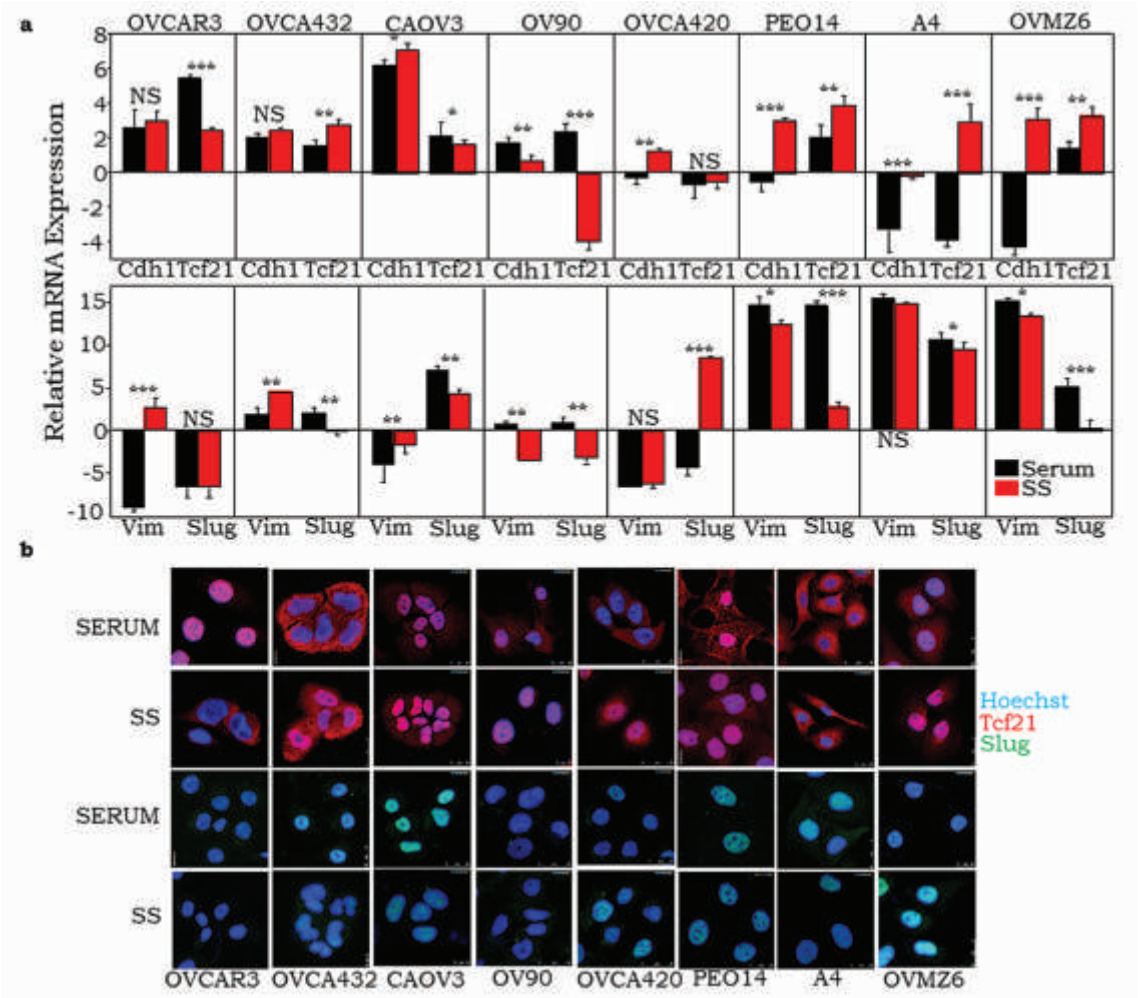


Fig. 2: Phenotypic plasticity of HGSC cell lines post growth factor depletion. a. Expression profiles of Ecadherin (Cdh1), Tcf21, Vimentin (Vim) and Slug in a panel of eight HGSC cell lines in the presence and absence of serum (n=3). GAPDH was used as an endogenous control **b.** Immunostained images depicting the expression and localization of Tcf21 and Slug in the eight HGSC cell line panel either in the presence and absence of serum(n=3). Nuclei were stained with Hoechst 33342.

4. Intermediate mesenchymal - A4, and
5. Mesenchymal - OVMZ6

Independent studies in the lab had earlier established a negative correlation between two transcription factors (TFs) viz. Tcf21 and Slug in HGSC. Further, these TFs were enriched in 2 molecular tumor types each of which associated with discrete biological pathways including an association with MET and EMT, thereby suggesting their epithelial and mesenchymal phenotypes respectively (Gardi et al. 2014). A recent further validation including these TFs in the panel of biomarkers for stratification of clinical samples into these classes also effectively segregated tumors on the basis of these phenotypes besides identifying the intermediate and hybrid states (data not shown).

To further ascertain the involvement of these TFs in driving a definitive phenotype at a cellular level, profiling of the same was undertaken in the above cell line panel (Fig. 2a). Expression profiles were generated in the presence and absence of serum to account for the variation in migratory and invasive properties observed previously. Mesenchymal cells A4 and OVMZ6 at steady state were associated with high Slug and vimentin expressions that inversely correlated with Tcf21 and E-cadherin expression. On the contrary, cell lines associated with the epithelial phenotype including OVCAR3, OVCA432, CAOV3 and OV90, exhibited a strong positive correlation with E-cadherin and Tcf21 expression accompanied by low expression of mesenchymal markers. The dual positive cell lines viz. PEO14 and OVCA420, expressed a complex correlation between marker profiles and phenotype. Protein, and specifically TF localization is earlier reported to have different influences on the phenotype due to altered target profiles. We thus attempted to understand the differential localization of the two TFs in our cell line panel. While most cell lines expressed Tcf21, its

localization was highly varied. Epithelial cells demonstraed nuclear Tcf21 with low to negligible levels of cytoplasmic/ nuclear Slug, while mesenchymal cells exhibited an almost complete absence or cytoplasmic localization of Tcf21 with a strong nuclear Slug localization (Fig. 2b). Interestingly, PEO14 exhibited a dual nuclear localization of Slug, and Tcf21.

Serum deprivation in the cell line panel further perturbed phenotypic profiles and improved corroboration with functional analysis. Most cell lines except OV90 acquired enhanced expression of E-cadherin and to a lesser extent Tcf21 that however exhibited nuclear cellular localization, and was coupled with lowered Slug expression. This implies a negative feedback loop that could thereby lead to an intermediate epithelial phenotype (Fig. 2a]. Serum deprivation also triggered a dual positive transcription factor profile in OVCA420 and OVMZ6 wherein both Slug and Tcf21 exhibited nuclear localization (Fig. 2b).

To conclude, our assessment of the phenotypic spectrum in HGSC cell lines led to the identification of five distinct states that exhibit a correlation with levels of expression and localization of Tcf21 and Slug, which correlates with an epithelial and mesenchymal phenotype respectively. These states are dyanamically determined as is noted by their modulation in response to serum deprivation that results in a phenotypic switch towards the epithelial state.

Future Research Plans

- Modulation of cellular plasticity by studying the effect of growth factors such as TGF and BMP7 on HGSC cells to further delineate micro-environmental effects on cellular phenotype.
- The above predicted role(s) of Slug and Tcf21 in maintenance of cellular plasticity will be affirmed by establishing knockout and over-expressing clones.

Manoj Kumar Bhat

manojkbhat@nccs.res.in

Cancer, Chemotherapy, and Metabolic disorders

Participants

Mr. Abhijeet Singh, *JRF*
Mrs. Bhavana Deshmukh, *JRF*
Mr. Mayengbam Shyamananda Singh, *JRF*
Ms. Ankita Deb, *JRF*
Mr. Pranay L. Ramteke, *SRF*
Ms. Dipti Athavale, *SRF*
Ms. Snahlata Singh, *SRF*
Ms. Surbhi Chouhan ,(Submitted thesis February 2016)
Dr. Vijayakumar M.V., *Technical officer B*

Collaborators

Dr. Mahesh J. Kulkarni, *National Chemical Laboratory, Pune, India*
Dr. Bipin Nair, *Amrita Vishwa Vidyapeetham University, Kollam, India*
Dr. Rajashekar Mohan, *SDMMHS, Dharward, Karnataka*
Dr. Vasudevan Seshadri, *NCCS*
Dr. Mohan R. Wani, *NCCS*
Dr. Jomon Joseph, *NCCS*
Dr. Jeet Singh, *IISER, Pune*
Dr. Amit Agarwal, *CRF, Pune*

Background

Metabolic disorders diabetes and obesity alter the risk of developing variety of cancers, and the associations are biologically plausible. The diabetes-cancer link has been hypothesized to rely on factors such as hormones (insulin, IGF-1, adipokines), immunoresponse (inflammation) or metabolic features (hyperglycemia). So far, insulin has been considered as a major link between diabetes and cancer, while high glucose or hyperglycemia has been considered as a subordinate cause. However, recent epidemiological studies strongly link high glycemic index to HCC risk which suggests that glucose homeostasis directly affects cancer associated pathways.

Hyperglycemia is a common feature to all types of diabetes. Recent studies report that aberrant Wnt signaling is present in 40–90% gastrointestinal cancers including HCC. These are the organ specific cancer sites which are more tightly associated with metabolic parameters altered in diabetes. Moreover, mutations in the CTNNB1 gene (encodes β-catenin) and atypical accumulation of β-catenin protein has been reported in human HCC tumors. Growing number of evidences suggest that canonical Wnt signaling which is modulated by β-catenin may serve as a pathway that links enhanced cancer risk with altered metabolic state such as hyperglycemia. However, direct involvement of high glucose induced Wnt signaling in association with HCC growth is least explored.

Canonical Wnt signaling is suppressed by dickkopf (DKK) family of secretory glycoproteins namely DKK1, DKK2, DKK3 and DKK4. DKK proteins bind to low-density lipoprotein receptor-related protein-5, which enhances GSK3β mediated degradation of β-catenin complex in the cytoplasm, thus reducing

transcription of target genes. Contradictorily, a report suggests that DKK1 is associated with increased β-catenin accumulation while DKK2 and DKK3 genes are inactive in HCC tumors because of epigenetic modification. Although, reduced expression of DKK4 has only been reported in HCC cell lines and a human HCC tumor, its functional relevance under hyperglycemia is still unexplored. Present study investigates the role of DKK4 in glucose induced proliferation of HCC cells through modulation of canonical Wnt signaling pathway.

Aims and Objectives

- To study the hyperglycemia promoted molecular events associated with growth of HCC cells.

Work Achieved

Results

High glucose enhances proliferation in HCC by increasing percent of cells in S phase and suppresses expression of Wnt antagonist DKK4

We first investigated whether glucose directly affects HCC growth by determining percent change in proliferation of HepG2, SK-HEP-1, Chang Liver and WRL 68 cells under varying glucose culture conditions for 48 hr and 96 hr. We observed that treatment with high glucose significantly increases proliferation of HCC cells (Fig. 1A). To rule out the possibility that this effect is due to differences in the osmolarity, cells were cultured in NG along with mannitol (Mntl) (19.5 mM), as an osmolarity control. No significant change in proliferation of cells cultured in NG medium, with or without Mntl was detected, as assessed by MTT assay (Fig. 1A). Also, in the colony formation assay, significantly increased numbers of colonies were detected in HepG2 and SK-HEP-1 cells cultured in HG as compared to NG. These results indicate that HG enhances proliferation of HCC cells. Rapidly proliferating cells require more glucose to meet their energy requirements. Active uptake of glucose is mainly dependent on the level and activity of glucose transporters. Thus, we probed into the status of glucose transporter-1 (Glut-1) and glucose transporter-2 (Glut-2) by western blotting. Although, no change in their levels was detected, an increase in radioactive glucose uptake and utilization was noted in HepG2 cells in a time and concentration dependent manner. The maximum uptake of glucose occurred at 12 hr. These results indicate that cells cultured in HG uptake more glucose and rapidly utilize it, than cells in NG or HG treated with cytochalasin B (CytoB), an inhibitor of receptor mediated glucose uptake.

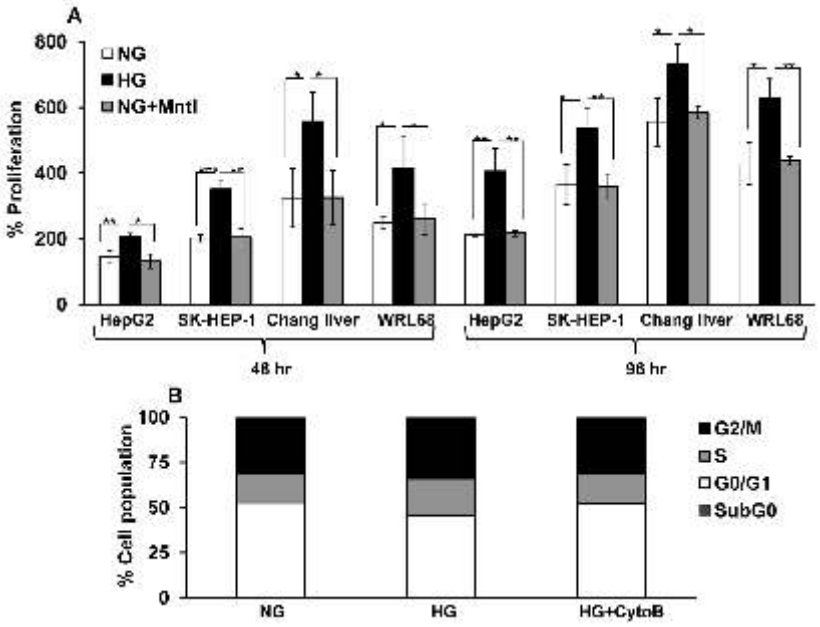


Fig. 1: Glucose enhances proliferation in hepatocellular carcinoma cell lines.

(A) HCC cells (HepG2, SK-HEP-1, Chang liver and WRL 68) were cultured in HG and NG conditions for 48 hr and 96 hr. Thereafter, percent proliferation was determined by MTT assay. Mannitol (Mntl) treated NG conditions served as an osmolarity control. (B) Cell cycle profile of HepG2 cells cultured in NG, HG and HG+CytoB for 16 hr. Bar graphs represent percentage of cells in different phases of cell cycle by flow cytometry of an experiment done in triplicate.

All the bar graph represents the mean±SD of an experiment done in triplicate (*P<0.05, **P<0.001, ***P<0.0001).

Enhanced cellular proliferation involves changes in cell cycle phases and is associated with maximum uptake of nutrients, such as glucose, in the synthesis phase (S- phase). Implication of increased glucose uptake and its utilization by cells was evaluated by performing cell cycle analysis. We observed that in HepG2 cells, the percentage of cells in S-phase of cell cycle was more in HG than in NG, which was curtailed by CytoB (Fig. 1B). Additionally, we checked the levels of regulatory proteins such as CDK6, CDK4, Cyclin D1 and c-Myc, specific to G0/G1/S phases of cell cycle, by western blot analysis. In HCC cells cultured in HG, the levels of these proteins were elevated compared to NG and in HG treated with CytoB. These results indicate that, HCC cells cultured under variable glucose conditions show enhanced glucose uptake and proliferate more in HG than in NG or HG with CytoB, which parallels with increase in percentage of cells in S-phase of cell cycle. Since changes in proliferation of HCC cells in response to variable glucose conditions were observed, we investigated the effect of glucose on various proliferative signaling cascades. In response to variable glucose culture conditions, the levels of proliferation associated proteins such as pMEK, MEK, pAKT, AKT, pERK, ERK and Raf-1 remain unaltered. However, c-Myc (a target of canonical Wnt signaling) levels were prominently increased in both HepG2 and SK-HEP-1 cells. The enhanced level of c-Myc because of glucose induced activation of canonical Wnt signaling has been previously reported.

Abrupt oncogenic activation of canonical Wnt signaling pathway by Wnt3a ligand is one of the comprehensive events in hepatocarcinogenesis. Also, it has been reported that the activity of Wnt ligands is suppressed by the presence of antagonists such as DKK secretory proteins. Therefore, to check for correlation if any, between DKK4, β–catenin, Wnt3a and c-Myc expression in HCC, we searched the ONCOMINE human cancer genomics database. Data available from 210 samples from TCGA cohort suggest that the expression level of DKK4 was decreased while c-Myc and Wnt3a levels were increased in HCC in comparison to normal liver. Analysis of grade wise distribution

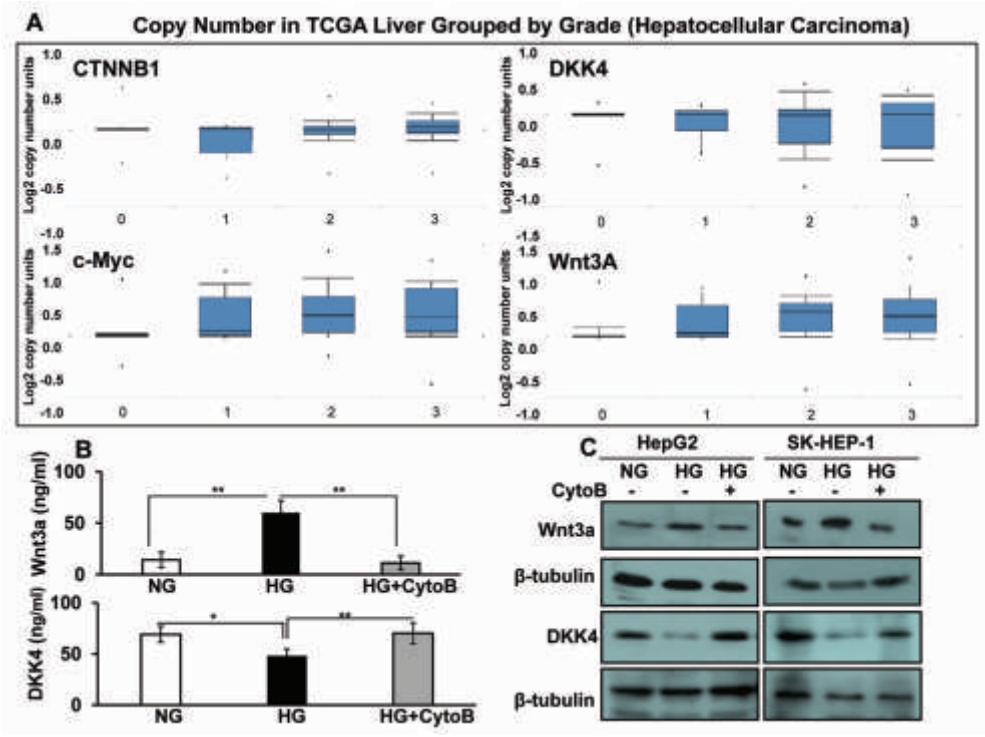


Fig. 2: TCGA analysis of normal liver v/s HCC for expression and grade wise distribution of CTNNB1, DKK4, c-Myc and Wnt3a, and effect of glucose.

(A) Grade wise expression of CTNNB1, DKK4, c-Myc and Wnt3a in HCC from TCGA cohort study obtained from ONCOMINE database. (0) no value (n=173), (1) grade 1 (n=14), (2) grade 2 (n=62) and (3) grade 3 (n=37). All datasets were filtered by threshold criteria by P value 1E-4, Fold change 2 and gene rank top10%.

(B) ELISA measurements of Wnt3a and DKK4 secretory proteins in culture media collected after 16 hr from HepG2 cells in NG, HG and HG+CytoB.

© HepG2 and SK-HEP-1 cells were cultured in NG, HG and HG+CytoB for 16 hr.Whole cell lysates were subjected to western blotting and levels of Wnt3a and DKK4 proteins were detected. All the bar graphs represent the mean±SD of an experiment done in triplicate (*P<0.05, **P<0.001, ***P<0.0001).

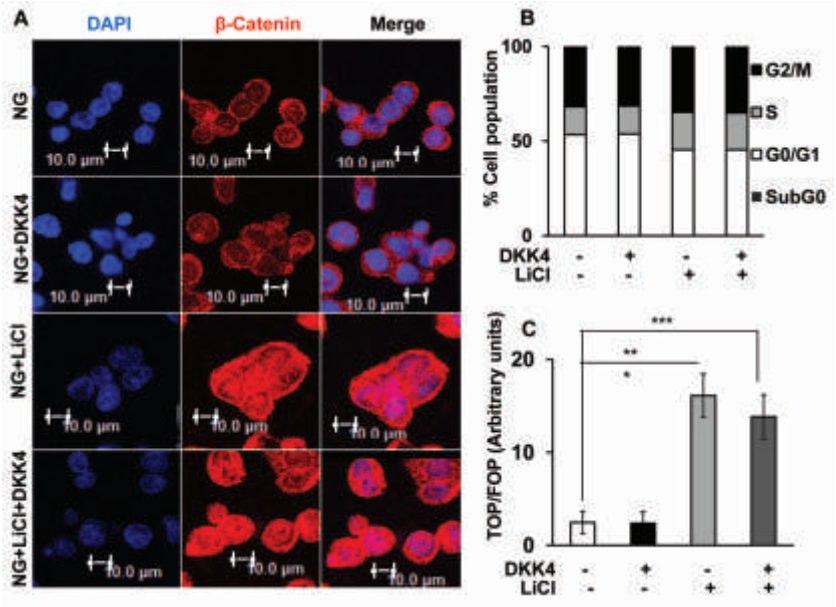


Fig. 3: β-catenin stabilization in NG reverses effect of DKK4.

(A) Immunofluorosence based confocal imaging of β-catenin protein in HepG2 cells cultured under indicated conditions for 16 hr. Bars represent 10μm. (B) Cell cycle profile of HepG2 cells cultured in NG, NG+LiCl, NG+DKK4 and NG+LiCl+DKK4 protein, for 16 hr. Bar graphs represent percentage of cells in different phases of cell cycle by flow cytometry. (C) TCF reporter activity assay in HepG2 cells cultured in NG, NG+LiCl, NG+DKK4 and NG+LiCl+DKK4, protein for 16 hr. The luciferase intensities were normalized with Renilla intensities and data is represented as ratio of TOP/FOP. Bar graphs represent mean±SE of three independent experiments (*P<0.05, **P<0.001, ***P<0.0001).

(grade 1-4) of these samples showed reduction in DKK4 expression and increase in expression of c-Myc and Wnt3a (Fig. 2A), whereas, CTNNB1 expression remain unchanged. These observations prompted us to investigate the alterations in the levels of Wnt3a and DKK4 proteins in response to glucose.

In response to variable glucose concentrations we examined the status of Wnt3a and DKK4 ligand. Interestingly, Wnt3a level was significantly elevated in culture medium and in whole cell lysate of HepG2 cells cultured in HG compared to NG or in HG with CytoB (Fig. 2B upper panel and 2C upper panel). Also, we screened the mRNA levels of DKK genes. We observed that under HG condition, the level of only DKK4 mRNA significantly decreased than in NG. DKK4 mRNA levels increased again upon inhibition of glucose uptake by treating cells in HG with CytoB. Also, the level of secreted form of DKK4 decreased in HG compared to NG culture medium. Interestingly, in HG cells, upon treatment with CytoB, DKK4 secretory protein level increased than in HG alone (Fig. 2B lower panel). The level of DKK4 protein in whole cell lysate of HCC cells diminished in HG compared to NG and in HG with CytoB (Fig. 2B lower panel). On the contrary, when HepG2 cells were cultured for varying time points in glucose free medium, the level of DKK4 protein was increased with increase in time duration of glucose deprivation. Additionally, supplementation of other carbon sources to cells, such as glutamine or glucose analogue such as L-glucose did not affect DKK4 protein level. These results suggest that in HG condition DKK4 expression is suppressed whereas, Wnt3a level is increased, which might be involved in activation of Wnt signaling pathway.

Stabilization of β-catenin reverses the proliferation suppressive effect of DKK4 in HCC cells cultured in NG

So far, we observed that under NG culture condition, DKK4 is constitutively expressed which diminishes β-catenin induced cell proliferation. We therefore, investigated whether stabilization of β-catenin in NG by LiCl, which primarily acts by inhibiting GSK3β inside the cell, interferes with the proliferation inhibitory effect of DKK4 and allows activation of canonical Wnt signaling. No changes were detected in GSK3β protein level in response to glucose. However, HCC cells in NG subjected to LiCl treatment proliferate rapidly than cells in NG alone, as determined by MTT assay. Also, more number of colonies were detected upon LiCl treatment in NG cells, and the number of colonies did not decrease upon treatment with DKK4 protein. Moreover, treatment of cells with LiCl causes an increase in β-catenin protein level in HepG2 cells cultured in NG, which was not reduced, even upon DKK4 protein treatment (Fig. 3A). Upon

LiCl treatment, the level of DKK4 protein in whole cell lysate remains unchanged, whereas the level of c-Myc protein was increased under NG condition. This correlates with increase in percentage of cells in S-phase of cell cycle under NG cultured conditions, in presence of LiCl (Fig. 3B). TOP/FOP ratio was also significantly enhanced in LiCl treated HepG2 cells in NG together with DKK4 protein, than in NG alone (Fig. 3C). Furthermore, upon treatment of HepG2 cells cultured in NG with 6-Bromoindirubin-3'-oxime (BIO), a specific inhibitor of GSK3β, cell proliferate rapidly, which parallels with increase in β-catenin protein level and transcriptional activity, as indicated by elevated TOP/FOP ratio. Collectively, these results indicate that DKK4 induced suppression of proliferation in NG can be abrogated by stabilizing β-catenin through inhibition of GSK3β either by LiCl or BIO.

Hyperglycemia enhances the progression of HCC xenograft tumor

Up till now, we demonstrated that high glucose causes proliferation of HCC cells in vitro. Further, these findings were evaluated in vivo, and we investigated the effect of hyperglycemia on the progression of HCC xenograft tumors. NOD/SCID mice were grouped as Group I and Group II. Group II animals had access to 15% glucose in drinking water. After 58 days, glucose levels in blood increased in mice supplemented with 15% glucose in drinking water (Group-II) as compared to control mice (Group-I) ($P<0.001$) (Fig. 4A). No significant difference in the body weight was detected in mice from both the groups. Following two months of glucose supplementation, equal numbers of HepG2 cells were subcutaneously injected into the right flank of each animal and these mice were observed for initiation and progression of tumors. Tumors progressed rapidly and tumor dimensions in glucose supplemented mice were at least twice the size of tumors in control fed animals on day 27 (Fig. 4B). Tumor weight was significantly higher in Group-II animals than in Group-I (Fig. 4C). In the lysate of tumors from Group-II mice, the levels of c-Myc and β-catenin proteins were more as compared to levels in the tumors from Group-I. Interestingly, DKK4 protein was barely detectable in lysates of tumors from glucose supplemented mice whereas in tumors from control group, DKK4 protein was abundantly present (Fig. 4D). These results suggest that glucose rich environment enhances progression of HCC tumor in vivo. In rapidly growing tumors DKK4 level is diminished whereas β-catenin level increases which are in concurrence with our in vitro findings.

In summary, our findings suggest that high glucose facilitated HCC proliferation is a consequence of the expressional and

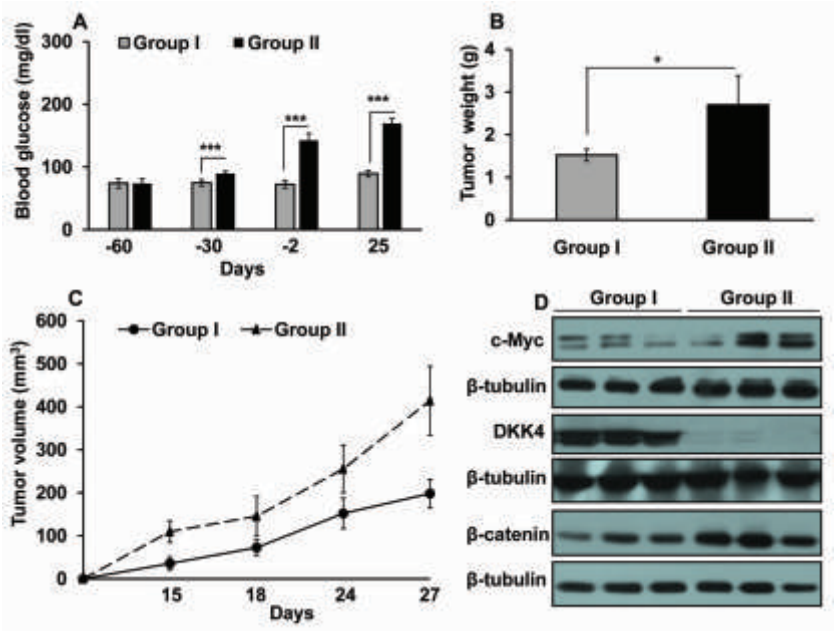


Fig. 4: High glucose enhances HepG2 xenograft tumor growth in vivo.
(A) Blood glucose (mg/dl). (B) Tumor weight (g). (C) HepG2 cells (5 × 106/mice) were injected s.c. on the right flank of each mouse. Tumor initiation and progression in Group-I and Group-II mice were recorded for 27 days. Data is represented as mean of five mice+SD (*$P<0.05$, **$P<0.001$, ***$P<0.0001$). (D) Protein level of c-Myc, β-catenin and DKK4 in representative three tumor samples each from mice of Group-I and Group-II were detected by western blotting.

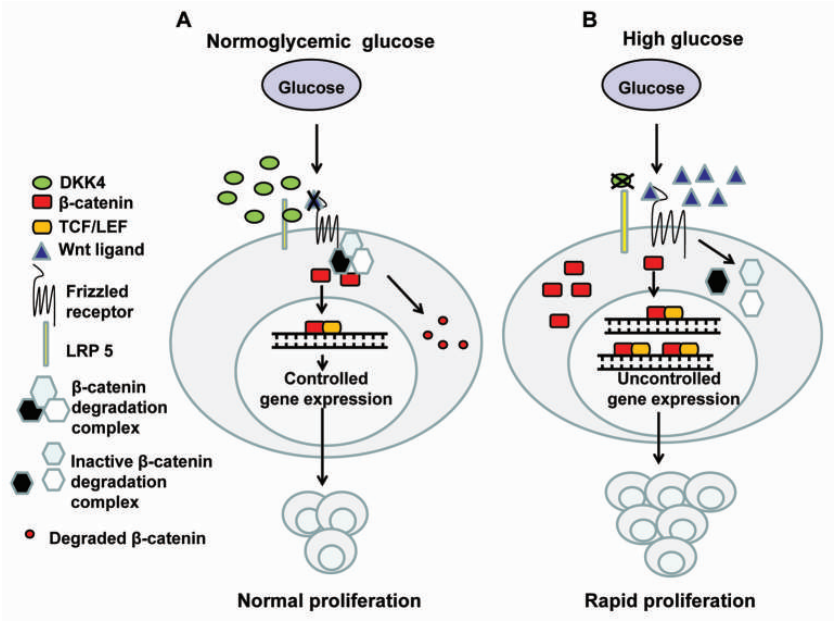


Fig. 5: Schematic representation of glucose induced regulation of DKK4 and effect over HCC proliferation.

(A) Normoglycemic glucose promotes sustained expression of DKK4 protein. DKK4 antagonizes activation of canonical Wnt signaling by facilitating degradation of β-catenin in cytosol and thus reducing its transcriptional activation thereby causing decrease c-Myc level. Increased DKK4 expression affects progression of cells at S-phase of cell cycle and therefore limits proliferation of HCC cells. (B) High glucose diminishes DKK4 expression allowing activation of canonical Wnt signaling because of inactivation of β-catenin degradation complex, by Wnt3a proteins. Increase in β-catenin level enhances its transcriptional activity and promotes c-Myc expression which causes uncontrolled proliferation of HCC cells.

functional interplay between DKK4, β-catenin and c-Myc. In HCC cells, under normoglycemic conditions, DKK4 antagonizes activation of canonical Wnt signaling and thus checks proliferation specifically at G0/G1/S phase of the cell cycle. Hyperglycemia diminishes DKK4 protein which causes activation of canonical Wnt signaling pathway through Wnt3a ligand mediated enhanced translocation of β-catenin into nucleus, thereby promoting proliferation of HCC cells.

Future Research Plans

The World Health Organization (WHO) predicts that the diabetic and obese population will double from the year 2000 to 2030 and the epidemiological data clearly establish a link between metabolic disorders and cancer. The 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. By applying appropriate in vitro as well as in vivo models, the future objectives of our laboratory are to explore:

- Hyperglycemia-induced alterations at gene level as well as signaling cascade in solid tumors cells and the role of drugs causing hypoglycemia on the growth, proliferation and survival of solid tumors.
- The interrelation-ship between metabolic abnormalities and cancer; Influence on growth and therapy.

Samit Chattopadhyay

samit@nccs.res.in
samit@iicb.res.in

Understanding novel functions of the tumor suppressor protein, SMAR1

Participants

Aritra Das, *CSIR-SRF*
Nandaraj Taye, *CSIR-SRF*
Aftab Alam, *UGC-SRF*
Shruti Joshi, *CSIR-SRF*
Sonal Patel, *CSIR-SRF*
Apoorva Parulekar, *UGC-SRF*
Arpankumar Choksi, *CSIR-SRF*
Priyanka, *DBT-JRF*
Richa Pant, *NCCS-JRF*
Vibhuti Kumar Shah, *DBT-JRF*
Tanaya Roychowdhury, *UGC-JRF*

Collaborators

Jeff Dilworth, *Ottawa Hospital Research Institute, Canada*
Tanya Das, *Bose Institute, Kolkata*
GaurisankarSa, *Bose Institute, K*olkata
Amitava Das, NCL, Pune
KishorPaknikar, *ARI, Pune*
Saumitra Das, *IISc, Bengaluru*
Siddhartha Roy, *IICB, Kolkata*
Subhrangshu Chatterjee, *Bose Institute, Kolkata*
Ramanamurthy Bopanna, *NCCS*
Manas K Santra, *NCCS*
AbhijitDey, *ACTREC, Navi Mumbai*
Balaram Ghosh, *IGIB, New Delhi*

Background

The nuclear matrix provides structural framework to the nucleus, tethering several proteins which are important for many processes like transcription, splicing, DNA repair etc. The nuclear chromatin is organized in loops by the nuclear matrix, thus modulating the chromatin architecture. Scaffold Matrix Attachment Binding Protein 1 (SMAR1) is a nuclear matrix-binding protein and belongs to a family of BEN domain proteins. This BEN domain is crucial for the DNA binding and protein binding function of these proteins. Earlier studies from our lab have shown that SMAR1 is a chromatin modifier which recruits HDAC1 to the promoter and brings about modulation of the activity of promoter like that of Cyclin D1. SMAR1 was also reported to regulate apoptosis and survival by regulating the expression of Bax and Puma. Recently, SMAR1's role as a stress response protein was elucidated, wherein SMAR1 was reported to modulate the acetylation status of Ku70 by interacting with HDAC6 (Chaudhary et. al. 2014 Cell death and Disease). Additionally, SMAR1 was reported to negatively regulate alternative splicing by modulating the acetylation status of Sam68 by recruiting HDAC6 (Nakka et. al. 2015 PNAS). Recently we also showed that the switch between effector T cells and regulatory T cells is governed SMAR1. T cell polarization is controlled by SMAR1, as SMAR1 allows the T cells to commit to Th2 lineage and suppresses the Th1 and Th17 lineage commitment. FoxP3, a major factor in Treg cell differentiation, is controlled by SMAR1 and this maintains the fine balance between the Treg and Th17 phenotypes (Mirlekar et.al. 2015 Mucosal Immunology; Mirlekar et. al. 2017 Frontiers in Immunology). 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 the p53 status (Mathai et. al. 2016 Scientific Reports).

Aims and Objectives

- Studies on the regulation of antigen processing and presentation by SMAR1 and its implication in tumorigenesis.
- Determination of the role of SMAR1 in the Wnt signaling pathway.
- Studies to understand the metabolic regulation of epigenetic changes in the tumor suppressor gene, SMAR1.
- Determination of the role of the nuclear matrix binding protein, SMAR1, in vertebrate embryogenesis.

Work Achieved

Regulation of antigen processing and presentation by SMAR1 and its implication in tumorigenesis

Cancer immune evasion is a major problem in designing effective anti-tumor therapy. Cancer cells evade immune surveillance by down-regulating the antigen processing machinery affecting the major histocompatibility complex (MHC) I pathway. Proteins with chaperone activity, like calnexin and calreticulin, play a pivotal role in the MHC I pathway. SMAR1 is a nuclear matrix protein having repressor function and targets a set of specific genes in response to various physiological and environmental conditions. The effect of SMAR1 knockdown on the proteome of colon carcinoma cell-line carrying wild-type p53 (HCT116) was determined by 2D gel electrophoresis. In this screen, one of the target proteins of SMAR1 was found to be calnexin. Further, the regulation of calnexin expression by SMAR1 was studied in details [figure 1 (a)]. To delineate the mechanism of how SMAR1 regulates calnexin gene expression, a bioinformatics analysis of the calnexin promoter was performed. Interestingly, SMAR1 and GATA2-binding sites were observed proximal to each other on the calnexin promoter. Chromatin immunoprecipitation confirmed the binding of SMAR1, GATA2 and HDAC1 on the calnexin promoter [Figure 1 (b)]. We found that SMAR1 forms a triple complex with GATA2 and HDAC1. Recruitment of HDAC1 by SMAR1 on the calnexin promoter results in deacetylation of GATA2. Under deacetylated condition, GATA2 acts as a repressor, resulting in downregulation of the calnexin gene. This study mechanistically highlights the co-ordinated regulation of the calnexin gene by SMAR1 and GATA2 [figure 1 (c)]. We further checked if SMAR1 can regulate MHC1 gene expression by regulating calnexin gene expression in cancer cells. We showed that SMAR1 down regulates calnexin gene expression, resulting in increased expression of MHC1 [figure 1(d)]. MHC1 expression is directly

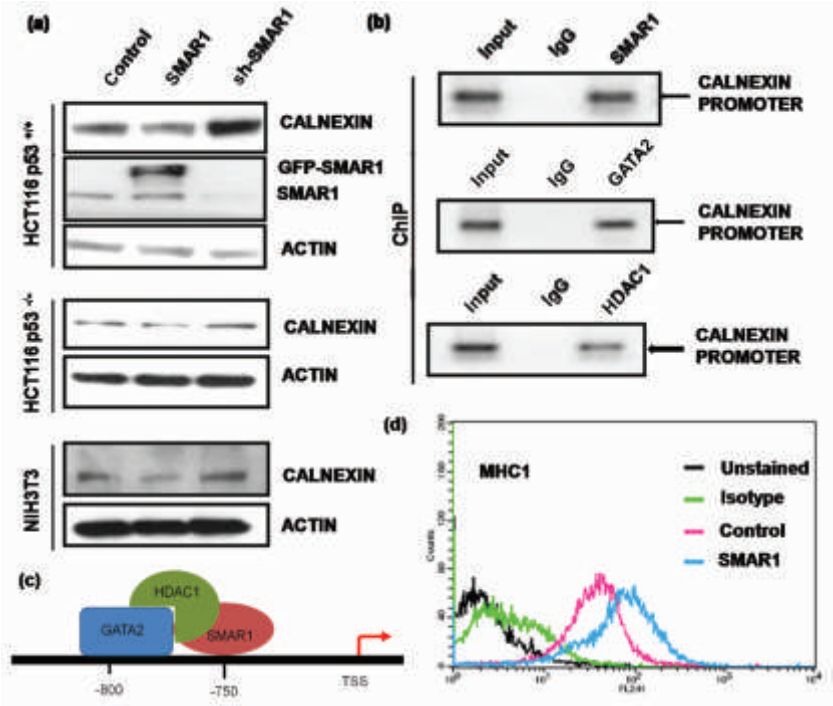


Fig. 1: SMAR1 modulates MHC1 surface expression on cancer cells by downregulating Calnexin: (a) Western blotting of HCT116 $p53^{+/+}$, HCT116 $p53^{-/-}$ and NIH3T3 cells upon SMAR1 overexpression and downregulation. (b) Chromatin immunoprecipitation (ChIP) to check the binding of SMAR1, GATA2 and HDAC1 on calnexin promoter containing the MAR sequence. (c) Model showing SMAR1 and GATA2-mediated coordinated regulation of calnexin promoter by recruiting repressor complex. (d) Flow cytometry to check the surface expression of MHC1 on HCT116 $p53^{+/+}$ cells upon SMAR1 overexpression.

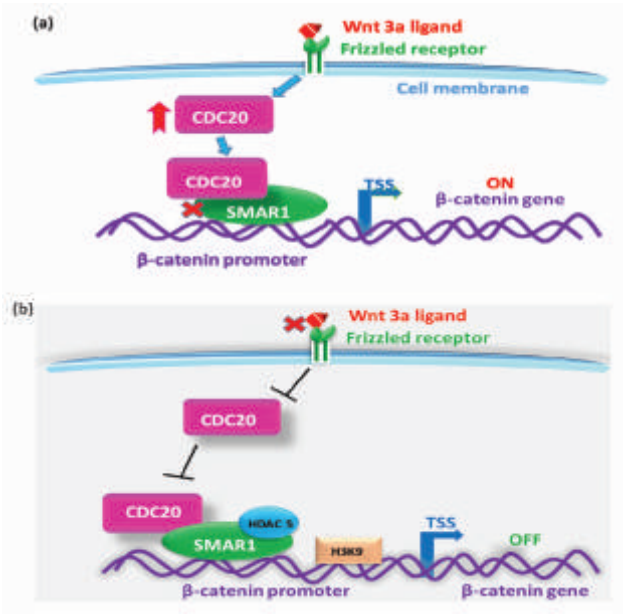


Fig. 2: Model of SMAR1 regulation upon Wnt signaling activation. (a) In the presence of active Wnt 3a ligand binding to Frizzled receptor the levels of CDC20 increases. Increased interaction of CDC20 with SMAR1 mediates more SMAR1 proteasomal degradation. SMAR1 occupancy in the β-catenin promoter decreases leading to increased transcription of β-catenin mRNA. (b) In the absence of Wnt 3a ligand, the endogenous level of CDC20 is not able to increase SMAR1 proteasomal degradation. Thus, increased occupancy of SMAR1 in the β-catenin promoter increases along with HDAC5. This effect leads in the decreased promoter activity of β-catenin leading to its decreased expression.

related to cancer antigen presentation, which is part of the immune editing phenomenon. In the majority of cancers, including breast and colon cancer, SMAR1 and MHC1 expression was found to be downregulated, leading to poor prognosis and disease outcome. Here we showed that SMAR1 has a positive correlation with MHC1 by downregulating calnexin expression. We are working on small compounds that can stabilize SMAR1 expression and MHC1 expression in cancer cells, which will have potential in therapeutic intervention by modulating the immune editing phenomena.

Role of SMAR1 in the Wnt signaling pathway

Wnt/β-catenin signaling is a hallmark of various diseases including cancers. The Wnt ligands bind to Frizzled receptors and disrupt the "destruction complex" consisting of Axin, Adenomatous polyposis Coli, GSK-3β and Caesin kinase Iα. This allows β-catenin to accumulate in the cytoplasm and translocate into the nucleus. Inside the nucleus, β-catenin binds to the TCF/LEF family of transcription factors and activates the transcription of β-catenin target genes. However, in normal conditions when Wnt ligands are absent, the "destruction complex" mediates the phosphorylation of the β-catenin protein. Phosphorylated β-catenin undergoes proteasomal degradation by β-TrCPI, and thus the down-stream target genes are prevented from transcription. We found that aberrant Wnt/β-catenin signaling favours oncogene expression and deregulates tumor suppressors. Here we report that active Wnt signaling using Wnt 3a CM stimulation promotes SMAR1 protein degradation. SMAR1 is a well reported tumor suppressor that antagonizes the expression of various oncogenes like p300, TGFβ and CD44. It also stabilizes the expression of p53 and regulates cell cycle stage at G2/M phase. Two E3 ubiquitin ligase recognition sequences, called D-box elements, viz. "RQRL" and "RCHL", which are present in the SMAR1 amino acid sequence, are responsible for SMAR1 protein degradation. Mutation of arginine and leucine to alanine in these D-box elements completely abrogated Wnt 3a-stimulated SMAR1 protein degradation. Human colorectal tissue sections (harbouring polyps) expressed more β-catenin than SMAR1 in the basal region of the crypt, where stemness is highest, due to active Wnt signaling. Polyp tissues from mouse colon expressed more β-catenin and diminished SMAR1 levels than the adjacent tissues. Later, we found that CDC20 (Cell-Division Cycle Protein 20) associates with SMAR1 and mediates proteasomal degradation of SMAR1 [figure 2(a)]. However, restoration of SMAR1 led to attenuation of Wnt signaling by controlling the expression of the β-catenin gene. SMAR1 occupies the β-catenin promoter and recruits HDAC5, which keeps the promoter in a deacetylated state. In the absence of SMAR1, HDAC5 recruitment fails, leading to active transcription of the β-catenin gene. The activity of the promoter of β-catenin is enhanced by H3K9 acetylation in the β-catenin promoter [figure 2 (b)]. Therefore, overexpression of SMAR1 in HCT116 cells downregulates β-catenin, whereas SMAR1 knockdown results in increased expression of β-catenin. Increased β-catenin levels have been found to be involved in enhancing colorectal cancer tumorigenesis. Hence, downregulation of β-catenin by SMAR1 could have therapeutic potential against colorectal cancer.

Metabolic regulation of epigenetic changes in the tumor suppressor gene, SMAR1

Rapidly proliferating cells show a significant increase in glycolysis, known as the "Warburg effect". To survive and continue proliferation, rapidly proliferating cancer cells have to circumvent many stresses, one of which is metabolic stress. Since these cells have a much higher glucose requirement than

normal healthy cells, it would be really interesting to study the effects of glucose deprivation on them. Reports from previous studies have shown that the levels of SMAR1 in malignant cells are constitutively low, probably because SMAR1 being a tumor suppressor, it is essential for cancerous cells to keep it in a suppressed state. We observed that the SMAR1 promoter is methylated at eight CpG dinucleotides in hepato-adenocarcinoma cell lines. This might explain how the levels of SMAR1 remain at low, basal levels in these cells.

We have shown for the first time that in HepG2 cells, SMAR1 levels are kept low by the promoter hypermethylation and that this methylation can be erased and re-introduced just by changing the metabolic conditions. We have further shown the mechanism by which the promoter is methylated and de-methylated. This change is seen when the cell is given metabolic stress by depriving the cells of glucose. We observed that this not only causes de-methylation, but it also causes the loss of histone methylation marks. We also confirmed the methylation of the *SMAR1* promoter in murine liver cancer samples. Our observations showed that the promoter is methylated in higher grades of cancer, which also reduceds the transcript levels of *SMAR1* in these murine tumor samples. Apart from methylation status of the promoter and different histone modifications, we have also investigated the possible role of the STAT3-Dnmt1 axis in this entire mechanism and we have found some significant leads. Moreover, we have shown for the first time that, SMAR1 controls the acetylation of GAPDH, which is required for the enzymatic function.

Role of the nuclear matrix binding protein, SMAR1, in vertebrate embryogenesis

SMAR1 has been shown to have a multifaceted role. It acts as a tumor suppressor by virtue of its interactions with p53, in modulation of cell cycle, in inhibition of migration by modulating the TGFβ pathway, etc. However, the role of SMAR1 in vertebrate development remains unclear. Here, we report the presence of SMAR1 in zebrafish, which shares 66% homology with the mouse version of SMAR1. The expression of SMAR1 in zebrafish was validated both at the transcript and protein levels by semi-quantitative PCR and western blotting. Whole mount RNA in-situ hybridization revealed the spatial distribution pattern of SMAR1 mRNA in the developing embryo, showing localization of the probe around the brain ventricles and in the posterior region, which is considered to be the hematopoietic part. Cloning of the ORF and protein expression, followed by its

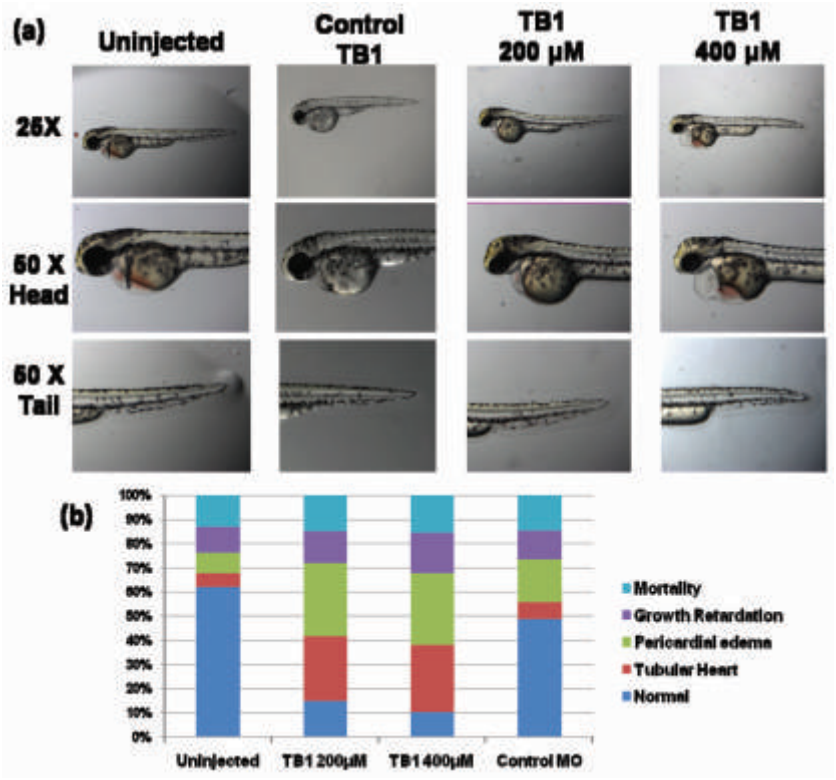


Fig. 3: Phenotypic changes observed in zebrafish embryos upon MO-induced SMAR1 knock-down. (a) Visible phenotypic changes including pericardial edema, tubular heart and smaller cranial size. (b) Quantitation of the phenotypic changes upon SMAR1 knockdown as compared to normal embryos.

purification by affinity chromatography has been achieved. This purified protein was confirmed as SMAR1 from zebrafish using MALDI.

Morpholino antisense oligonucleotide against the SMAR1 transcript was used to block the translation of SMAR1 and knockdown was achieved in zebrafish embryos. Knock-down of SMAR1 was marked by embryonic malformations - smaller head size, pericardial edema and a linear heart tube phenotype [figure 3 (a)]. Taken together, these results indicate that SMAR1 might be regulating embryogenesis, particularly cardiogenesis, in zebrafish. To pinpoint how SMAR1 is involved in any of these developmental pathways, a holistic approach was used and differential whole transcriptome analysis was performed. This revealed several transcripts showing variations in the absence of SMAR1. Genes with different molecular functions involved in various biological pathways, markedly cell cycle regulation, mRNA surveillance and FoxO signaling, were altered [figure 3(b)]. The molecular mechanism underlying such malformations might be interesting to study.

Future Research Plans

- Studies on regulation of the catalytic subunit of telomerase by SMAR1.
- Determination of the role of SMAR1 in tumor cell metabolism via regulation of PKM alternative splicing.
- To decipher the role of SMAR1 in adipogenesis: Its implication in obesity-related cancer.
- To gain mechanistic insights into LPS-regulated cancer progression: Fine-tuning of the tumor suppressor SMAR1.
- Studies on chromatin remodeling protein SMAR1 in $CD4^+$ memory T cell differentiation.

Radha Chauhan

radha.chauhan@nccs.res.in

Structural and Functional Studies on Components of the Nuclear Pore Complex

Background

The nuclear pore complexes (NPCs) embedded in the nuclear membrane bilayer solely mediate transport of all kinds of macromolecules between the nucleus and the cytoplasm, and regulate most cellular processes such as gene expression, mitosis, cell differentiation etc. Additionally, alternations in NPCs and their 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 the NPC assembly formed by these 30 Nups, and its versatile functions, high-resolution structures are highly desired but complexity and 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 research group 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 to understand how Nups participate in nucleocytoplasmic transport, gene regulation and cell differentiation.

Aims and Objectives

- Reconstitution of minimally interacting regions of the Nup93 subcomplex to understand their roles in assembly of the NPC.

Participants

Parshuram Sonawane, *RA*
Somnath Dutta, *RA*
Pravin Dewangan, *SRF*
Kriti Chopra, *SRF*
Bhawna Burdak, *SRF*
Sangeeta Niranjan, *SRF*
Pankaj Kumar Madhesiya, *JRF*
Shrankhla Bawaria, *JRF*
Virashree Jamdar, *Technician B*
Jyotsna Singh, *Project JRF*

Collaborator

Manidipa Banerjee, *India Institute of Technology, New Delhi, India*

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

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

Various native subcomplexes, such as Nup62•Nup54•Nup58, Nup93•Nup205, Nup93•Nup188, were purified and characterized for stability at the biochemical level. Further, these complexes are being analyzed by electron microscopic analysis to characterize their structural features and stoichiometry.

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

The minimal interacting region of Nup93 is identified to interact with the central transport channel. Then the Nup62•Nup54•Nup58•Nup93 quaternary complex is reconstituted in heterologous expression system and purified to homogeneity. EM based reconstruction of 3D model was performed and the ~20 angstrom resolution 3D structure revelaed how Nup93 anchors the central channel of the NPC.

We also analyzed the structure and function of individual Nups to understand their role in NPC assembly. In this regard, our studies on the Nup62 coiled-coil domain have demonstrated that its plasticity plays an important role in the formation of three helix bundles with distinct partners, such as Nup54, Nup58 and Exo70 (Figure 1).

Through in-silico evolutionary analysis of all Nups from various species, we observed that there are siginificant variations in the NPC compostion in many species. Even in Nups where the composition is conserved among diverse species (about 30 Nups), we observed differences at the level of secondary structure and domain organization. For example, the human Nup58 phylogenetic tree (Figure 2) shows that higher

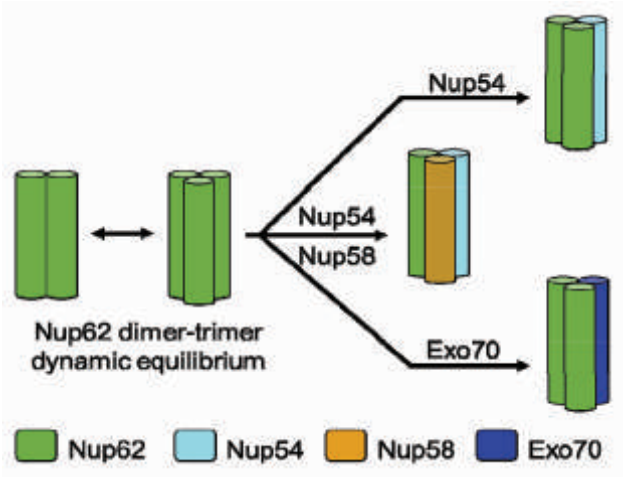


Fig. 1: The Nup62 coiled-coil motif provides plasticity for three helix bundle formation.
Rat Nup62(362-425) helical region exists in hodimeric to homotrimeric parallel helical bundles (shown as gree cylinders). A chain of Nup62(362-425) can be displaced with either Nup54, Nup58 or Exo70 coiled-coil containing helical region, thus yielding Nup62•Nup54, Nup62•Nup54•Nup58 and Nup62•Exo70 helix bundles.

organisms have distinct Nup58 from their neighbors such as unicellular yeast species. Such complexity is likely to be related with more complicated functions of Nups in specific species.

Future Research Plans

- Crystallization of the Nup93•Nup62•Nup54•Nup58 quaternary complex.
- Structural characterization of native complexes such as Nup93•Nup62•Nup54•Nup58 and Nup93•Nup205, Nup93Nup188.
- Mapping the interactions of Nups based on immuno-precipation assays and in-silico approach.

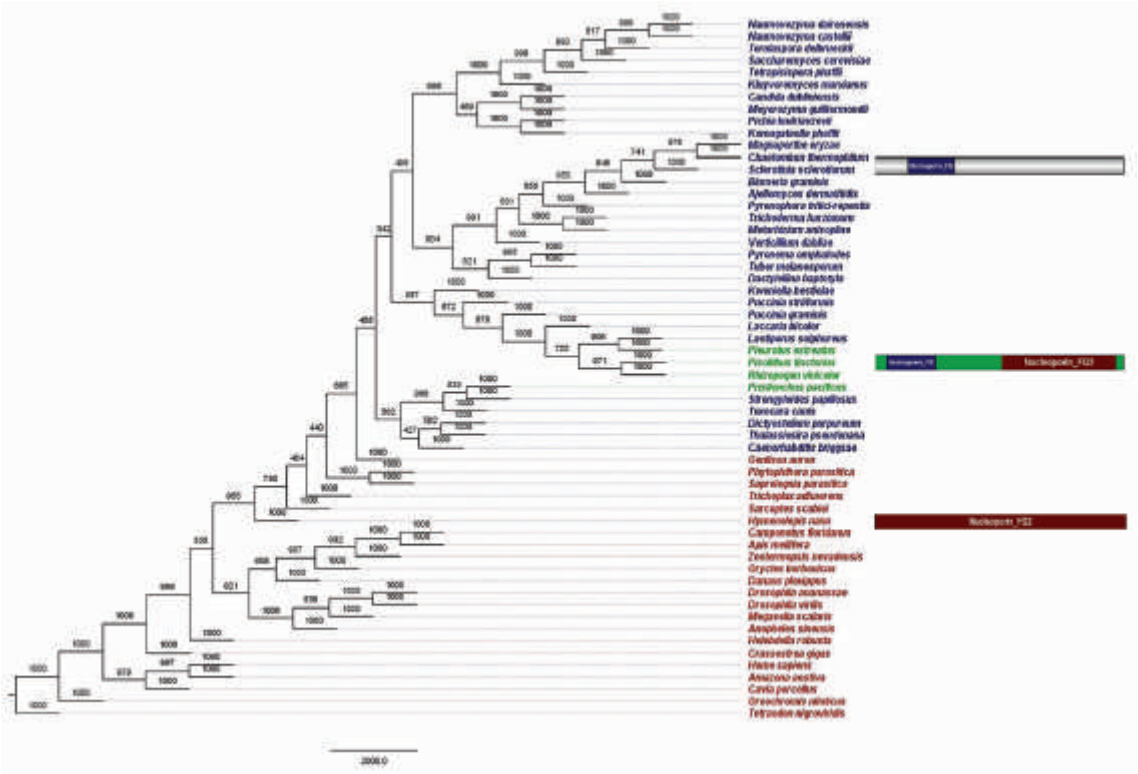


Fig. 2: Phylogentic analysis of Nup58 homologues.
Based on structural multiple sequence alignment, a phylogenetic tree is generated. The branch labels represent the boot strap values. Species marked in blue were predicted to have Nucleoporin_FG domain, species in red showed Nucleoporin FG2 domain and those in green showed both the domains. The representative diagram for domain organization is shown along each class of species found in this phylogenetic tree.

Jomon Joseph

josephj@nccs.res.in

SUMO-interacting motif (SIM) is a new Argonaute-interacting motif (AIM)

Participants

Santosh Kumar Yadav, *SRF*
Aditi Singh, *SRF*
Indrasen Magre, *SRF*
Prachi Deshmukh, *SRF*
Poulomi Banerjee, *JRF*
Sakalya Chavan, *JRF*
Jyoti Pawar, *JRF*
Misha K.R., *JRF*
Aparna Salunke, *Technician*

Collaborators

Mahendra Sonawane, *TIFR, Mumbai*
Madhusudhan, M.S., *IISER Pune*
Gayathri Pananghat, *IISER Pune*
Vasudevan Seshadri, *NCCS*

Background

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 some studies suggest a role for this nucleoporin in specific receptor- and cargo-dependent transport. Nup358 has been identified as a small ubiquitin-like modifier (SUMO) E3 ligase and is shown to mediate in vivo SUMOylation of substrates such as topoisomerase II, borealin and Ran.

SUMO is a small protein that gets covalently conjugated to target proteins through specific lysine residues and modulates their function. SUMO pathway is shown to be involved in multiple cellular processes. In humans, there are four SUMO isoforms; SUMO1-4. In addition to the covalent interaction, SUMO associates with other proteins through directly binding to specific SUMO-interacting motif (SIM), which is characterized by a conserved set of hydrophobic amino acids. Multiple SIMs have been identified in many SUMO interacting proteins and functionally validated. Presence of a stretch of negatively charged amino acids adjacent to the N or C terminus of the hydrophobic sequence (SIM), is shown to contribute to the strength, orientation and paralog specificity of SUMO binding.

SUMO conjugation to the substrate lysine requires concerted action of SUMO specific E1 (Aos1/Uba2 heterodimer), E2 (Ubc9) and multiple E3 ligases. The nucleoporin Nup358 acts as one of the SUMO E3 ligases. RanGTPase-activating protein (RanGAP) is the first SUMO substrate identified. SUMO gets attached to lysine 524 of human RanGAP, which targets it to the NPC through binding to Nup358. Structural and functional analyses showed that SUMO-RanGAP

interacts with Nup358 through a region having internal repeats (IR) harbouring two SIMs. Nup358-IR also possesses the SUMO E3 ligase activity. Each of the two repeats, IR1 and IR2, has a SIM and a Ubc9-binding domain. However, studies have shown that IR1 (SIM1) is involved in SUMO~RanGAP1 interaction, which is also stabilized by Ubc9 that directly binds to IR1, RanGAP1 and SUMO. In vitro studies have illustrated that SUMO-RanGAP and Ubc9 form a stable complex with IR1, and not with IR2. Although no conclusive evidence exists, it is believed that SUMO-dependent binding of RanGAP1 to Nup358 would enhance RanGAP's ability to activate the hydrolysis of GTP on Ran in the export complex. Endogenously, bulk of RanGAP is SUMO-modified and has been shown to associate with Nup358 throughout the cell cycle.

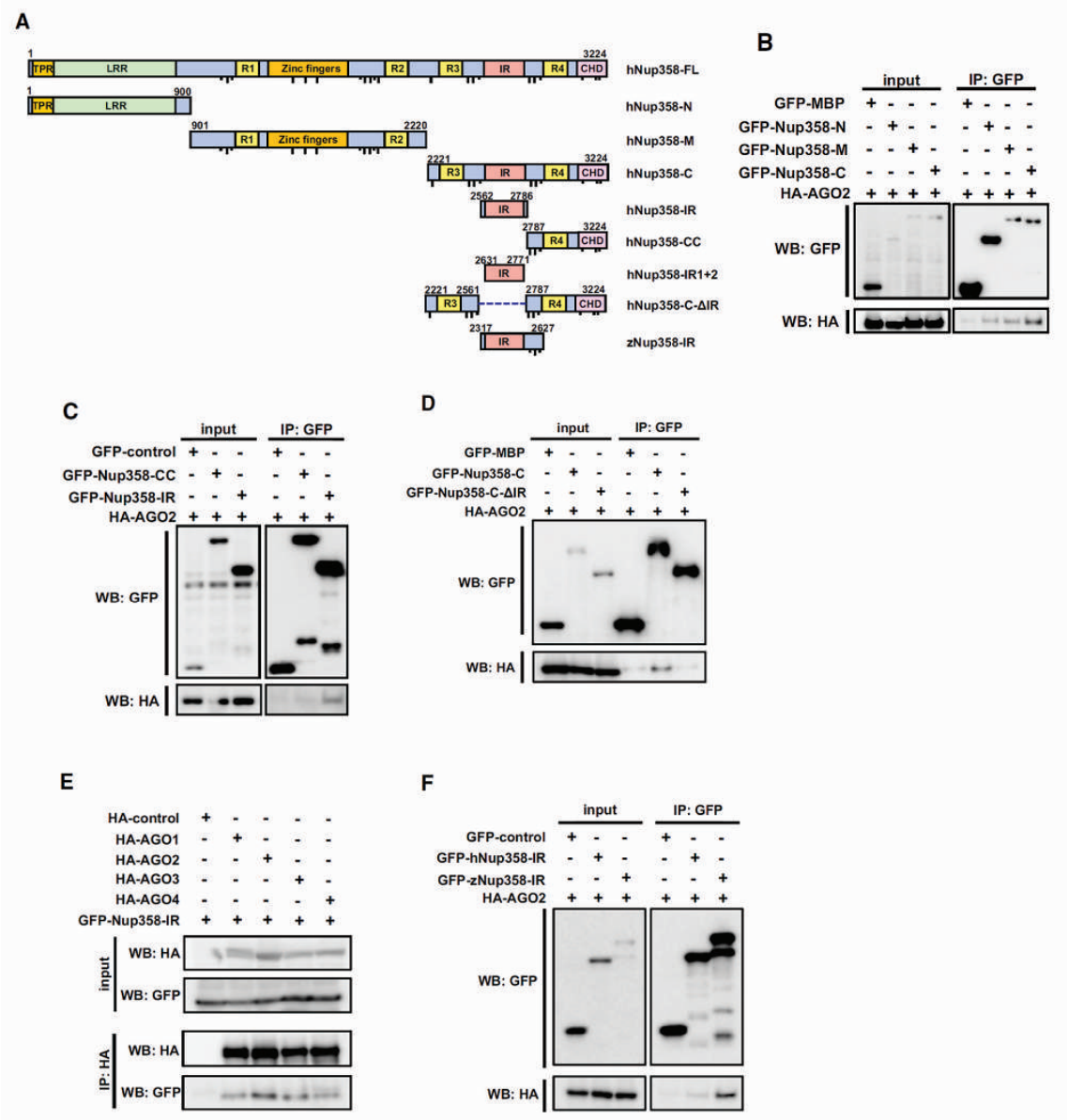


Fig. 1: AGO proteins interact with IR region of Nup358. (A) Schematic diagram representing the domains and constructs of human (h) and zebrafish (z) Nup358 used in this study. TPR, tetratricopeptide repeat; LRR, leucine - rich region; R1–R4, RanGTP - binding domain; ZnF, zinc finger domains; IR, internal repeats; CHD, cyclophilin - homology domain. Dotted line indicates the deleted region in the indicated construct. FG and F × FG sequence positions are represented as short and long black lines, respectively. Amino acid positions are indicated in numbers. FL, full - length; N, amino - terminal region; M, middle region; C, carboxy - terminal region. (B) HEK293T cells were co - transfected with GFP - maltose binding protein (MBP) control or GFP - tagged version of indicated Nup358 fragments along with HA - AGO2. Immunoprecipitation (IP) was performed using GFP antibodies and the presence of AGO2 was detected by Western blotting (WB) using HA antibodies. (C) Cells were transfected with indicated constructs and IP and WB were performed as described for (B). (D) HEK293T cells transfected with GFP - MBP (control), GFP - Nup358 - C, or GFP - Nup358 - CΔIR (a mutant devoid of IR region) and HA-AGO2. IP and WB analyses were performed as indicated. (E) Cells were transfected with HA - tagged version of indicated AGO subfamily member and GFP - Nup358 - IR. IP and WB analyses were performed using indicated antibodies. (F) Lysates from cells expressing GFP - control, GFP - human (h) Nup358 - IR, or GFP - zebrafish (z) Nup358 - IR and HA - AGO2 were immunoprecipitated using GFP antibodies, and the immunoprecipitates were analyzed for the presence of HA - AGO2 by Western blotting.

Previous study from our lab indicated a role for Nup358 in microRNA pathway. We also found that Nup358 physically interacts with the components of miRISC-AGO and GW182. Here we have attempted characterization of the interaction between Nup358 and AGO.

Aims and Objectives

- To delineate the region of Nup358 involved in interaction with AGO proteins
- To understand the functional significance of Nup358-AGO interaction in miRNA pathway

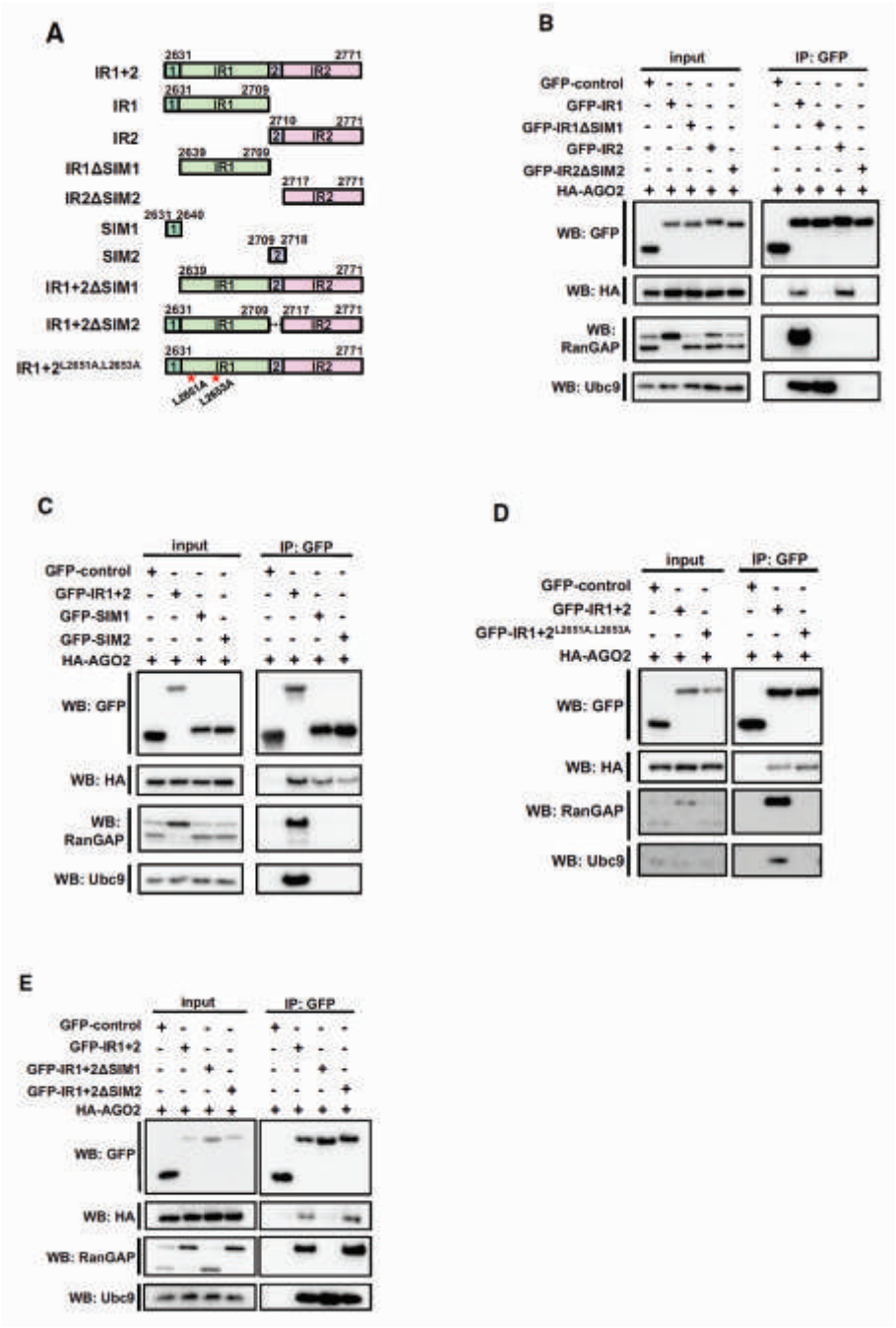


Fig. 2: SIM is the minimum region in Nup358 IR required for binding to AGO2. (A) Schematic representation of human Nup358 - IR region and the constructs used in this study. IR, internal repeats; SIM, SUMO - interacting motif; 1, 2, SIM1 and SIM2. Amino acids substituted in Ubc9 mutant are indicated with red asterisks. (B) HEK293T cells were transfected with indicated constructs, and immunoprecipitation (IP) was performed using GFP - specific antibodies and probed for HA - AGO2 by Western blotting (WB) using HA antibodies. Endogenous RanGAP and Ubc9 were probed with specific antibodies. (C) Lysates prepared from cells expressing the indicated constructs were subjected to IP and WB using indicated antibodies. The presence of endogenous RanGAP and Ubc9 was determined by WB. (D) GFP - control, GFP - IR1 + 2 wild type, or mutants were co - expressed with HA - AGO and IP and WB analyses were performed to detect the interaction using indicated antibodies. (E) Lysates prepared from cells expressing the indicated proteins were immunoprecipitated with GFP - specific antibodies, and the presence of specific proteins in the immunoprecipitates was detected by WB with indicated antibodies.

Work Achieved

Nup358 interacts with AGO proteins through the IR region

Nup358 is a large nucleoporin with multiple domains (Fig 1A). We wished to characterize the interaction between Nup358 and AGO proteins in detail. To delineate the region in Nup358 that is involved in the interaction with AGO, GFP-tagged N-terminal (Nup358-N), middle (Nup358-M) or C-terminal region (Nup358-C) of Nup358 was expressed along with HA-AGO2 in HEK293T cells. Co-immunoprecipitation assays revealed that AGO2 specifically interacted with all three fragments of Nup358, and more prominently with the C-terminal fragment (Fig 1B). Further, we proceeded to identify and characterize the minimum region in Nup358-C required for interaction with AGO2. Experiments with deletion constructs of Nup358-C indicated that the IR region was sufficient to mediate the interaction with AGO2 (Fig 1C). Consistent with this, a deletion mutant of Nup358-C lacking the IR region failed to interact with AGO2 (Fig 1D).

To examine the conservation of interaction between Nup358 and AGO subfamily proteins, GFP-IR was co-expressed with HA-tagged AGO1-4 in HEK293T cells. Co-immunoprecipitation assay confirmed that IR region physically associated with all four AGO proteins (Fig 1E). Moreover, a fragment encompassing the IR region derived from zebrafish Nup358, also showed specific interaction with human AGO2 (Fig 1F). Collectively, these data demonstrated that IR provides a conserved region for interaction with AGO subclade of proteins.

SIM is a conserved motif for AGO interaction

As the results suggested that IR region of Nup358 is involved in binding to AGO proteins, we wished to investigate this molecular interaction in detail. Nup358-IR possesses two internal repeats, each of them having a SIM and a Ubc9-binding region (Fig 2A). Interestingly, we found that both IR1 and IR2 could independently interact with AGO2 in a SIM-dependent manner (Fig 2B). However, as known earlier, IR1, and not IR2, specifically interacted with endogenous SUMOylated RanGAP and Ubc9. Also, deletion of SIM1 from IR1 abrogated the interaction with SUMO~RanGAP, but not with Ubc9 (Fig 2B). To test if SIM is sufficient for AGO interaction, we co-expressed HA-AGO2 with GFP-SIM1 or GFP-SIM2. Co-immunoprecipitation assay clearly indicated that SIM1 and SIM2 independently were capable of interacting with AGO2, but not with SUMO~RanGAP or Ubc9 (Fig 2C).

It has been shown previously that Ubc9 preferably binds to IR1 and stabilizes the interaction between IR1 and SUMO~RanGAP. We wished to test if Ubc9 is required for the interaction between IR (IR1+2) and AGO2. Interestingly, a mutant of IR that is defective in binding to Ubc9 (IR1+2^{L2651A,L2653A}) still efficiently associated with AGO2, whereas as expected, it failed to interact with SUMO~RanGAP (Fig 2D). Moreover, deletion of SIM1, but not SIM2, from IR disrupted its interaction with AGO2, indicating that in the context of intact IR, AGO2 interaction is mainly dependent on SIM1 (Fig 2E). As expected, SIM1 deletion also impaired the ability of IR to associate with SUMO~RanGAP, and not with Ubc9 (Fig 2E). Taken together, these results suggested that AGO-IR interaction neither requires Ubc9 nor SUMO~RanGAP, and SIM is the minimum region in Nup358 required for the interaction with AGO2.

Earlier studies indicated that within Nup358-SIM1, the hydrophobic amino acids V2632, I2634 and L2635 contribute to SUMO-binding. We found that substituting these residues with alanine, compromised the SIM's ability to bind AGO protein (Fig 3A), indicating that the binding mode of SIM with SUMO and AGO proteins could be similar. The finding that both SIM1 and SIM2 from Nup358-IR independently interacted with AGO2 prompted us to investigate if SIMs derived from other SUMO-interacting proteins are capable of binding to AGO. Towards this, GFP-fused SIMs from PIAS1 and TTRAP (Fig 3B) were individually co-expressed with HA-AGO2 in HEK293T cells, and assessed for their ability to interact with AGO2 by co-immunoprecipitation assays. It has already been shown that PIAS1-SIM interacts with both SUMO1 and SUMO2, whereas TTRAP-SIM shows greater preference for SUMO2. We found that irrespective of the proteins from which they were derived, both the SIMs interacted with AGO2 (Fig 3B). Moreover, SIM could associate with both AGO1 and AGO2 (Fig 3C). Collectively, these results indicated that SIM provides a binding platform for conserved interaction with AGO subfamily of proteins.

To examine whether SIM directly binds to AGO proteins, we resorted to bacterially-expressed recombinant proteins. Mixed bacterial lysates expressing maltose-binding protein (MBP, control) or MBP-AGO2 along with GST (control) or GST-SIM1 were used for performing GST pull down assays. The results suggested that GST-SIM1 specifically interacted with AGO2 in vitro, indicating that SIM can directly bind to AGO2 (Fig 3D). Collectively, these experiments suggested that SIM provides a direct binding platform for AGO proteins.

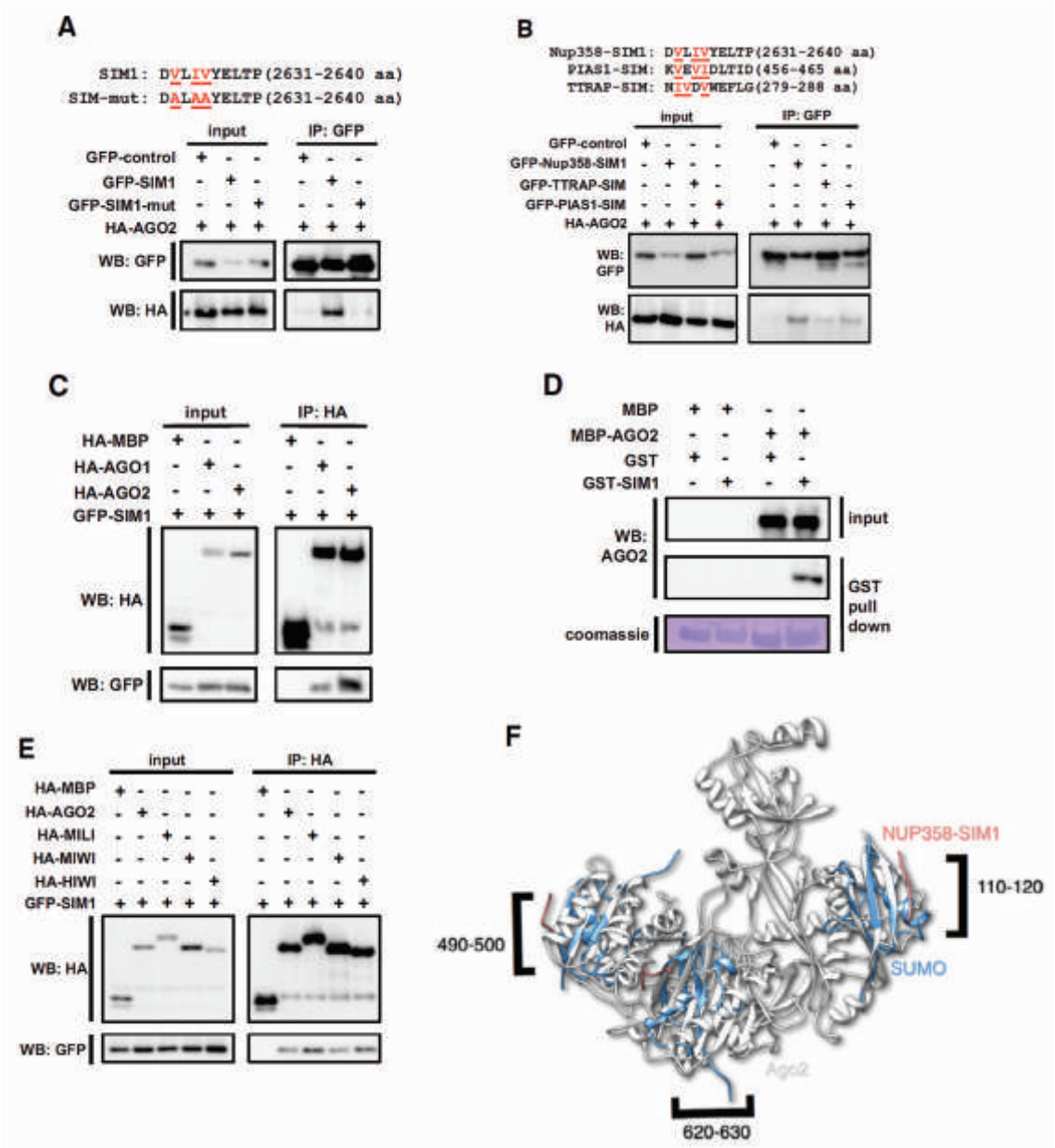


Fig. 3: SIM provides a conserved platform for interaction with AGO family of proteins. (A) Top panel: depiction of the amino acid sequence corresponding to the Nup358 - SIM1 region and substitutions introduced in the SIM1 mutant. Bottom panel: HEK293T cells were co - transfected with GFP - control, GFP - SIM1, or GFP - SIM1 - mut along with HA - AGO2 and immunoprecipitation (IP) was performed with GFP - specific antibodies, and Western analysis (WB) of the input lysate and immunoprecipitates was carried out using indicated antibodies. (B) Top panel: depiction of the amino acid sequence corresponding to the SIMs in the indicated proteins used in this study. Hydrophobic residues involved in SUMO binding are indicated in red. Bottom panel: Constructs expressing GFP - control or GFP - fused SIMs of indicated proteins were co - transfected with HA - AGO2 in HEK293T cells, and IP and WB were performed to determine the extent of interaction. (C) HA - MBP - control, HA - AGO1, or HA - AGO2 was co - expressed with GFP - SIM1 and the cell lysates were subjected to IP with HA antibodies and WB analysis with indicated antibodies to detect the presence of proteins in the immunoprecipitates. (D) SIM1 directly interacts with AGO2. Bacterial lysates expressing MBP control or MBP - AGO2 and GST or GST - SIM1 were mixed and GST pull - down assay was performed. The presence of proteins in the pull - down samples was analyzed by WB using specific antibodies. The extent of GST pull - down was monitored by Coomassie staining of the membrane (Coomassie). (E) SIM1 interacts with PIWI subfamily of proteins. HEK293T cells were co - transfected with indicated constructs expressing HA - tagged version of PIWI proteins and GFP - SIM1. IP was performed using HA antibodies. The immunoprecipitates were probed for the presence of GFP - SIM1 using GFP - specific antibodies by WB. (F) Putative SIM - binding regions in AGO2. Three distinct superimpositions of AGO2 (gray) and SUMO (blue), in complex with Nup358 - SIM1 (red), are shown in cartoon representation rendered with Chimera. The residue numbers on the AGO2 that could be the putative SIM - binding regions are labeled. To identify possible Nup358 binding sites on AGO2, the 3D structure of AGO2 (PDB ID: 4W5O) was compared with that of SUMO (PDB ID: 1Z5S).

Nup358 contains 5 potential SIMs as predicted by GPS-SUMO program at medium SUMO interaction threshold values (http://sumosp.biocuckoo.org). These include one SIM in the N-terminal region (Nup358-N), two within the middle region (Nup358-M) and two in the C-terminal region (Nup358-C). Presence of SIMs in all the three regions provides an explanation for the association of all these fragments with AGO2 (Fig 1B).

SIM can bind to PIWI clade of proteins

As PIWI and AGO subfamilies of proteins share similarity in domain architecture and functions in terms of small RNA mediated gene silencing, we tested if SIM could also bind to PIWI proteins. To address this, co-immunoprecipitation assay was performed using HEK293T cells co-expressing GFP-SIM1 and HA-tagged MBP (control), AGO2, MILI, MIWI or HIWI proteins. The results clearly indicated that PIWI clade proteins, similar to AGO subfamily members, specifically interacted with SIM (Fig 3E). We conclude that SIM provides a general binding platform for interaction with AGO family of proteins.

Potential SIM-binding sites in AGO2

The finding that AGO proteins bind to SIM raised the possibility that AGO has SUMO-like domains found to be present in a few proteins. However, analysis suggested that such domains are absent in AGO proteins. The other possibility included that there could be regions in AGO proteins that are structurally similar to the regions in SUMO that are involved in interaction with SIM. Using a recently developed algorithm, CLICK, probable regions on AGO2 were recognized by structural similarity to the SUMO regions that interact with SIM. This structural analysis identified three distinct regions on AGO2 (Fig 3F). The structures of SUMO and AGO2 in the superimposed regions were between 70-87% geometrically similar with RMSD values ranging from 1.6-1.9 Å. The predicted binding sites comprised amino acids belonging to the N, MID and PIWI domains of AGO2. These analyses point to the presence of multiple putative SIM-binding sites in AGO proteins.

Ectopically Nup358-SIM1 functionally interferes with miRNA pathway

As SIM was identified as the minimum region in Nup358 required for binding to AGO proteins, we overexpressed GFP-MBP (control), GFP-Nup358-SIM1 or GFP-Nup358-SIM1-mutant (defective in interaction with AGO) in HeLa cells along with RL-3xBulge reporter to monitor the let-7a miRNA activity. SIM1, but not SIM1 mutant, specifically interfered with the miRNA mediated suppression of the reporter RNA, indicating that Nup358-SIM1 has the ability to act in a dominant negative fashion, possibly by interfering with interaction of endogenous Nup358 with AGO proteins.

Artificial tethering of AGO proteins to the 3'-UTR of reporter mRNA has been shown to suppress the reporter mRNA. We wished to test if Nup358-IR, could be tethered to mRNAs to suppress their expression, presumably by recruiting AGO proteins. As we found that IR1+2 mutant (IR1+2^{L2651A,L2653A}) was incapable of binding to endogenous SUMO~RanGAP or Ubc9, but retained the ability to bind to AGO proteins (Fig 2D), we used this mutant in tethering studies. HEK293T cells were transfected with Nλ-peptide-HA-tagged IR1+2L^{2651A,L2653A} (N-HA-IR1+2L^{2651A,L2653A}) along with a reporter construct engineered to express the renilla luciferase mRNA with five BoxB hairpins at its 3'UTR, which provides binding site for N-HA-tagged proteins. In addition to N-HA-MBP, HA-IR1+2L^{2651A,L2653A} that is incapable of binding to BoxB hairpin was used as control. The results from tethering assays clearly suggested that N-HA-IR1+2L^{2651A,L2653A} could significantly suppress the expression of the reporter mRNA. Collectively, these results support the notion that IR region, and particularly SIM, acts functionally as an AGO interacting motif.

Future Research Plans

- Characterize the molecular details of AGO-SIM interactions

Vaijayanti P. Kale

vpkale@nccs.res.in

Mesenchymal stromal cells primed with nitric oxide donors augment the functionality of hematopoietic stem cells via intercellular transfer of microvesicles enriched with *Jagged-1*- and *Vegf-A*-specific mRNAs.

Background

Patients with leukemia, lymphoma, severe aplastic anemia, etc. are frequently the targets of bone marrow transplantation, success of which critically depends on effective engraftment by transplanted hematopoietic stem cells (HSCs). Ex vivo manipulation of HSCs to improve their engraftment ability may become necessary when the number or quality of donor HSCs is a limiting factor. Due to their hematopoiesis-supportive ability, bone marrow-derived mesenchymal stromal cells (MSCs) have been traditionally used as feeder layers for ex vivo expansion of HSCs. MSCs have also been shown to form a special niche for HSCs in vivo. Their close apposition with HSCs in vivo as well as in vitro implies that signaling mechanisms operative in the MSCs would have direct implications in the HSC fate. Previous reports have shown that Nitric oxide (NO) is expressed in the BM compartment by both, blood cells and niche cells, suggesting that NO could have both, direct and indirect, i.e. niche-mediated, effects on the HSCs' fate.

Previously, we have shown age-dependent cell-autonomous effects of NO on HSCs (Jalnapurkar et al. Stem Cell Res Ther., 2016), wherein we demonstrated that NO regulates CD34 expression on the murine HSCs both, at transcriptional and translational levels. This up-regulated CD34 leads to increase in the engraftment ability of juvenile HSCs. In sharp contrast, such increase in CD34 expression leads to decrease in the engraftment ability of adult HSCs. The molecular mechanism behind this phenomenon involved NO-mediated differential induction of various transcription factors involved in commitment vis-à-vis self-renewal in adult and juvenile HSCs, respectively.

Participants

Ms. Sapana Jalnapurkar, *SRF*
Ms. M. Ranjita Devi, *SRF*
Ms. Suprita Ghode, *SRF*
Ms. Shweta Singh, SRF
Mr. Rohan Kulkarni, *SRF*
Ms. Sheetal Kadam, *SRF*

Collaborators

Dr. Mrs. Lalita S. Limaye, *NCCS*

Since, MSCs are an important part of the BM niche, NO derived from them also plays important role in haematopoiesis. Previous reports showed that treatment of MSCs with NO donors improves their ability to repair kidney fibrosis and also their vasculogenic ability. Pre-activation of MSCs with NO together with TNFα and IL1b enhances their paracrine effects on radiation-induced intestinal injury. NO-releasing hydrogels are known to enhance therapeutic efficacy of MSCs for myocardial infarction. Based on these reports we conceptualized that treatment of MSCs with NO donors might augment their HSC-supportive ability and these primed MSCs may boost the engraftment ability of adult HSCs.

In the present study we examined stromal cell-mediated effect of NO on hematopoietic stem cells isolated from bone marrow of both, adult and juvenile mice.

Aims and Objectives

- To study effect of Nitric oxide donor SNP (Sodium Nitroprusside)-treated mesenchymal stem cells (MSCs) on HSCs.
- To elucidate the molecular or signaling pathway involved in this process.

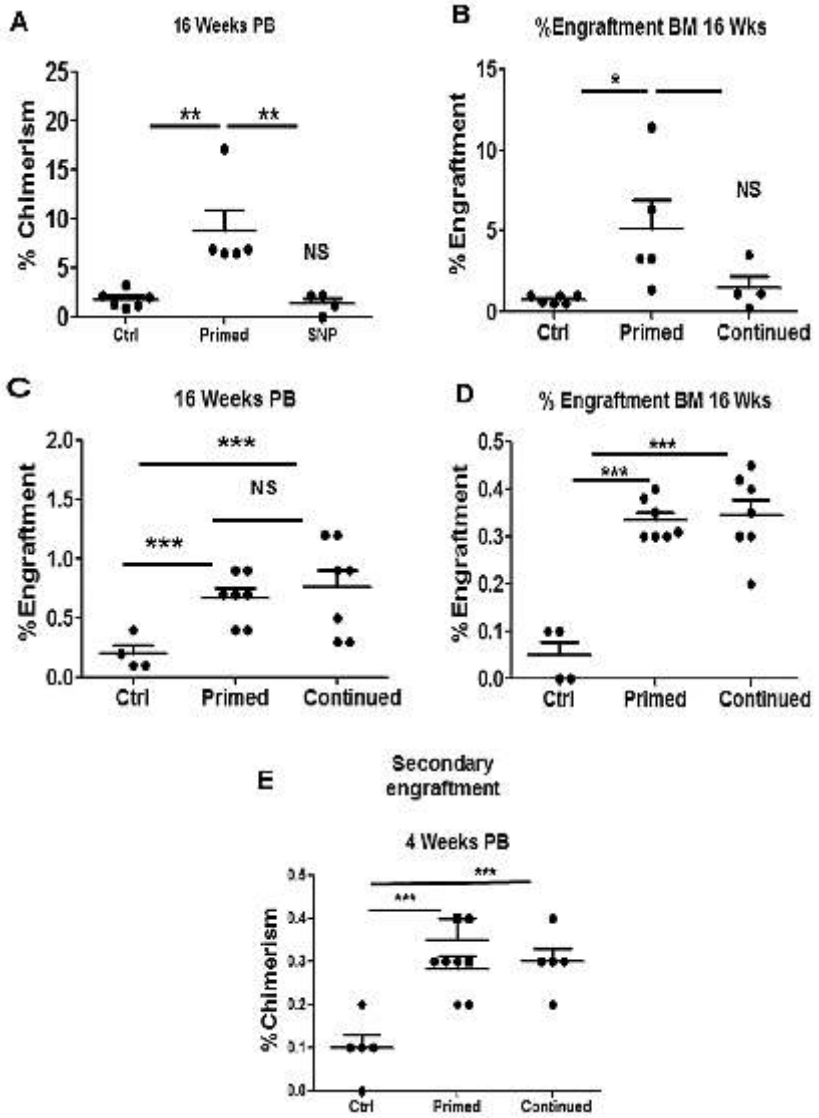


Figure 1: Adult as well as juvenile HSCs co-cultured with SNP-primed BMSCs exhibit higher engraftment potential:
HSCs (CD45.1) were co-cultured with control, primed or continued BMSCs for 3 days and infused into CD45.2 irradiated recipients ($1x10^6$/ mouse). (A-B) % peripheral blood (PB) chimerism (A) and % engraftment in BM (B) established by adult HSCs co-cultured with various BMSCs. (C-D) % PB chimerism (C) and % engraftment in BM (D) established by juvenile HSCs co-cultured with various BMSCs. (E) Engrafted juvenile donor cells were sorted from the bone marrow of primary recipients and infused in equal numbers into irradiated secondary recipients ($5x10^5$/cells/mouse). PB chimerism was assessed at 4 weeks post-transplant. The graph shows that juvenile HSCs co-cultured with both, primed and continued BMSC show significantly higher level of PB chimerism in secondary recipients, indicating their long-term functionality. The data are represented as mean ± SEM. N=6 to 8 * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Work Achieved

SNP-primed MSCs boost engraftment potential of both adult and juvenile HSCs

To understand the indirect or stromal cell-mediated effects of NO on HSCs, we used an NO donor, SNP (100μM) to treat the BMSCs. Lineage negative (Lin^-) cells isolated from adult (10-12 weeks old) mice were co-cultured with primed (pre-treated with SNP for 12 hrs.; SNP discontinued in co-culture) or continued BMSCs (pre-treated with SNP for 12 hrs; SNP continued in co-culture) for 3 days. When the engraftment ability of these co-cultured HSCs was examined in transplantation assays, we found that adult HSCs (10-12 weeks) co-cultured with primed BMSCs established a significantly higher level of chimerism in the peripheral blood of the recipients, as compared to control

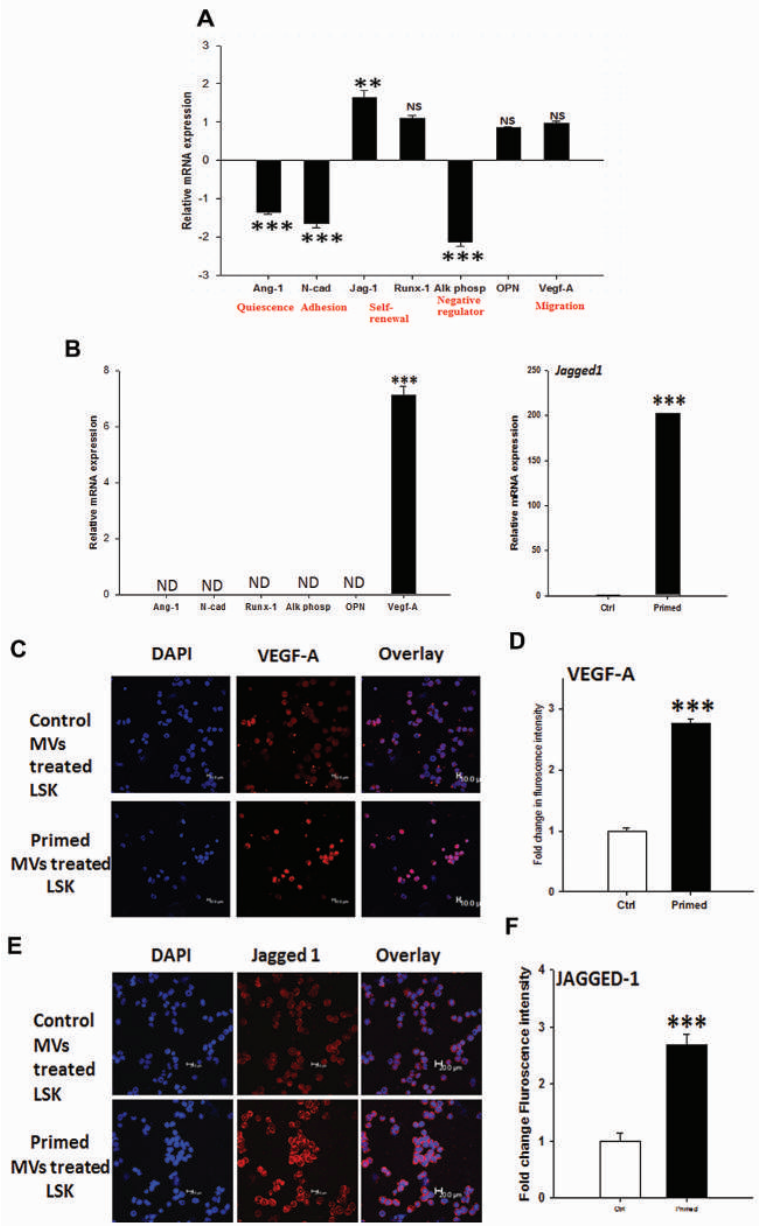


Figure 2: Primed BMSCs boost engraftment potential of adult HSCs via transfer microvesicles (MVs) enriched with HSC-supportive transcripts: (A) BMSCs primed with SNP for 12 hrs and further cultured for 12 hrs without SNP, were subjected to qRT-PCR analysis for quantification of mRNAs from HSC-supportive genes. The data were normalized with β-actin as endogenous control. Expression levels in control BMSCs were used as calibrator. (B) BMSCs were primed or not with SNP for 12 hrs and further cultured for 12 hrs without SNP. The conditioned media were collected and MVs were isolated by ultra-centrifugation. MVs were lysed and cDNA was prepared. qRT-PCR was done to quantify mRNAs for various HSC-supportive genes. (C-F) Sort-purified LSK HSCs were cultured with primed or control MVs and analyzed for the expression of VEGF-A and JAGGED-1 by immuno-fluorescence. Images were captured on a confocal microscope (Zeiss). (C) Confocal microscopy images of VEGF-A expression in LSK HSCs is shown. (D) Graph represents fold change in fluorescence intensity of VEGF-A expression as analysed by ImageJ software (NIH). (E) Image illustrates JAGGED-1 expression in LSK HSCs cultured with control or Primed MVs.(F) Fold change in fluorescence intensity quantified by ImageJ software is graphically represented. 20 cells were counted per field and 5 independent fields were scored. The data are represented as mean ± SEM. N=3 * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

and continued sets at 16 weeks post-transplant (Fig.1A). When % engraftment in bone marrow (Fig 1B) was analysed, we found that HSCs cultured with primed BMSCs showed significantly higher engraftment level, as compared to other two groups. These data show that engraftment potential of adult HSCs can be improved if they are co-cultured with primed BMSCs, as opposed to continued BMSCs, where they get exposed to NO directly.

To examine whether NO has an age-dependent effect on the HSCs in the presence of BMSCs as well, Lin^- HSCs from juvenile mice (6- 8 weeks) were also co-cultured with control, primed or continued BMSCs for 3 days. Harvested cells were analyzed for their repopulation ability. We found that the juvenile HSCs co-cultured with primed as well as continued BMSCs showed higher engraftment ability (Fig.1C). This also correlated with higher number of donor engrafted cells in the BM (Fig.1D). To confirm that the juvenile HSCs co-cultured with both, primed and continued BMSCs possess long-term repopulation ability we carried out secondary transplantation assay. We found that the HSCs co-cultured with primed or continued BMSCs had acquired significantly higher long-term repopulation potential (Fig.1E).

Collectively, these data show that primed BMSCs regulate HSC functions via indirect mechanisms i.e. through the NO-mediated signaling evoked in them and a continuous presence of NO in the co-cultures has a dominant negative effects on adult HSCs.

Primed BMSCs boost engraftment potential of adult HSCs via transfer of microvesicles (MVs) enriched with transcripts of HSC-supportive genes

To elucidate the mechanism involved in the indirect i.e. BMSC-mediated effects of NO on HSC-functionality, primed and control BMSCs were subjected to qRT-PCR analysis to quantify mRNAs of some of the known HSC-regulatory genes. Angiopoietin1 *(Ang1)* is known to maintain HSC quiescence and self-renewal through Tie2/Ang1 signaling axis .We found that Ang-1 levels were down-regulated in primed BMSCs, as compared to the control BMSCs (Fig. 2A). N-cadherin protects the HSCs against stress and limits the HSC pool through enhanced cell adhesion. We found that levels of *Cdh2* (N-cadherin) were down-regulated in the primed BMSCs (Fig.2A). Thus, reduced expression of *Ang1* and *Cdh2* may have resulted in an increase in the HSC pool in the primed set.

Importantly, we observed a significant increase in the expression of Jagged 1 in the primed BMSCs. The levels of Runx-1 transcripts were comparable in both sets (Fig.2A). Jagged-1 known to enhance self-renewal of HSCs, and thus, its up-regulation in primed BMSCs could be one of the principle reasons behind the increased transplantation ability of HSCs co-cultured with them.

Expression of Alkaline phosphatase *(Alkphosp)* is inversely associated with HSC proliferation and function. Alkphosp was found to be down-regulated in the primed BMSCs (Fig.2A). Opn (Osteopontin), which is a negative regulator of HSC pool and which also restricts excessive stem cell expansion under niche-stimulation, also showed a decreased expression in primed BMSCs (Fig.2A).

Vegf-A is an important angiogenic growth factor, which regulates homing of HSCs after myelosuppression. We found comparable levels of *Vegf-A* expression in primed and control BMSCs (Fig.2A).

These data show that increase in *Jagged-1* expression, reduction in quiescence axis and down-regulation of negative regulators in the primed BMSCs enhance self- renewal in HSCs co-cultured with them, leading to their enhanced engraftment ability.

Co-culturing of HSCs with BMSCs is known to increase their engraftment potential. Elucidating the factors that contribute to the ability of MSCs to maintain HSCs in cultures remains one of the most relevant, but unresolved issue, in the field. In recent years, the attempts to identify HSC-supportive mediators secreted by MSCs have shifted from growth factor and cytokines to extra-cellular vesicles secreted by them. Since we found that primed BMSCs increase the repopulation ability of HSCs, we examined whether culturing of HSCs with microvesicles (MVs) derived from primed BMSCs (primed MVs) also does the same. To examine whether primed MVs also show the mRNA profile of primed BMSCs, we subjected them to qRT-PCR analysis. We found that the transcripts of *Ang-1*, *Cdh2*, *Runx-1*, *Alkphosp* and *OPN* could not be detected in the MVs isolated from both, control and primed BMSCs. However, *Vegf-A* m-RNA was present in both MVs, but its expression was 7-folds higher in primed MVs, as compared to the control MVs (MVs isolated from control BMSCs).Most importantly, the *Jagged-1* m-RNA, which is required for self-renewal of HSCs, was ~200 fold higher in the primed MVs (Fig.2B, right panel).

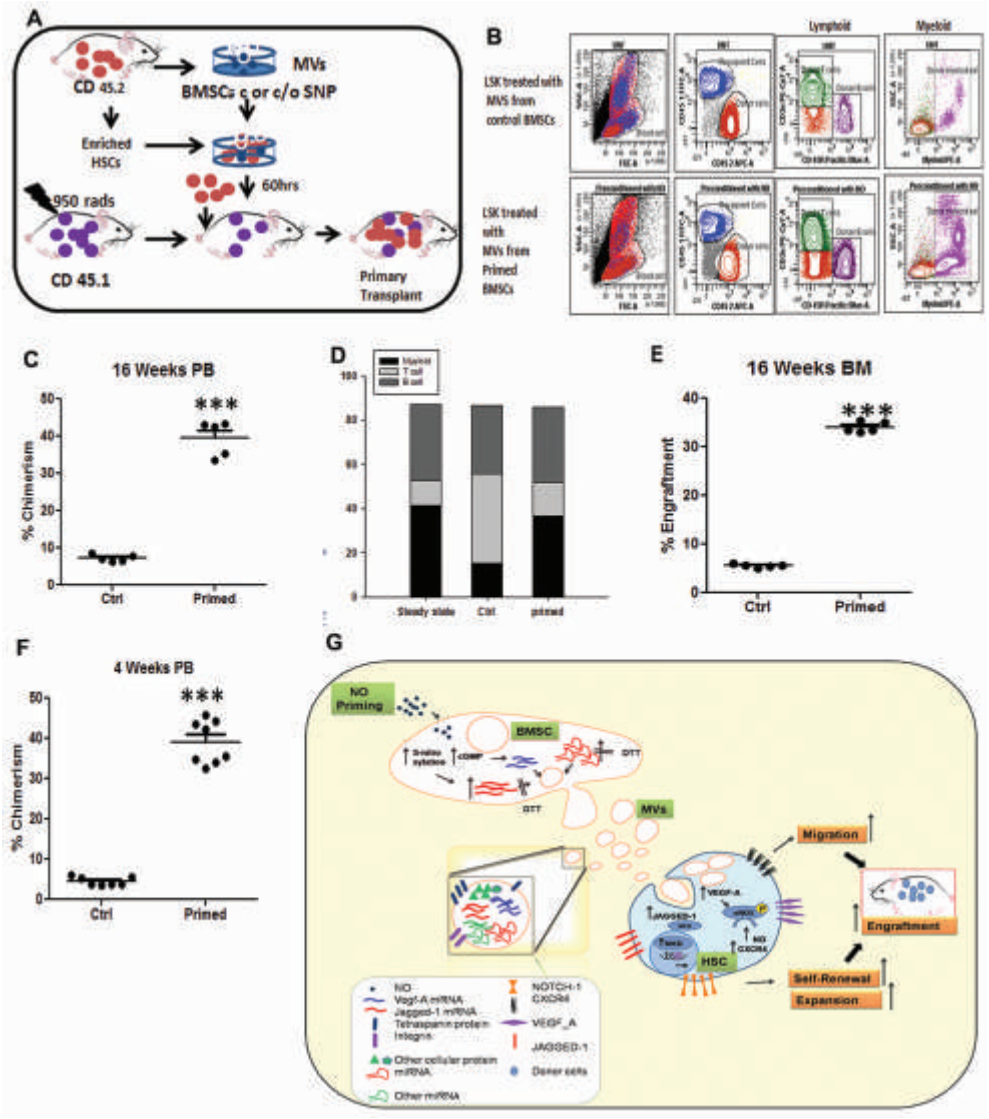


Figure 3: Primed MVs boost engraftment potential of HSCs:

Lin$^-$ cells isolated from adult mice (10-12 weeks; CD45.2) were cultured with control or primed MVs for 36 hrs. 10^6 cells were infused into irradiated recipients (8–10 weeks; CD45.1); 7–8 mice were used per group. (A) Schematic representation of the experimental protocol. (B) Gating strategy used for flow cytometry analysis of peripheral blood (PB) and bone marrow (BM) of the recipients. (C) PB chimerism established by the donor cells at 16 weeks post-transplant is depicted. (D) Myeloid and lymphoid cell formation from the donor cells was determined using lineage markers. (E) Percentage of engrafted donor cell population in BM of recipients is depicted. (F) The engrafted donor cells were sorted from the bone marrow of primary recipients and infused in equal numbers into irradiated secondary recipients ($5x10^5$/cells/mouse). The percent chimerism established by these primary engrafted donor cells was determined by flow cytometry analysis of the PB of secondary recipients. Percentage chimerism produced by the primary engrafted cells in the PB of secondary recipients at 4 weeks is illustrated. 6-7 secondary recipients were used per group. The data are represented as mean ± SEM. N=3 * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. G) **Graphical abstract.** NO-primed BMSCs boost the engraftment ability of HSCs co-cultured with them via intercellular transfer of MVs harbouring *Jagged-1* and *Vegf-A* mRNAs.

These data showed that the primed BMSCs might be exerting their salutary effects on the HSCs via secretion of MVs harboring *Jagged-1*- and *Vegf-A*-specific mRNAs. Sort-purified LSK HSCs from adult mice (10-12 weeks) were cultured with primed or control MVs in IMDM supplemented 20% FBS and murine specific growth factors (mIL3, mIL6 and mSCF) for 24 hrs. Cells were harvested and subjected to immuno-fluorescence analysis to examine the expression of VEGF-A and JAGGED-1 protein in them. We found that LSK HSCs treated with primed MVs showed significantly higher expression of VEGF-A (Fig. 2C and 2D) and JAGGED-1 (Fig. 2E and 2F), as compared to those incubated with control MVs .

These data demonstrate that MV-mediated intercellular transfer of *Jagged-1* and *Vegf-A* mRNA resulted in their translation in the LSK HSCs, leading to an increased expression of these proteins in them.

Primed MVs boost engraftment potential of HSCs:

Since treatment of HSCs with primed MVs resulted in increased expression of CXCR4 (through VEGF-A-eNOS axis; data not shown) and JAGGED-1 in them, we speculated that these primed MV-treated HSCs would also show higher engraftment potential. To examine this possibility, Lin^- cells (adult 10-12 weeks; CD45.2) were incubated with primed and control MVs for 60 hrs (Fig.3A). The incubated cells were infused in irradiated recipients (CD45.1 X 10^6 cells/mouse). The engraftment levels were assessed at 16 weeks post-transplant. We found that the HSCs cultured with primed MVs showed significantly higher peripheral blood chimerism at 16 weeks post- transplant (Fig.3B, 3C). Lineage analysis of the donor cells showed that cells incubated with primed MVs maintained the balance of myeloid to lymphoid cells ratio similar to that seen in the steady state (Fig.3D, comparison between first and third bars). As against this, the cells cultured with control MVs showed a distinct lymphoid bias (Fig.3D, middle bar). This may be an important factor to be considered in allogeneic bone marrow transplantations, where GvHD is a significant problem. HSCs cultured with primed MVs showed strikingly higher engraftment in the recipients' bone marrow at 16 weeks post-transplant (Fig 3E).

To assess the long-term engraftment ability of these MV-treated HSCs, we sort-purified the engrafted donor cells from the marrows of primary recipients and infused them in equal numbers in irradiated secondary recipients. We found that the HSCs cultured with primed MVs established a significantly higher PB chimerism in the secondary recipients at 4 weeks post-transplant (Fig.3F), clearly indicating their long-term functionality.

Collectively, these data demonstrate that primed MVs serve as an efficient tool to increase the engraftment potential of HSCs. Since these MVs can be cryopreserved, they can form a "ready-to-use" biologic for clinical transplantations (Fig.3.G).

Future Research Plans

- To elucidate the mechanisms involved in NO-mediated sorting of RNAs in the MVs secreted by BMSCs.

Musti Krishnasastry

mvks@nccs.res.in

Endemic like simulation using *Plasmodium berghei* ANKA evokes IgA specific to region VI of EBA ligands

Background

Plasmodium falciparum, the causative agent of malaria fever, can cause fatal and life-threatening complications such as cerebral and placental malaria. P. *falciparum* employs several ligands to invade the erythrocyte membrane to either evade immune surveillance and/or as a strategy to increase its survival. Among many ligands, EBA-175, employed for invasion by merozoites, has several regions referred to in the literature as I-VI. Overall, regions I and II of this ligand contain the Duffy Binding Domains, while regions III-V have variable functions, and the carboxy terminus has a cysteine-rich region VI followed by a transmembrane domain (TMD). This is schematically shown in Figure 1A. This carboxy terminal region also contains a vital cleavage site for ROM4, which helps in completion of the invasion cycle. Infection by *P. falciparum* is difficult to model in laboratory animals mainly due to its specificity towards the human host. Although a great degree of progress has been achieved by in vitro culture of the parasite, some aspects in the areas of immunology and pathophysiology can only be addressed appropriately by using an in vivo animal model. In this regard, primates have proved useful for modeling of *P. falciparum* malaria. However, their use has proven to be difficult due to both economic and ethical considerations, in addition to limited supply of subjects. Importantly, the narrow ranges of parasite lines adapted to primate infection are limited and the inferences drawn from their pathophysiology might not be directly correlated to human subjects. Overall, it is important to emulate an endemic-like situation using the appropriate *Plasmodium* species, to generate relevant knowledge that can be applied to human subjects.

Aims and Objectives

- To investigate the nature and utility of the immune responses generated by region VI of EBA-175 in an endmic-like situation.

Participants

Anil Kumar, *SRF*
Sapna Deore, *SRF*
Shikha Nag, *SRF*
Santosh Kumar, *SRF*
Ekansh Mittal, *SRF*
Raj Kumar Gour, *SRF*
Mahendra Kumar, *JRF*
Ankita Durge, *JRF*
Tanuja Bankar, *Technician*
Anil Lotke, *Technician*

Work Achieved

Cloning, expression and purification of the PfrVI domain

The cloning and expression of region PfrVI (Fig. 1A) of *P.falciparum* was achieved in pET32a^{+} in fusion with a Trx tag (thioredoxin), which provides the 6x his-tag for easy purification. In contrast, both PfrVI and its equivalent in *P.berghei ANKA* were found to be extremely toxic to *E.coli* despite numerous attempts, for reasons that are not clear. This domain was confirmed by MS-MS analysis of the peptides obtained after trypsin digestion. PfrVI-specific serum has detected the extracellular domain of EBA175 protein (3D7 parasite line). Upon immunization, we observed that IgG3, followed by IgG_1, were the dominant IgG in the PfrVI-specific antiserum, and this might be very obvious. However, the interesting feature observed was the presence of IgA in the serum. This gave rise to curiosity about, whether this IgA has any utility, since very little is known about the role of malaria-specific IgA in malarial infection.

Simulation of endemic-like infection reveals presence of malaria-specific IgA

Although limited, malaria-specific IgA is not elicited during first infection of the host, and multiple infections are needed to let the IgA evolve within the host. This puts an enormous limitation on the experimental approaches to study malaria-specific IgA. Hence, we attempted to mimic endemic-like situation in malaria infection using the rodent model of malaria, by repeated cycles of infection and cure to observe the emergence of IgA that is specific to malaria. This approach, which is depicted in Fig. 1B, is anticipated to provide us with "semi-immune mice" to examine the presence of the IgA^{+} B-cell population. 1- cure, 2- cure and 3- cure semi-immune mice were obtained by subjecting them to 1, 2, and 3 cycles of infection and treatment, respectively, to analyze the serum IgA levels and IgA^{+} B cells at the end of each cycle of infection. ELISA revealed that, in comparison to controls (0-cure mice), serum from 1- cure, 2- cure, and 3- cure mice showed higher levels of IgA specific to PfrVI by about 7.5, 11.6 and 12.83 fold, respectively.

The splenocytes isolated from each of these groups *i.e.* 1- cure, 2- cure, and 3- cure mice, showed an increase in the CD19^{+}IgA^{+}PfrVI-specific B cells as 2.1 %, 2.7 % and 4.1 % respectively, compared to the control mice, which showed only 0.4 % of this population (Fig. 1C). In addition, we also examined the secreted IgA specific to PfrVI under *in vitro* culture conditions in the presence of antigen without any supplements, by incubating the splenocytes in antigen (PfrVI, Pb1 and Pb4)-coated or uncoated wells. After 72 hrs, the culture supernatants were examined by ELISA for the secreted IgA, using appropriate controls. We observed an increase in the IgA specific to PfrVI and *P. berghei* ANKA-derived peptide-1, with multiple cycles of infection and recovery, whereas in case of another mixed sequence, no significant increase in IgA was apparent. Several other approaches, viz., ELISPOT, culture of spelnocytes, and characterization of the monoclonals specific to the IgA described here, suggest that immunization of the host with the antigens identified by us result in a B-cell population that secretes the IgA. Deeper studies involving these B-cell populations are expected to enhance our understanding of the evolution of IgA memory and its utility.

These observations suggest that it is possible to mimic the infection and recovery cycles akin to the endemic scenario in which IgA was found to increase progressively with multiple infections.

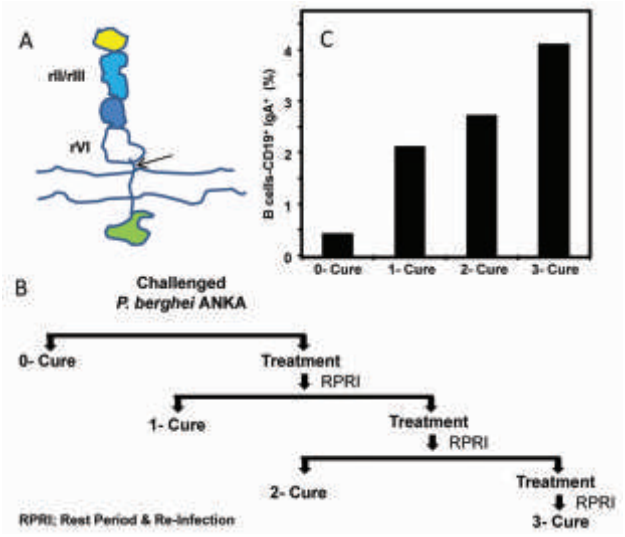


Fig. 1: (A) Pictorial depiction of domains of PfrVI: The region VI (rVI) of EBA ligand lies close to the membrane anchoring where the cleavage by PfROM4 enzyme takes place (marked with arrow). As a result of this cleavage, the extra-cellular domain of EBA ligand is shed into circulation/medium.

(B) Schematic representation for generation of semi immune mice: Briefly, the 1- cure, 2- cure and 3- cure mice were generated by infection with 1x10^{5} *P.berghei* ANKA-infected RBCs, followed by treatment with 20 mg/kg/wt of chloroquine for 7 days post 5 days of challenge. The group of mice without any cure (0 cure) was used as control.

(C) Surface IgA expression on splenocytes of C57BL/6 mice: Splenocytes were isolated from 1- cure, 2- cure and 3- cure mice along with age matched unimmunized control (0- cure). Isolated splenocytes were labeled with FITC (IgA) and APC (CD19). The bar graph shown is a summary after gating out total CD19^{+} B cells. Representative dot plots for one mouse of each group showing the proportion of IgA^{+} cells before and after gating for CD19^{+} cells.

It is highly relevant to note that the IgA is the second most abundant antibody i.e. ~ 1-4% of total serum protein in humans as well as mice. Moreover, earlier work by Biswas *et al* revealed that the IgA detected among the endemic Indian population was significant only among individuals who had multiple clinical attacks of *Plasmodium* infection. It was also reported that the IgA titer were found to increase with age among seropositive individuals. This observation should help us understand the evolution of IgA in malaria endemic populations. Though malaria endemic populations contain IgA specific to malaria, the antigen that elicits the IgA is not known. Our earlier study has clearly revealed that this IgA can inhibit the in vitro invasion of *P. falciparum* merozoites. Hence, the present study forms a rational basis for understanding the evolution of IgA, memory, and their utility in the context of malaria infection.

To summarize, our study will serve as an important starting point for the development of IgA- based therapy against malaria infection. Monoclonal antibodies against the antigens identified by us may serve as a potent antibody-mediated therapy, as an alternative approach to combat malaria.

Future Research Plans

- Investigation of IgA-specific memory, its maintainance in the system, and the characteristics of the outcome will be the course of future investigations.

Janesh Kumar

janesh@nccs.res.in

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 basic nervous system functions, including learning and development, and are involved in a remarkable range of neuronal diseases. iGluRs have been shown to be regulated by a family of transmembrane auxiliary proteins that exist as macromolecular complexes at synapses. Auxiliary subunits not only regulate the gating properties of iGluRs but also their distribution and traffickingto the synapse. However, till-date the molecular mechanisms and structural insights into these processes is unknown due to lack of atomic structures of auxiliary subunits or their complexes with cognate iGluRs.

Our research goals include structure-function analysis of the iGluR auxiliary subunits targeting both, isolated extracellular domains as well as intact full-length proteins, and their complexes with cognagte iGluRs, to elucidate their mechanisms of action. Structure-based functional and mutational analysis will also be carried out to test the veracity of the structural findings.

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

Jyoti Kumari, *SRF*
Pratibha Bharati, *SRF*
Ananth Prasad Burada, *SRF*
Anshul Assaiya, *JRF*
Surbhi Dhingra, *JRF*
Monika Jaiswal, *Project-JRF*
Rajesh Vinnakota, *PDF*
Prachi Chopade, *Technician B*

Work Achieved

Expression and purification of Kainate and Delta-subtype ionotropic glutamate receptors and Neto1

In the last year the primary goals planned and fulfilled were cloning of all the auxiliary proteins, their optimization and screening for overexpression. We were also able to optimize Kainate receptor auxiliary proteins, NETO1 and NETO2. We overexpressed and purified both rat and human NETO1 ectodomains from transiently-transfected mammalian cells to homogeneity. Cyrstallization trials for structure determination are ongoing.

For complex formation between iGluRs and their auxiliary proteins; we went on to overexpress and purifiy GluR6, GluR7 and GluD1 receptors (Figure1). Briefly, Rat GluR6 and GluR7 cDNA sequences were cloned into pFastBac1 and pEGBacMAM baculovirus expression vectors for protein expression in Sf9 insect and HEK293 GntI- cells respectively. Cells were harvested 72 hours after infection, collected by low speed centrifugation, and frozen at -80°C. Cell pellets, lysed by sonication were clarified by low speed centrifugation, and membranes collected by ultracentrifugation were solubilized in n-dodecyl-β-D-maltopyranoside (DDM) and cholesterol hemisuccinate (CHS). The supernatant was purified using a cobalt-charged TALON metal affinity resin (Figure 1a & d). Peak fractions were digested with thrombin to remove the C-terminal sequence containing the affinity and fluorescence tags, and receptors were purified by gel filtration chromatography in buffer containing DDM and CHS (Figure 1b & e). The final FSEC profile show well behaved and stable tetrameric protein suitable for complex formation and structural studies (Figure 1c & f). We also optimized constructs for orphan Delta receptors GluD1 and GluD2 via FESC Optimized constructs with C-terminal deletions alongwith mutations showed higher expression as well as sharp and symmetrical peak in comparision to wild type GluD1 & GluD2 receptors (Figure 1g-h). We went on to overexpress and purify and optimized GluD1 receptors to homogeniety (Figure 1i) which we have sent for structure determination via single-particle cryo-EM.

Interaction studies of GluR6 and Neto1 and complex formation

Our colocalization analysis using confocal microscopy showed that both the GluR6-EGFP and rat Neto1-mRuby2 are surface expressed and have very good spatial overlap when coexpressed suggesting interaction between them (Figure 2a). Our pull-down assays also indicate interations between purified GluR6 receptor and Neto1 ectodomain (NETO1-ECD) (Figure

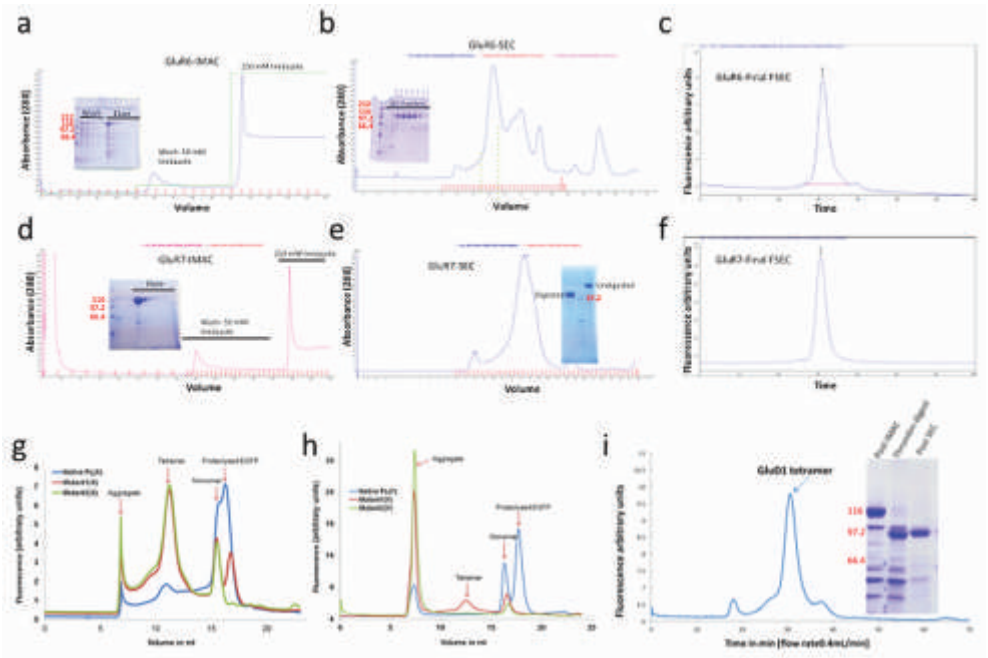


Fig.1: Full-length receptor constructs for GluR6 (a-c), GluR7 (d-f) and delta receptors GluD1 and GluD2 (g-i) were optimized using FSEC; they has been overexpressed and purified to homogeneity from baculovirus infected HEK293 or SF9 cells using a combination of IMAC and size exclusion chromatography. Panels (a-c) show IMAC, SEC and final FSEC profiles for GluR6 and GluR7 (d-f). Panel (g) shows FSEC profiles for GluD1 (h) GluD2. The peak profiles of the optimized constructs are larger in amplitude and more symmetric when comared to native receptors. (i) shows final FSEC profile of purified GluD1 and SDS-PAGE profile of various stages of purification

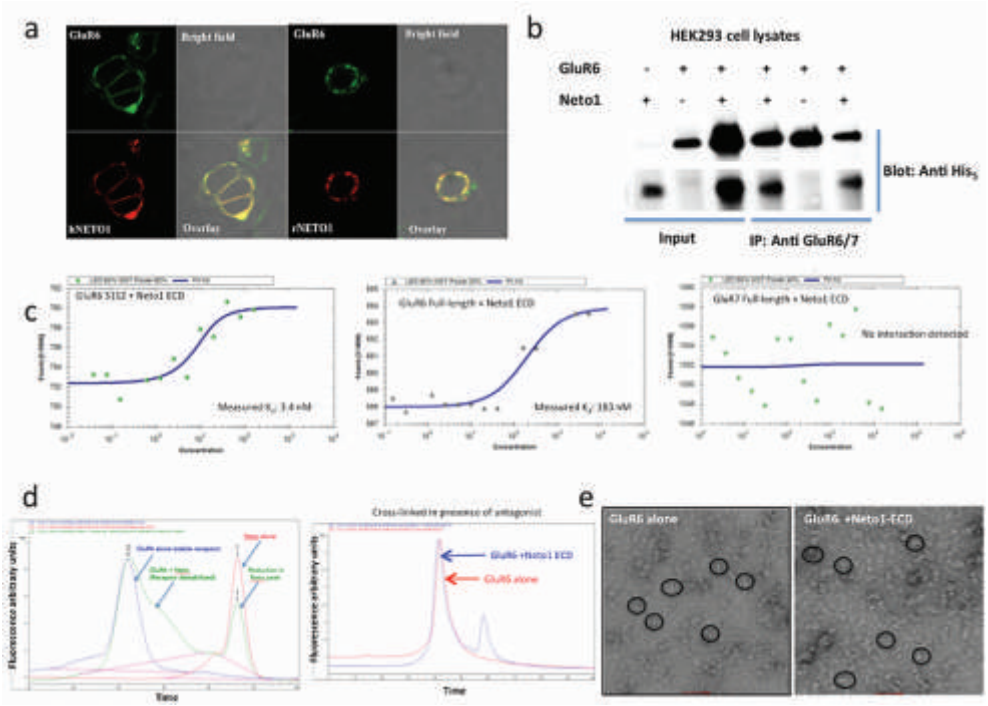


Fig.2: (a) Panel shows confocal images of transiently transfected HEK293 cells with GluR6 (green) and Neto1 (red) and their overlay (yellow) for both the rat and human neto proteins showing good spatial colocallization. (b) Western blot of the pull-down assays show interaction between GluR6 and Neto1ECD construct. (c) Shows thermophoresis binding curve for the GluR6-S1S2 (ligand binding domain) with Neto1 ECD with an estimated K_d value of 3.4 nM while GluR6-full length receptor and Neto1-ECD had a binding affinity of ~183nM. No interaction was observed between GluR7 receptor and Neto. Panel (d) shows FSEC screening for GluR6 and Neto1ECD complex formation. The crosslinked receptor alone and receptor Neto complex show well-behaved, symmetrical SEC profiles. Same complexes were subjected to negative stain electron microscopy (e) and show uniform receptor particles in both GluR6 alone and GluR6+Neto1-ECD complex images.

2b). To further evaluate interactions between purified full-length receptor and NETO1 auxiliary protein, we performed preliminary microscale thermophoresis based assays that detect changes in the hydration shell, charge or size of molecules by measuring changes of the mobility of molecules in microscopic temperature gradients. For Neto1 ECD and GluR6 S1S2 (ligand binding domain dimer) the estimated K_d for interaction was 3.4nM, while for Neto1 ECD and full-length GluR6 receptor it was estimated to be ~183nM. However, we did not find any interaction between Neto1-ECD and GluR7 receptor, which is consistent with the reports that Neto interaction is specific to GluR6 subunit containing kainate recptors only (Figure 2c). We also went on to screen for conditions suitable to formation of a stable complex between GluR6 and Neto1-ECD. For this, we extensively utilized Fluorescence-detection Size Exclusion Chromatography (FSEC) to screen multiple ligands, detergents etc. We found that the Neto1-ECD when added to apo-GluR6 receptor leads to destabilization of the receptor tetramer (Figure 2d). However, in presence of antagonist UBP301 that traps the receptor in a closed state we found a more stable receptor complex (data not shown). We further optimized glutaraldehyde based chemical cross-linking conditions to further stabilize and trap the GluR6 receptor and Neto1ECD complex (Figure 2d). This cross-linked GluR6+Neto1-ECD complex also eluted before GluR6 receptor alone indicating complex formation. We performed negative stain electron microscopy (in collaboration with Dr. Manidipa Banerjee, IIT, Delhi) on these complexes. The images clearly show uniform particles of the size of receptor, however, we also observe lot of background noise, possibilly due to high detergent concentration. Further, optimization for reducing the detergent background to enable single-particle cryo-electron microscopy on these complexes for their structure determination is ongoing.

GluR6/GluR2 receptor chimera design for interaction and electrophysiological assays

We intend to exploit the specificity of iGluR and auxiliary protein interaction and their effects on the gating properties of iGluRs to understand their mode and site of interaction. Modular

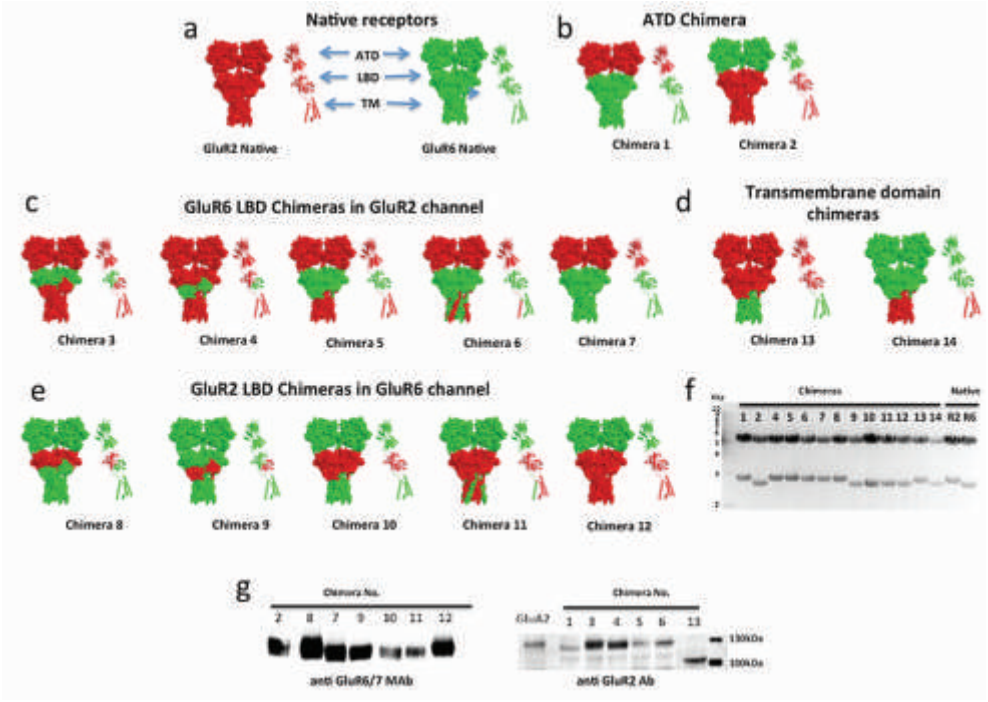


Fig.3: (a) shows GluR2 (red) and GluK2 (green) receptor tetramer in surface representation alongwith a single subunit in cartoon representation. Various extracellular and transmembrane domains are indicated. Panel b-e shows various chimera designs between GluR2 and GluK2 for a(b) ATD domain, (c) GluR6 LBD swapped in GluR2 channel, (d) transmembrane domain and (e) GluR2 LBD chimera in GluR6 channel. Panel (f) shows RE digest confirmation of the chimeras and (g) shows Western blot showing expression of various chimeras probed with GluR6 and GluR2 antibodies against appropriate domains.

architecture of iGluRs allows for receptor chimeras to be made by exchanging different domains between iGluR subtypes. We designed fourteen (14) receptor chimeras between kainate receptor GluR6 and AMPA receptor GluR2 to be used as tools for pull-down, immunoprecipitation and electrophysiology assays (Figure 3a-e). The chimeras were confirmed via a diagnostic RE digest and sequencing of the complete coding region (Figure 3f). We also transiently transfected HEK293 cells with these chimeras and checked for their expression via Western blot with monoclonal antibody against GluR6/7 receptor and polyclonal antibody agaist GluR2. All the chimeras except chimera no 13 and 14 show expression with bands at expected sizes (Figure 3g). However, we still need to check the surface expression of each of these constructs and normalize them with respect to surface expression of wild type GluR2 and GluR6 receptors. This will enable more precise interpretation of electrophysiology data.

Future Research Plans

- **Construct optimization, expression and purification of AMPA auxiliary proteins:** We have optimized constructs of AMPA receptor auxiliary subunits CKAMP44 and cornichons. We intend to optimize their overexpression and purification protocols. Once purified we would be reconstituting their complexes with purified GluR2 receptors and subject them to structure determination via cryo-EM.
- **Crystallization trials of Neto1 ectodomains and AMPA interacting proteins neuritin and Brorin:** A more extensive search of the crystallization space of NETO1 ecto domain, neuritin & brorin is planned so that we are able to grow diffraction quality crystals of the same. If that happens we intend to determine atomic structure of the same.
- **Structure determination of GluR6-Neto1 complex via single particle cryo-electron microscopy:** One of the immediate goals is to solve the structure of GluR6-Neto1 complexes which we have reconstituted via single particle cryo-electron microscopy.
- **Functional assays via electrophysiological recordings:** We intend to setup electrophysiology unit this year so that we may test our GluA2, GluK2 chimeric constructs. After that control experiments in presence of auxiliary subunits will also be carried out.

Gopal C. Kundu

kundu@nccs.res.in
gopalkundu@hotmail.com

Tumor-derived Osteopontin Regulates Trans-differentiation of Fibroblasts into Myofibroblasts and Controls Breast Tumor Growth

Participants

Dr. Dhiraj Kumar
Ms. Pompom Ghosh, *SRF*
Mr. Amit Singh Yadav, *SRF*
Mr. Ramesh Butti, *SRF*
Ms. Deepti Tomar, *SRF*
Mr. Totakura V. S. Kumar, *SRF*
Ms. N. Naga Venkata Radharani, *SRF*
Mr. Nimma Ramakrishna, *SRF*
Mr. Sumit Das, *JRF*
Mr. Pinaki Banerjee, *JRF*
Ms. Shamayita Roy, *Project JRF*
Mr. Shailesh Bhalara, *Project JRF*
Ms. Anuradha Bulbule, *Technical Officer-A*
Dr. Mahadeo Gorain, *Technician B, IVIS*
Mr. Jiten Sharma, *Project Trainee*
Mr. Sahadev Choudhary, *Project Trainee*

Collaborators

Dr. H. V. Thulasiram, *Senior Scientist, National Chemical Laboratory (NCL), Pune*
Dr. Absar Ahmed, *Professor, Aligarh Muslim University, Aligarh*
Dr. Rohit Shrivastava, *Professor, IIT Bombay, Mumbai*
Professor Akhilesh Pandey, *IOB, Bangalore / John Hopkins University, MD, USA*
Dr. Sudeep Gupta, *TMH, Mumbai*
Dr. Soma Bamerjee, *PGIMER, Kolkata*

Background

Breast cancer is a highly complex disease and one of the largest causes of morbidity and mortality among women. Complexity of breast tumor is dictated by clonal evolution, resident stromal cell activation and different types of cellular infiltration. Various reports have showed that breast carcinomas are heterogeneous in nature and not only composed of cancerous epithelial cells but also of fibroblasts, macrophages, endothelial, immune and bone marrow derived cells (BMDCs). As tumor progresses from neoplastic stage to higher grades, establishment of reactive "tumor stroma" takes place. Major part of breast tumor stroma consists of cancer associated fibroblasts (CAFs). CAFs are reminiscent of myofibroblasts and show myofibroblast-specific gene expression such as expression of α-smooth muscle actin (α-SMA), FAPα, FSP1 and exhibition of SDF-1/CXCR4 autocrine loop. Various studies have revealed that CAFs have pivotal role in inducing tumor growth, angiogenesis and metastasis by secreting array of growth factors and chemokines. However, source and generation of CAFs in the tumor microenvironment is still under debate. Osteopontin (OPN) is a sialic acid-rich, ECM associated-RGD motif containing chemokine-like protein. It is highly expressed in cancer cells and crucial regulator of various hallmarks of cancer. OPN shows dual role in the context of tumor progression by directly regulating cancer cells as well as triggering stromal cell recruitment and activation. However, evolution of reactive tumor stroma in response to OPN is not studied well. In this study, we address the role of OPN in regulating mesenchymal to mesenchymal transition (MMT) of fibroblasts to myofibroblasts in the tumor microenvironment leading to breast tumor progression.

Dr. Susanta Roychoudhury, *Saroj Gupta Cancer Centre and Research Institute, Kolkata*
Professor Tanya Das, *Bose Institute, Kolkata*
Professor A. Dharmarajan, *Curtin University, Australia*
Professor Deirdre Coombe, Curtin University, Australia
Dr. Gautam Sethi, *Assistant Professor, National University of Singapore, Singapore*
Professors Shanti Nair & K. Manzoor, *AIMS, Kochi*
Dr. S. Gosavi, *Professor, Dept of Physics, Savitribai Phule Pune University, Pune*
Dr. Vipin Kumar & Dr. Pawan Singh, *NIF, Ahmedabad*
Prof. Siegfried Weiss, *Helmholtz Centre for Infection Research, Germany*
Prof. Bjarne Bogen, *University of Oslo, Oslo*
Prof. Aditya Koley, *BITS Pillani, Goa*

Aims and Objectives

- To establish fibroblast primary cultures (NAFs and CAFs) from human breast cancer specimens and peripheral tissues
- To delineate the molecular mechanism by which tumor derived OPN regulates mesenchymal to mesenchymal transition (MMT) of fibroblasts to myofibroblasts
- To study how OPN-activated myofibroblasts controls breast tumor growth and metastasis.

Work achieved

Fibroblasts are trans-differentiated into myofibroblasts during patho-physiological processes. CAFs are reminiscent of myofibroblasts, show multifaceted role in tumor progression by secreting array of growth factors and cytokines which regulate several hallmarks of cancer. Accumulated evidences suggest that CAFs are derived from resident fibroblasts upon activation in the tumor microenvironment. However, critical regulators of fibroblast activation in the tumor microenvironment need to be systematically investigated. To examine this, primary cultures of cancer associated fibroblasts (CAFs) and normal associated fibroblasts (NAFs) were established from human breast tumor

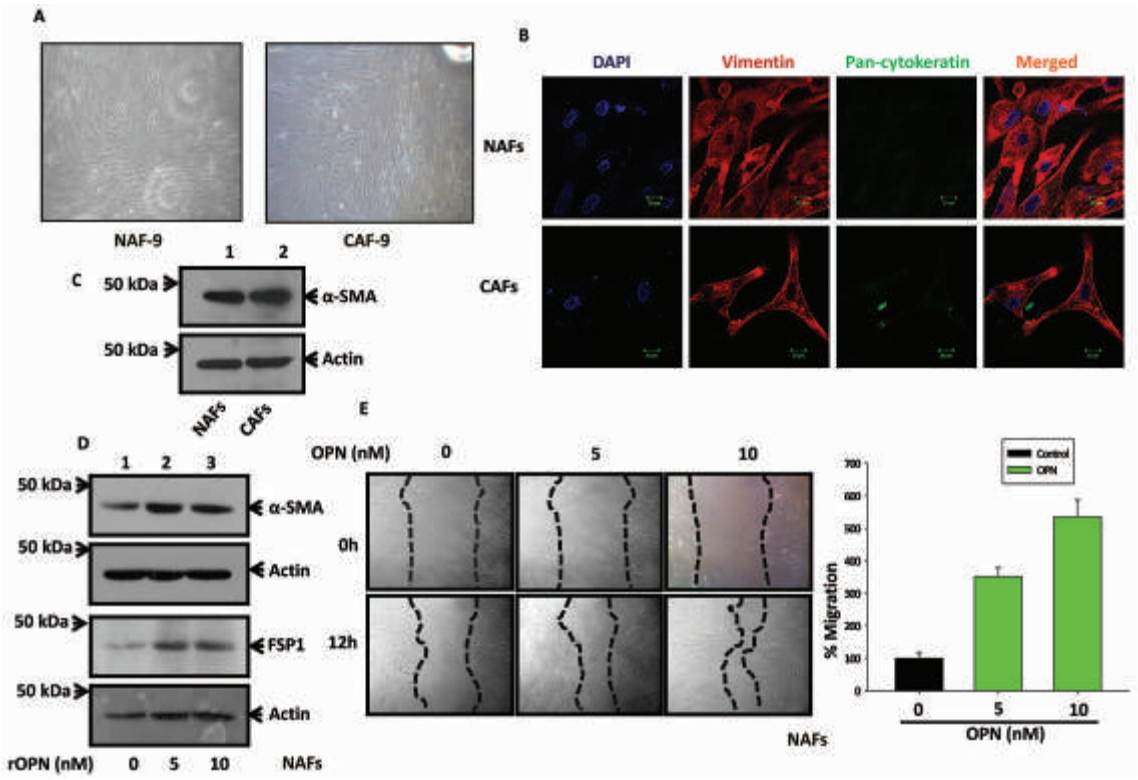


Fig. 1: OPN regulates fibroblast activation to myofibroblasts

(A) Morphology of NAFs and CAFs isolated from peripheral normal and human breast tumor specimens respectively. (B) Immunofluorescence analyses of vimentin and pan-cytokeratin expression in NAFs and CAFs. (C) Western blot analysis of α-SMA expression in NAFs and CAFs. (D) Western blot analyses of α-SMA and FSP1 expression in OPN-treated NAFs. (E) Wound closure assay of control and OPN-treated fibroblasts and the results were analysed and represented in the form of bar graph.

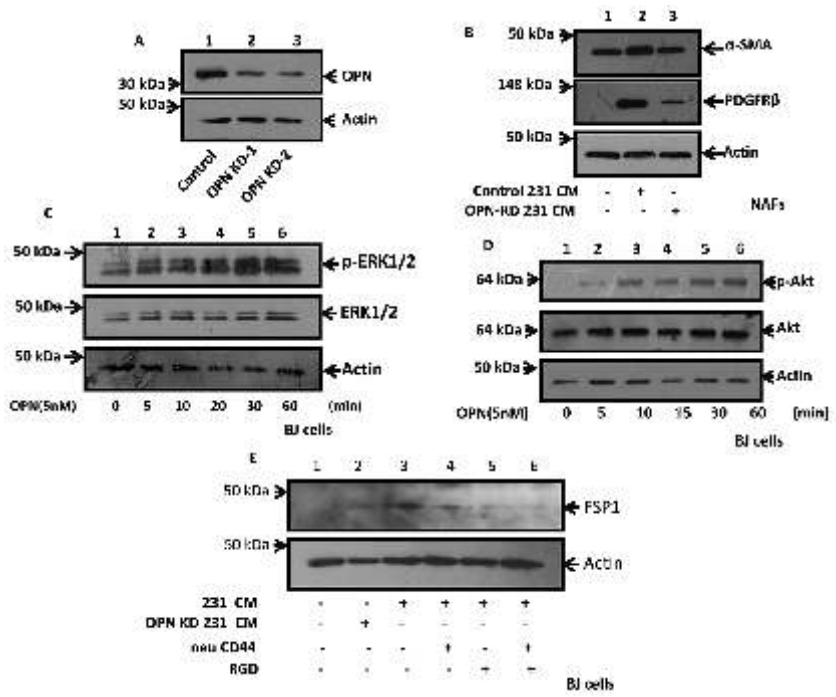


Fig. 2: OPN activates ERK and AKT signalling pathway in NAFs

(A) Western blot analysis of OPN expression in control (MDA-MB-231) and OPN-KD MDA-MB 231 cells. (B) Western blot analyses of α-SMA and PDGFRβ expression in control or OPN-KD MDA-MB-231CM-treated NAFs. (C) Western blot analysis of ERK phosphorylation in OPN-treated BJ cells. (D) Western blot analysis of AKT phosphorylation in OPN-treated BJ cells. (E) Western blot analysis of FSP1 expression in control or OPN-KD MDA-MB-231 CM-treated BJ cells in the absence or presence of RGD peptide or CD44 blocking antibody.

specimens and peripheral tissues respectively (Fig. 1A). To study the purity of fibroblasts, fibroblast specific primary cultures were stained with vimentin and pan-cytokeratin antibodies. Immunofluorescence analysis showed that these primary cultures were positive for vimentin (mesenchymal specific marker) and negative for pan-cytokeratin (epithelial specific marker) (Fig. 1B). Over-expression of α-SMA in CAFs was observed that confirms their activated state in tumors (Fig. 1C). To investigate the effect of OPN in fibroblast activation, NAFs were incubated with rOPN. The results showed that OPN augmented α-SMA and FSP1 expression in NAFs (Fig. 1D). Previous reports have suggested that activated fibroblasts show higher migratory potential under various pathophysiological conditions. To investigate the migratory potential of OPN-activated NAFs, wound closure assay was performed. OPN activated fibroblasts showed significantly higher migratory potential as compared to untreated NAFs (Fig. 1E). The above results revealed that OPN stimulates mesenchymal to mesenchymal transition (MMT) of mammary fibroblasts to myofibroblasts.

To further examine whether cancer cell-derived OPN has any role in fibroblast activation, we have silenced OPN in OPN high expressing cells, MDA-MB-231 using Lenti-viral system (Fig. 2A). CM-derived from MDA-MB-231 cells (as control) or OPN knocked down (KD) MDA-MB-231 cells were added to NAFs and myofibroblast-specific gene expression was examined. Western blot analysis has shown that myofibroblast markers such as α-SMA and PDGFRβ are expressed in high level in CM-treated NAFs whereas knocking down of OPN abrogated this effect (Fig. 2B). We also found that activating fibroblasts with control CM increased migratory potential of fibroblasts while incubating with OPN-KD CM attenuated this effect. The above data showed that cancer cell-derived OPN has role in the regulation of trans-differentiation of normal fibroblasts to myofibroblasts. Previous reports have demonstrated that OPN interacts with integrins and/or CD44 and activates ERK and AKT signalling pathways in breast and other cancer cells. To understand the indepth mechanism by which OPN regulates fibroblasts activation, BJ fibroblasts (human normal fibroblasts) were treated with rOPN and phosphorylation of ERK and AKT were studied. Our results showed that OPN activates both ERK

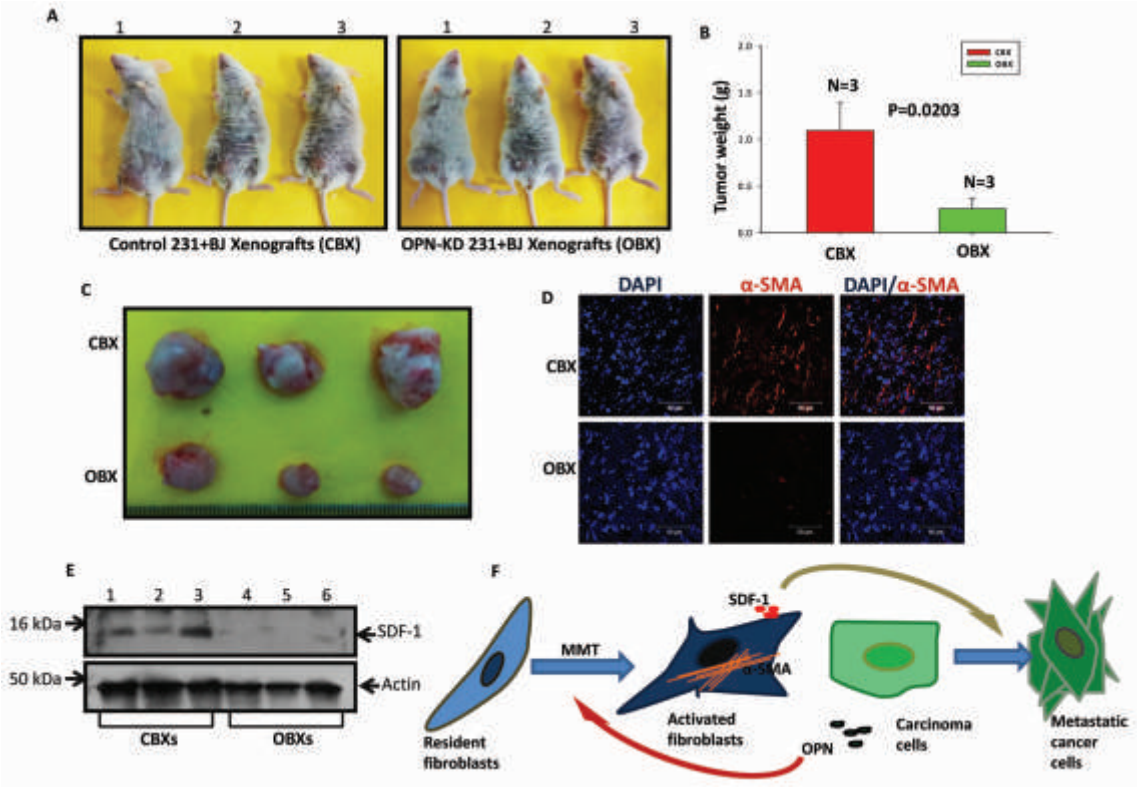


Fig. 3: OPN activated fibroblasts augment SDF-1 expression and tumor growth in co-implanted xenograft models.
(A, C) Typical images of co-implanted xenograft tumors in mice. (B) Tumor weights are represented in the form of bar graph; error bars, SD (P=0.0203, N=3 for both CBX and OBX). (D) Immunofluorescence analysis of α-SMA expression in co-implanted xenograft tumors. Scale bar=50 μm. (E) SDF-1 expression in tumor lysates derived from CBXs and OBXs as shown by immunoblots. (F) OPN activated fibroblasts secrecte SDF-1 leading to enhancement of tumor growth.

and AKT pathways in fibroblasts (Fig 2C, D). To further examine whether these OPN and its receptor regulated pathways have any role in the fibroblast trans-activation to myofibroblasts, the function of integrin and CD44 (OPN receptor) were blocked by RGD peptide and CD44 blocking antibody respectively and activated state of fibroblasts was studied under MDA-MB-231CM- stimulation. The results showed that blocking these pathways reduced myofibroblastic phenotype (Fig. 2E).

To further validate in vitro results, in vivo co-implantation human xenografts were developed by injecting admixture of BJ fibroblasts and control or OPN KD MDA-MB-231 cells into mammary fat pad of NOD/SCID mice. Tumor growth was significantly reduced in OPN KD MDA-MB-231 and BJ co-implanted xenografts (OBXs) as compared to control MDA-MB-231 and BJ co-implanted xenografts (CBXs) (Fig. 3A-C). To investigate the status of myofibroblast phenotype in these tumors, tumor sections were stained with α-SMA antibody and immunofluorescence analysis was performed. The results showed that fibroblasts were activated to myofibroblasts (α-SMA expression) in CBXs while fibroblasts activation was considerably low in OBXs (Fig. 3D). Activated fibroblasts secrete high level of SDF-1 (stroma-derived factor-1) which is known to regulate tumor growth, angiogenesis and metastasis in various types of cancer including breast. Western blot analysis was performed to check expression of SDF-1 in CBXs and OBXs. As expected, SDF-1 expression in OBXs was significantly low as compared to CBXs (Fig. 3E). Taken together, the tumor-derived OPN is required for activation of normal fibroblasts to myofibroblasts (CAFs) and that ultimately controls breast tumor progression (Fig. 3F).

Future Research Plans

- To identify differentially expressed transcription factors (TFs) and TF-regulated downstream genes which drive OPN-mediated reprogramming of resident fibroblasts to myofibroblasts.
- To investigate the role of OPN-activated fibroblasts in tumor angiogenesis and metastasis.

Girdhari Lal

glal@nccs.res.in

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

Participants

Neeraja Kulkarni, *PhD Student*
Sourav Paul, *PhD Student*
Sandip Sonar, *PhD Student*
Shilpi, *PhD Student*
Priyanka Padghan, *PhD Student*
Heikrujam Thoihen Meitei, *PhD Student*
Manisha Pathak, *Research Associate*

Collaborators

Dr. P. K. Sharma, *AFMC, Pune*
Dr. Sanjeev Galande, *IISER, Pune*
Dr. Arvind Sahu, *NCCS, Pune*

Background

Inflammation is a complex set of reactions involving a set of cytokines, chemokines and adhesion molecules. Joint ventures of pro- and anti-inflammatory functions are initiated 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 tissue. These chemokines and cell adhesion molecules are well studied in the migration of cells. However, whether intrinsic signaling from these receptors perturbs the cell differentiation and function is not well understood.

Most of the chemokines and some of the adhesion molecules are G-protein coupled receptors (GPCRs). G-proteins are heterotrimers consisting of α-, β-, and γ-subunits and transduce signals from surface receptors to intracellular effectors. Upon receptor activation, the G-protein complex dissociates into α- and βγ-subunits, which in turn, recruit various signaling components at the inner surface of the plasma membrane, followed by production of an array of intracellular second messengers such as IP3, DAG, Ca2+, cAMP, and IP3. G-protein signaling regulates a number of cellular events such as induction of cell polarity, actin polymerization, reversible cell adhesion, myosin dynamics, transmigration, and cell activation, differentiation and functions.

CCR6 is a GPCR expressed on various immune cells, which interacts with its specific chemokine, CCL20. CCR6 plays 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. In autoimmune

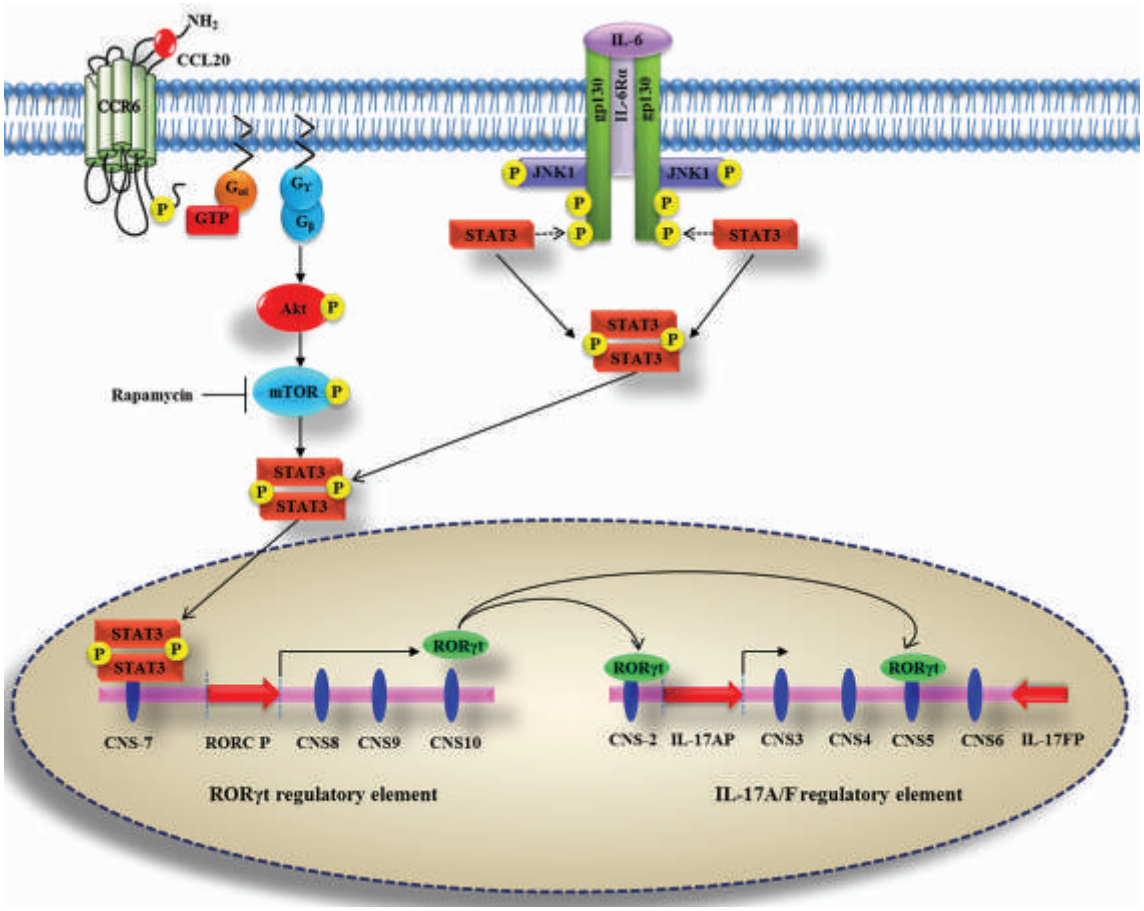


Fig. 1: Schematic representation of CCL20-CCR6 signaling in CD4 T cells

colitis, CCR6 plays a critical role in the migration of inflammatory Th17 cells into the gut. Genome-wide association studies have shown a strong association of single nucleotide polymorphisms (SNPs) in the CCR6 gene with the development of IBD. The genetic deficiency of CCR6 in animals was found to give protection against low-dose dextran sodium sulfate (DSS)-induced colitis, and blocking of CCL20 attenuates trinitrobenzene sulfonate (TNBs)-induced colitis. Most of these studies were focused on the migration function of CCR6 in CD4+ T cells and the attenuation of autoimmune colitis was proposed to be due to impaired migration of effector CD4+ T cells into the gut. The role of intrinsic signaling of CCR6 in the differentiation and plasticity of Th17 cells is not known.

Aims and Objectives

- To determine how chemokine activation, together with co-stimulatory molecules, affects the differentiation and function of CD4 T cells.
- To determine how chemokine receptor signaling perturbs the epigenetic marker in regulatory elements of the genes.

Work Achieved

We induced colitis in C57BL/6 mice by giving dextran sodium sulfate (DSS) in drinking water or in RAG1$^{-/-}$ mice by adoptive transfer of naïve CD4 T cells (CD4^{+}Foxp3rfp^{-}CD44^{-}CD45RB^{hi} cells). CCR6^{+}CD4^{+} T cells showed significantly more expression of RORγt during colitis, as compared to controls, and CCR6$^{-/-}$ mice were protected from colitis. To test the influence of CCR6 on RORγt, we injected CCR6$^{-/-}$ CD4 T cells (CD45.2^{+}) in CD45.1^{+} congenic mice, and animals were given DSS. Compared to the CD45.1^{+}(CCR6$^{+/+}$) CD4 T cells, CD45.2^{+}(CCR6$^{-/-}$) cells did not significantly increase the RORγt expression. To faithfully monitor the effect of CCR6 on RORγt expression in Foxp3^{+} Tregs, we adoptively transferred naïve CD4 T cells from CCR6gfp$^{+/-}$::Foxp3rfp$^{+/+}$ mice into RAG1$^{-/-}$ mice. During colitis, CCR6gfp^{+}Foxp3rfp^{+} Tregs showed significantly increased RORγt expression, as compared to the CCR6gfp^{-} Tregs. The addition of CCL20 (CCR6 ligand) during Treg and Th17 lineage differentiation significantly increased RORγt and IL-17 expression. Binding of CCL20 with CCR6 induced phosphorylation of Akt, mTOR, and STAT3, which in turn promoted the binding of RORγt specifically to the IL-17A

promoter and CNS5, and increased IL-17 reporter activity (Figure 1). CCL20-CCR6 signaling promotes differentiation of Th17 cells. Therefore, designing strategies to block CCR6 signaling could help in controlling gut inflammation and autoimmunity.

Future Research Plans

- To investigate the molecular plasticity induced by CCR6 signaling in Th17 and Treg cells.
- To understand the crosstalk between T cell receptor (TCR) signaling and CCR6 signaling in CD4+ T cell.

Nibedita Lenka

nibedita@nccs.res.in

Signaling cues guiding Mesoderm induction and its Derivatives

Background

The gastrulating embryo in vertebrate consists of three germ layers that execute the well orchestrated body plan culminating in the development of an organism. Mesoderm, the middle of the three embryonic germ layers, gives rise to cells of various vital systems including haematopoietic, cardiovascular, reproductive, excretory, urogenital etc. during early development. Mesoderm induction and cardiomyogenesis are carefully orchestrated processes that involve key modulators such as Wnt, activin/Nodal, Cripto, bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), Hedgehog, Notch etc. However considering the existence of conflicting reports during the same, the precise interplay among these signaling pathways remains elusive. Moreover, several post-translational modifications do impart additional modulations during the cell fate decision machinery. Ubiquitination is one of the major post-translational modifications in eukaryotes that controls expression of enzymes and structural proteins during cell fate acquisition, DNA damage repair and also targets proteins for degradation. To counter-balance the same, cells do express deubiquitinating enzymes [DUB or USP (Ubiquitin specific protease)] as well. Hence, normal cell fate choices are dependent on the balanced actions of ubiquitination and deubiquitination. Several studies do indicate the importance of USP during hematopoietic stem cells (HSCs) homeostasis, preserving HSC self-renewal and repopulation potential both *in vitro* and *in vivo*. Also Gene Expression database indicates higher expression of USP during mesodermal differentiation. Hence, we intended to investigate USPs and their role in exerting fine-tuned control, if any, during Wnt mediated mesoderm fate modulation.

Participants

Varun Haran M, *SRF*

Aims and Objectives

- Elucidating the role of USPs during mesoderm induction and subsequent differentiation to its derivatives.

Work Achieved

Our earlier and ongoing studies suggest temporal influence of Wnt during Mesoderm induction and also the downstream haemato-endothelial fate specification and cardiomyogenesis. Hence, we explored various Wnt targets that may have a critical modulatory role during the same. Interestingly USP was found to contain multiple TCF/LEF binding motifs and hence suggesting that, USP might be a direct target of Wnt signaling. To verify the same, ESCs were differentiated under canonical Wnt activation condition and USP transcripts were analysed at various stages of mesodermal differentiation. Gene expression analysis of USP showed its expression to be significantly higher at d5 of differentiation compared to that at d2, and with its further up-regulation seen under Wnt activation condition at both the time points tested. This result suggested that USP might function as a downstream effector molecule of canonical Wnt activation. Accordingly, we further investigated the role of USP in mediating mesoderm differentiation from ESCs *in vitro*. USP knockdown was carried out using Mesp-Venus ESCs clone and the same was validated at both transcriptional and translational levels. Our data revealed that, USP knockdown (USP-Sh) promoted the generation of $Mesp1^{+}$ cardiac progenitor cells when compared with that of control (Ctrl-Sh). The same was further authenticated by quantifying $PDGFR^{+}$ multipotent cardiac progenitors that showed about 2-2.5 fold increase upon USP knockdown thereby suggesting USP to have a negative influence on the generation of cardiac progenitors. To discern further the role of USP in cardiac differentiation, Ctrl-Sh and USP-Sh clones were differentiated into cardiomyocytes and the cardiac differentiation efficiency was quantified by counting the percentage of EBs that were beating and also the number of beating clusters/EB at d10 of ESCs differentiation. Although there was no difference noted in the percentage of beating EBs, the beating clusters/EB was seen to be higher upon USP knockdown and the opposite remaining true in case of USP overexpression. Both immunocytochemical and flowcytometry analysis also revealed increased cardiac Troponin+ cells upon USP knock down when compared with that of control. Together our data suggested a negative modulatory influence of USP during cardiac progenitor specification and their subsequent differentiation into cardiomyocytes.

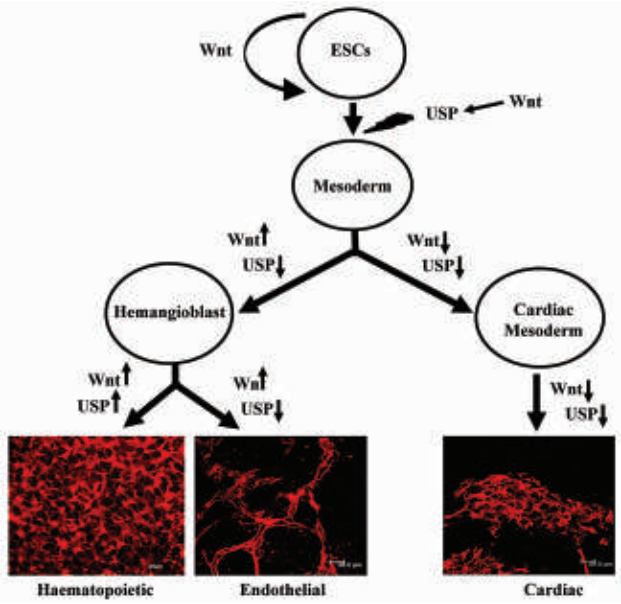


Fig. 1: Schematic prediction of Wnt-USP attribute during mesoderm specification and differentiation.

$Flk1^{+}$ mesoderm gives rise to endothelial cells during early development. Mice harbouring mutations in Flk gene have been reported to be defective for all the mesoderm derivatives. Hence to assess whether USP could regulate haemato-endothelial differentiation from ESCs *in vitro*, USP-Sh and Ctrl-Sh ESCs were differentiated and Flk1 expressing cells were quantified at d5 of ESCs differentiation by flow cytometry. Similar to cardiac progenitors and cardiomyocytes, increase in Flk^{+} mesodermal cell was also seen upon USP knockdown that further resulted in pronounced endothelial differentiation as discerned by increase in $PECAM^{+}$ population. On the contrary, $CD34^{+}$ and $CD45^{+}$ cells representing the haematopoietic stem cells were declined upon USP knowndown. Together our data suggested differential haemato-endothelial fate modulation by USP during mesodermal differentiation from ESCs. While USP may have a positive influence on haematopoiesis, it may interfere with endothelial and cardiac differentiation and Wnt might be exerting its influence through USP during the same (Fig. 1). We are further investigating the targets of USP that might be prevented from undergoing ubiquitination and thereby contributing to the call fate specification and differentiation during early development.

Future Research Plans

- We would like to investigate the mechanistic basis underlying Wnt and USP mediated differential fate modulation during early development.

Lalita S. Limaye

lslimaye@nccs.res.in

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

Participants

Darshana Kadekar, *UGC SRF*
Kedar Limbkar, *ICMR SRF*
Ankita Dhenge, *UGC SRF*
Sophia Fernandes, *UGC SRF*
Prajakta Shinde, *DBT SRF*
Rutuja Kuhikar, *DBT SRF*
Nikhat Firdaus Q. Khan, *Technical Officer A*

Collaborators

1. Dr. R.L. Marathe, *Jahangir hospital, Pune*
2. Dr. Sameer Melinkeri, *Deenanath Mangeshkar Hospital, Pune*
*3. Dr. Maj. Gen. Velu Nair, *AVSM, VSM Dean and Deputy Commandant, AFMC, Pune-411040*
**4.Dr. Raju Agarwal, *Prof. In Obstetrics and Gynaecology, Golden Jubilee Block, AFMC, Solapur Road, Pune-411040*
*Present address- *Director General Medical Services (Army) & Col Commandant, Dte Gen Medical Services (Army), Adjutant General's Branch Integrated HQ of MoD (Army), New Delhi-110001, India.*
**Present address- *Professor and Head of Department, Obstetrics and Gynecology, Command Hospital, Lucknow-226002, India.*

Name of collaborators within NCCS :

1. Dr.V .P. Kale, *Scientist G ,NCCS,Pune*
2. Dr.Manas Santra, *Scientist D, NCCS,Pune*

Background

Out of the five different ongoing projects from the lab., the project on "Studying the role of Valproic acid and polyunsaturated fatty acids in differentiation of megakaryocytes and biogenesis of platelets" is described in detail below.

Platelets are small (2-3 micron) enucleate particles that have highly organized cytoskeleton and have abundant secretory granules. Approximately 1011 platelets are released daily into the blood stream of an adult human on a steady state basis. They play a very important role in maintaining hemostasis by activation and aggregation at the site of vessel injury and formation of blood clot. The normal platelet count ranges from 1, 50,000 to 4, 00,000 per µl and any abnormality leads to either increase (Thrombocythemia) or decrease (Thrombocytopenia) in platelet count creating serious problems to human health. Various clinical conditions including immune thrombocytopenic purpura(ITP), myelodysplastic syndromes (MDS), chemotherapy-induced thrombocytopenia, continuous bleeding due to defect in blood clotting, aplastic anemia, human immunodeficiency virus (HIV) infection are accompanied by thrombocytopenia. Treatment for these conditions requires platelet transfusion. This procedure has certain drawbacks like - low shelf life of platelets, their efficacy goes down with storage time, shortage of donor availability, the procedure is expensive, time consuming and has high risk of carrying infections. To overcome these hurdles, there is a need to look for alternative sources of platelets and produce these cells *in vitro* in large quantities and search new pharmacological compounds that can enhance the platelet production. MKs and platelets have been derived from various sources like $CD34^+$ cells obtained from Umbilical cord blood, mobilized peripheral

blood and bone marrow. Earlier work from our lab has shown that incorporation of two nutraceuticals i.e. omega-3 - docosahexanoic acid (Docosahexanoic acid) and omega-6 - arachidonic acid (Arachidonic acid) in the culture medium leads to an enhancement in the megakaryocyte and platelets production from umbilical cord blood derived $CD34^+$ cells. In this study, we examined the effect of above mentioned PUFAs on MKs and platelet formation from yet another source of $CD34^+$ cells i.e. apheresis samples obtained from healthy allogeneic donors from BMT units. We have utilized this source as it is a clinically more relevant source in transplantation settings.

Megakaryocytes (MKs) comprises 1% population of all the hematopoietic cells in the bone marrow. MKs are giant multinucleated cells 100-150 micron in size , they undergo endoduplication , cytoplasmic maturation and form pseudopodia like extensions known as pro-platelets which release platelets from their ends into the blood stream and the remaining MK cell body then undergoes apoptosis. Thus platelet biogenesis is a very complex process involving multiple cellular and molecular events. Though worldwide many investigators are trying to understand this process still there are several aspects that remain unanswered. We have procured MEG-01 cell line from ATCC and used it as a model cell line to study the process of platelet biogenesis., This is a human megakaryoblast cell line derived from a CML patient, We used Valproic acid, a known differentiating agent. The cellular and molecular events during platelet formation and release were studied in depth by using this cell line model. Notch and Rho-A pathway – the two major signaling pathways were studied during PLT formation.

Aims and Objectives

- To study beneficial effect of nutraceuticals on generating Megakaryocytes and platelets from apheresis derived $CD34^+$ cells.

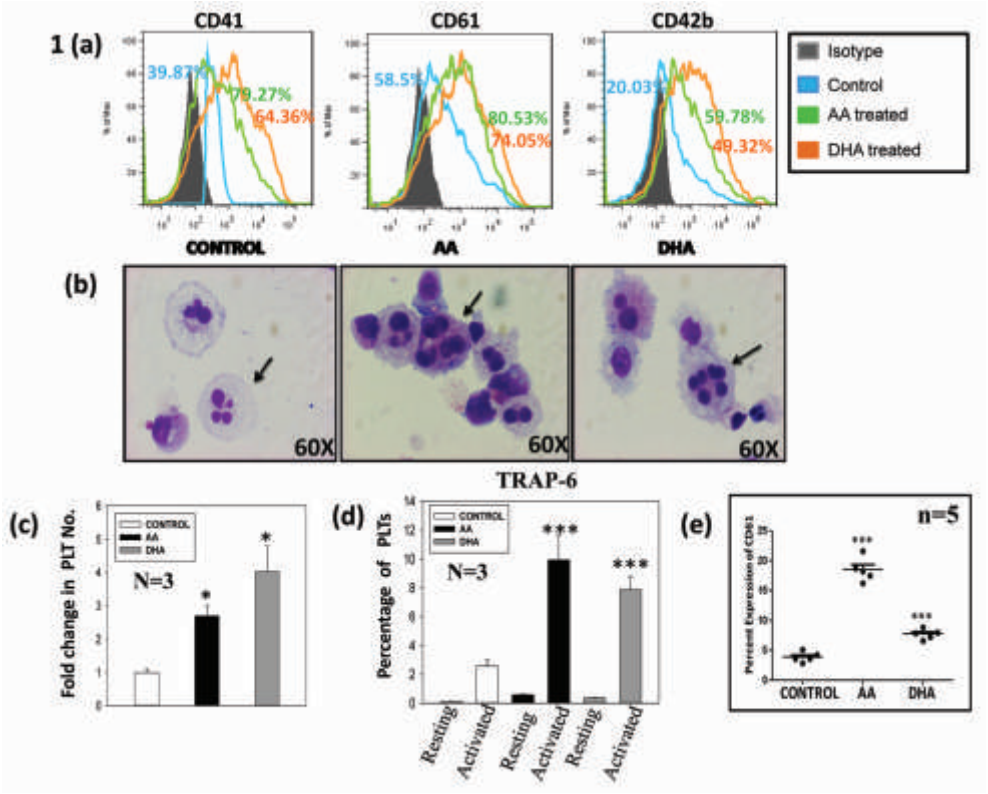


Fig.1. AA/DHA promotes Megakaryocyte differentiation - $CD34^+$cells from apheresis samples when grown in presence of AA/DHA favour megakaryocyte differentiation. (a) Representative overlay showing higher percentage of CD41/CD61 and CD42b in AA/DHA sets.(b) Wright's and Giemsa stain showing mature MKs, larger cells with polylobulated nuclei as shown by arrows (60X).(c) Total yield of culture derived platelets were found to be significantly higher in test set than control set.(d) The percentages of activated platelets are higher for test sets as compared to control set in the presence of agonist TRAP-6.(e) Scatter plot showing human CD61expression in NOD /SCID mice of different groups

- Elucidating the cellular and molecular events involved in Proplatelet, Platelet formation and release from Megakaryocytes derived from MEG-01.

Work Achieved

To address the first objective, $CD34^+$ cells were isolated from apheresis samples of healthy donors and were cultured in the presence of SCF and TPO ,supplemented with or without two nutraceuticals i.e. arachidonic acid(AA) or docosahexanoic acid (DHA). Megakaryocytes start expressing integrin CD41, CD61 and maturation marker CD42b on their surface upon differentiation from haematopoietic stem cells (HSCs).This specific marker expression was analysed by flow cytometry. The expression of these markers was higher in AA/DHA sets as compared to control as shown in representative FACS overlay (Fig.1a) The number of large cells with poly lobulated nuclei were evident in Wright's and Giemsa staining (Fig.1b). Culture derived platelets were harvested by centrifugation on day 14^{th} and counted by using neubauer chamber. The data shows 2.8 fold and 4.4 fold increase in platelet number in AA and DHA set than control set respectively (Fig.1c). Upon activation CD62P marker is expressed on the surface of platelets as detected by flow cytometry. When the culture derived platelets were activated by the agonist - TRAP-6 - CD62P expression was increased in AA/ DHA set as compared to control set. (Fig.1 d).

The engraftment of human CD61 positive cells in NOD/SCID mice was significantly higher in test sets than the control (Fig.1 e).

To achieve the second objective MEG-01 cells were grown in the presence/absence of Valproic acid (VPA) as test and control respectively. The culture was maintained for 21 days with complete media change on every 3^{rd} day. Ploidy levels increased up to 8N on treatment with VPA as compared to control as shown by FACS profile (Fig.2a). Details of morphology were further examined by scanning electron microscopy, Test cells show proplatelet structures that are not so evident in control (Fig.2b). Megakaryocytes undergo cytoskeletal rearrangements to form pro-platelet like structures i.e. elongation, bending, branching of extensions and further shedding of platelets from their ends.Valproic acid treated cells show increased fluorescence intensity for MK specific β-1 tubulin as compared to control (Fig.2 c).

We tried to elucidate notch signalling in VPA mediated differentiation of MEG-01 cells. Interestingly Notch 3,its ligand DLL4 and target gene PDGFR β were found to be upregulated at protein levels as detected by western blot (Fig.3a) Further we checked the levels of proteins involved in actin organization.We found an upregulation of Rac1/cdc42, P-Rac/cdc42, Arp-2/3,

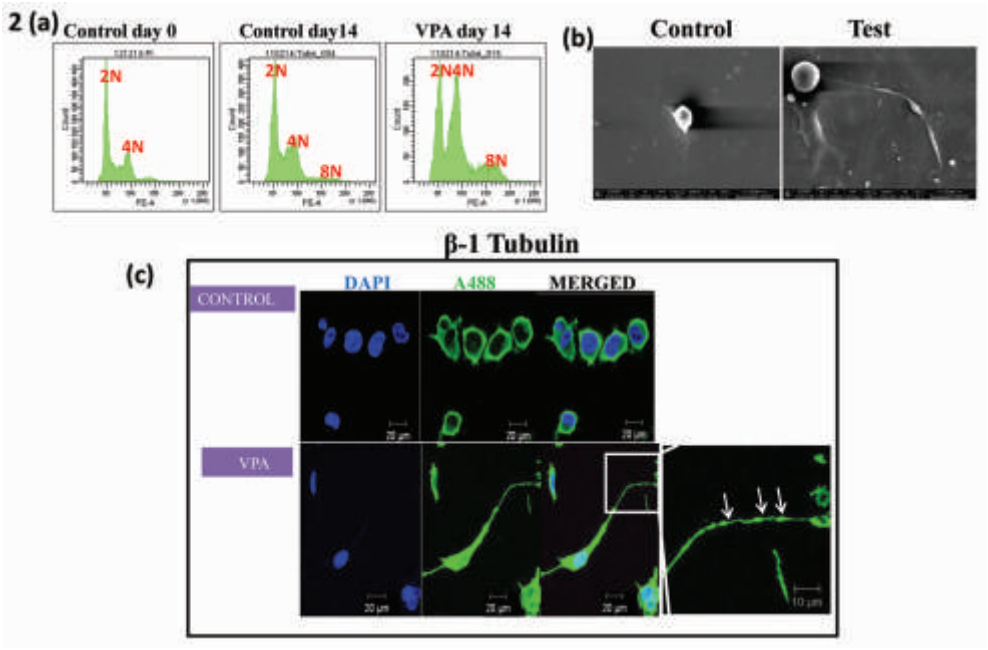


Fig.2. MEG-01 cells differentiated with Valproic acid show (a) Ploidy levels up to 8N as compared to control as shown by FACS profile.(b) proplatelet formation by Scanning electron microscopy.(c) immunofluoroscence images of MK specific cytoskeletal protein β1 tubulin.

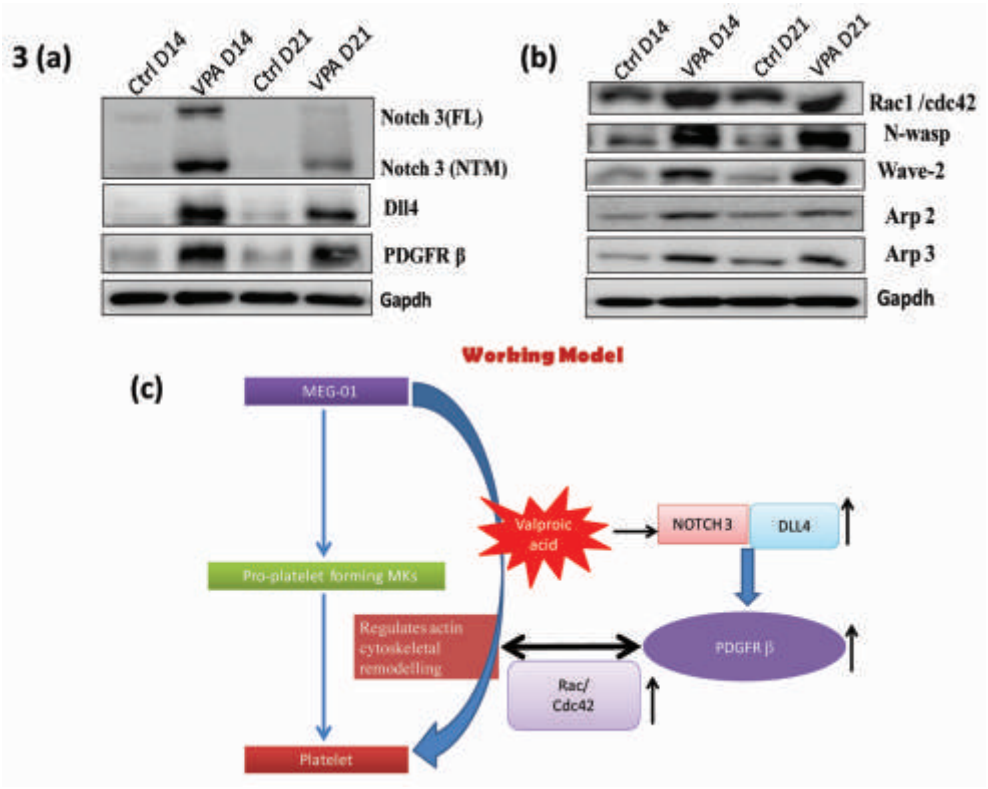


Fig.3. MEG-01 cells differentiated with VPA: (a) Expression of Notch-3, DLL4 and PDGFRβ was higher and (b) Proteins involved in actin cytoskeletal remodelling was also higher.(c) Working model showing involvement of Notch 3 in regulation of platelet biogenesis by controlling actin organisation via PDGFRβ.

N-wasp and wave-2 proteins (Fig.3b) which are the key players during actin nucleation and polymerization events. So, we speculate that valproic acid induces platelet formation through Notch-3 signalling which in turn modulates actin polymerization which is one of the crucial steps necessary for thrombopoiesis.The schematic model is depicted in Fig.3c.

Future Research Plans

- Our data shows that Notch 3 is upregulated in MEG-01 cells differentiated by VPA and is controlling platelet formation through PDGFRβ by actin polymerization. This needs to be further confirmed by Knock down of Notch3 by ShRNA approach to study its exact involvement in the process of platelet biogenesis.

Amitabha Majumdar

mamitava@nccs.res.in

Understanding the molecular mechanism of persistence of memory

Background

Our lab is attempting to understand how long term memory persists over time. We use Drosophila as our model system. We are focusing our research on the translation regulaor Orb2. Previously Orb2 has been shown to have functional prion-like nature, and using mutations to interfere with Orb2's prion like nature it was observed that long term memory could form but would not persist over time. This was one of the first examples of a prion-like protein being beneficial.

Aims and Objectives

Currently we are fousing on understanding the following questions:

- What regulates the prion-like oligomerization of Orb2.
- In neurodegenerative disorders is Orb2 effected?
- Orb2 being a translation regulator, is there any cross talk of Orb2 with other translation regulators like Fmr1 which re invoved with autism.

Progress

Through various ongoing screens we have found out some modifiers of Orb2 and are currently working on them. We also have found out a connection between Orb2 and Huntington's disease and are studying the interaction amongst them. We have noticed a genetic interaction between Orb2 and Fmr1 and are currently characterizing them.

Participants

Vighnesh Ghatpande, *SRF*
Meghal Desai, *SRF*
Sadhana Mutalik, *JRF*
Vishal Naik, *Project Assistant*
Maitheli Sarkar, *Project Assistant*
George Fernandes, *Technician Officer*

Shekhar Chintamani Mande

shekhar@nccs.res.in

Structural and Functional Studies on Mycobacterial Proteins

Participants

C. M. Santosh Kumar, *RA (left since March, 2017)*
Pratibha Tiwari, *SERB-NPDF*
Suhas Kharat, *RA (left since November, 2016)*
Lumbini Yadav, *SERB-NPDF*
Ankita Chatterjee, *SERB-NPDF*
Swastik Phulera, *SRF*
Ashwani Kumar, *SRF*
Vipul Nilkanth, *SRF*
Yousuf Ansari, *SRF*
Sapna Sugandhi, *SRF*
Nitin Bayal, *SRF*
Bithika Chatterjee, *JRF*
Malti Umrani, *TO*

Collaborators

Sharmistha Banerjee, *University of Hyderabad, Hyderabad, India.*
Rohini Suryavanshi, *Richardson Leprosy Hospital, Miraj, Sangli, India*
Amit Singh, *IISc, Bangalore, India*
Milind S. Patole, *NCCS*
Yogesh Shouche, *NCCS*

Background

One of the problems that my group has been exploring is in understanding latency and activation in the case of *Mycobacterium tuberculosis* (Mtb), the pathogen causing tuberculosis. Previously, via computational approaches we have shown that Mtb has a well defined response pathway for latency activation and reactivation. My laboratory has been involved in the characterization of such proteins.

Traditionally, hypoxia sensing mechanism of Mtb has been studied through genetic manipulations and comparative transcriptomics. Hypoxia activated DosR/DosS (DevR/DevS) and MprAB two-component signal transduction regulatory system are the ones that are commonly implicated in the regulation of dormancy. It has also been shown that besides DosR-mediated initial hypoxic response, sustained hypoxia spanning to 4-7 days induces a set of 230 genes, referred to as Enduring Hypoxic Response (EHR) that facilitated the shift to a persistent, metabolically inactive, but viable state.

A comprehensive study by Galagan *et. al.* combining ChIP-Seq data with system-wide transcriptomics, proteomics, metabolomics and lipidomics during hypoxia and re-aeration identified several transcriptional regulators outside that of the DosR or EHR response. They identified Rv0081, a part of the DosR regulon, as a major regulatory hub for multiple hypoxia induced pathways. This supported our earlier prediction that DosR and DosS, along with Rv0081 act as important regulators of latency.

Rv0081 is a non-canonical ArsR/SmtB family transcription factor. It is the first gene of the operon Rv0081-Rv0088. It belongs to ArsR/SmtB family of prokaryotic metalloregulatory transcriptional repressors. This family of

repressors is known to regulate the expression of genes linked to heavy metal ions stress, like those involved in metal uptake or efflux or detoxification. Rv0081 was established experimentally as a repressor by Zahrt *et. al.* who showed that Rv0081 self regulates its expression by binding to an inverted repeat element upstream to the locus Rv0081-Rv0088. Besides self-regulation, Rv0081 was shown to co-regulate the locus Rv0081-Rv0088, the genes of which are predicted to encode formate hydrogen lyase (FHL) enzyme complex. Classically, in bacteria, a shift from aerobic to anaerobic growth brings about cleavage of pyruvate predominantly to acetyl-CoA and formate by Pyruvate-formate Lyase. Formate is then metabolized into CO_2 and H_2 by Formate hydrogen lyase. As mycobacteria would require shifting metabolism to anaerobic conditions inside granuloma during latency, one may think Rv0081 as one of the key regulators deciding the fate of mycobacterial latency. Interestingly, Rv0081 has been explored for antigenic response in several population studies.

Further investigations by Zahrts' group led to the multi-layered regulation of Rv0081 by DosR/DevR and MprA, which acted as positive regulators. In the proposed model, conditions like hypoxia or NO stress induced DosR/DevR and MprA that promoted synthesis of Rv0081. While DNA binding property and the repressor activity of Rv0081 are well established now, the specific stimulus that regulates its DNA binding and dissociation remains unclear. Although ArsR/SmtB family of regulators are known to respond to toxic levels of metals by virtue of the conserved metal binding motif - ELCV(C/G)D. We observe that Rv0081 does not have the conserved metal binding residues but has DNA binding residues. This suggested that binding properties of Rv0081 may be metal independent and this protein thus represents a non-canonical ArsR/SmtB transcription factor. With the emerging significance of Rv0081 in dormancy and our own puzzling observations regarding its mode of activity, we solved the structure of Rv0081 and identified the possible stimulations that may regulate its DNA binding ability.

Structural information can aid in predicting host-microbe protein interactions

A comprehensive map of the human Mtb protein interactome would go a long way in understanding tuberculosis. Computational predictions are typically used to complement experimental studies towards this end. Several sequence-based *in silico* approaches use existing data on experimentally validated protein-protein interactions (PPIs). In the existing methods, experimentally validated PPIs serve as templates from which novel interactions between pathogen and host are inferred. Such comparative approaches typically make use of local sequence alignment, which, in the absence of structural details about the interfaces mediating the template interactions, could lead to incorrect inferences, particularly when multi-domain proteins are involved.

Aims and Objectives

- Exploring the structure and function of Rv0081.
- Use structural information in predicting host-microbe protein interactions.

Work Achieved

Crystal Structure of Rv0081 and its functional relevance

We determined the crystal structure of Rv0081 using Molecular Replacement. Each asymmetric unit contained four polypeptide chains. These four chains together constitute two biological dimers (Figure 1). Studies using Multi-Angle Light scattering coupled to size exclusion chromatography (SEC-MALS) reveal that this protein is dimeric in solution.

The overall structure is similar to other proteins in the ArsR/SmtB family. The overall structure is the archetypical winged helix-turn-helix motif DNA binding proteins of this family. Previous structures from this family are known to bind DNA in a metal-regulated manner. Further, we examined the Rv0081 structure for potential metal binding sites in the PAR3D program (Goyal and Mande, 2008), and found that there are no other potential metal binding sites. It therefore appears that the activity of Rv0081 binding to DNA might not be regulated by metals.

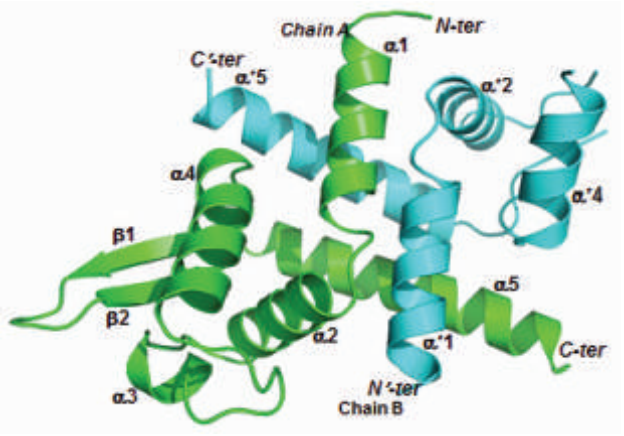


Fig. 1: Overall Structure of Mtb Rv0081. Cyan and Green cartoons represent the two polypeptide chains that form the functionally active dimer. The N-terminus and C-terminus have been labeled along with other labels for the corresponding helices and sheets.

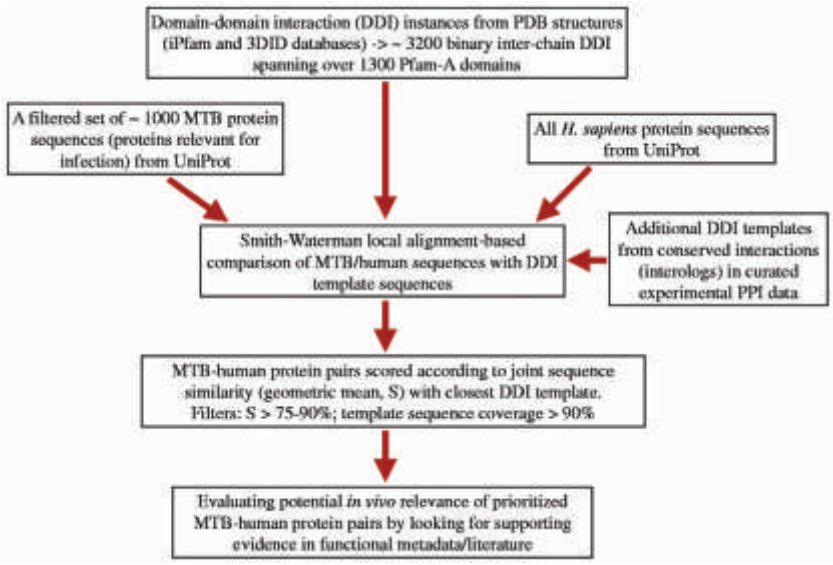


Fig. 2: Summary of the steps involved in our sequence/domain-based approach to the search for potentially novel (uncharacterized) human-microbial PPI.

Comparing Rv0081 structure, which is in complex with the DNA, we could locate the putative DNA-binding residues of Rv0081. The presence of Serine residues in DNA binding is suggestive of post-translation modification of these for regulating DNA binding. We therefore hypothesized that DNA binding activity of Rv0081 might be regulated by post-translational modifications- *i.e.* phosphorylation of Serines, rather than by metal ions.

We also carried out electrophoretic mobility assays with the oligonucleotide sequences containing the two sequence motifs proposed to bind to Rv0081. Further, we created mutants of Rv0081, where the Ser-residues are mutated to Ala or to Asp. The Ser to Asp mutants were created to mimic phosphorylation of Rv0081, where we could test the hypothesis that regulation of DNA binding activity was mediated by post-translational modification. We hypothesized that Ser to Ala mutants might bind DNA, with lower affinity, while Ser to Asp mutants might abolish binding completely. Results on these EMSA experiments show clearly that the Ser to Asp mutants fail to bind DNA, strengthening the hypothesis that the Serine residues if phosphorylated, would lead to a loss of DNA binding ability of Rv0081.

Finally, to test if the protein was really phosphorylated under normoxic and hypoxic conditions, we probed the protein from crude cell lysates with anti-pSer and anti-pThr antibodies. Cells grown under these two conditions showed no significant change in expression of Rv0081. However, under the hypoxic condition, there was a marked change in the phosphorylation of the protein. This thus confirmed that Rv0081 might be differentially modified under these two environmental conditions, and that its DNA-binding activity might be regulated by phosphorylation.

Using structural information in a targeted search for candidate host-Mtb protein interactions

The various steps involved in our methodology have been summarized as a flow chart in Fig. 2. We have arrived at a non-redundant, prioritized list of Mtb-human protein pairs, each of which contains at least one pair of subsequences closely resembling a known domain-domain interaction (DDI) instance on the sequence level. Although we have illustrated our methodology with the specific case study of Mtb, it is of general applicability, and should provide a useful data-driven approach to predicting and prioritizing potential PPIs between any pathogenic microbe and its host that leverages the existing genomic and structural datasets available in the public domain.

Future Research Plans

- Exploring the cellular consequences of Rv0081 overexpression and under expression under various laboratory conditions.
- Exploring phosphorylation status of Rv0081under various conditions and identifying its kinase.
- Exploring the pleiotropic effects of Rv0081 expression / phosphorylation in correlation with the cellular metabolism under different growth conditions.

(DST-INSPIRE Faculty -
Dr. Shekhar Mande's research group)

Jyoti Singh

jyotis@nccs.res.in

Project Title: A comparative whole genome sequence analysis leads to identification of several novel conserved miRNAs in *B. mori*

Background

Almost a decade ago, the discovery of founding members of miRNA has dramatically revolutionized our understanding of regulation of gene expression and since then, there is an exponential increase in number of miRNA identified from different species. Different experimental and computational approaches have been pursued for the prediction of miRNAs and all these approaches have their share of advantages and limitations. The main difficulties with the experimental identification methods based on cloning are that they require a considerable amount of small RNA as a starting material, and generally result in under-representation of differentially restricted temporal and meagerly expressed miRNAs sequences. On the other hand, computational methods rapidly identify miRNAs that have very low expression levels or that are expressed only in specific conditions, time period and cell type. But the main limitation is that many of these computation driven methods are often based on the known data sets.

The most conventional computational approach for the prediction of conserved miRNAs is the homology search of known miRNAs reported in miRBase (the primary repository for published miRNA sequences) on to the complete genome of the given species, followed by scanning of secondary structures of their precursor sequences for characteristic stem-loop structure. The primary hurdle with these kinds of approaches is the dynamic nature of the known dataset, i.e. miRBase; with every release the data keeps on changing because of addition of newly predicted miRNAs. Secondly, these approaches generally miss many of the conserved miRNAs not reported in the known dataset.

Participants

Uddhav Ambi, *Project Assistant*
Vartika Gurdaswani, *Project Trainee*

Collaborators

Dr Arun Kumar KP, *CDFD, Hyderabad*

Considering these limitations, we have developed an approach to predict all the possible conserved miRNAs among closely related species by analyzing the whole genome sequence for the tell-tale signs of experimentally validated miRNAs

Aims and Objectives

- To develop an algorithm to predict all the possible conserved miRNAs among closely related species by analyzing the whole genome sequence.
- Experimental validation of all the newly predicted miRNAs.
- Target prediction of newly predicted miRNAs.

Work Achieved

Identification of novel miRNAs: By employing the algorithm as described in Figure 1A, we identified 76 conserved miRNAs from *Bombyx mori*, by comparing the whole genome sequence with those of two closely related Dipteran species; *Drosophila melanogaster* and *Anopheles gambiae*. Out of these 76 miRNAs, 34 are novel ones as none of these have been reported from any other species till date. Most of these newly predicted miRNAs were also found to be conserved in other insect species i.e. *Apis mellifera* of order Hymenoptera, *Tribolium castaneum* of order Coleoptera and *Acyrthosiphon pisum* of order Hemiptera (Figure 1B).

Experimental Validation: A stem-loop RT-PCR was carried out for expression analysis of all the 34 novel miRNAs in different tissues as well as the ovary-derived BmN cell lines. As a result, 32 out of 34 newly predicted miRNAs were found to express in different tissues.

Target Prediction: Mapping all the experimentally validated miRNAs on to the 3'UTR sequences of B. mori using miRanda program along with different parameters yielded 173 hits. These hits were then scanned for the seed region complementarity, which restricted the number of hits to 83. To give an extra strength to the local miRNA::mRNA duplex, at least two base-pairing at 3'end of the miRNA and less than three continuous gaps were taken into consideration. Finally, we obtained 55 target hits of 23 miRNAs on 47 distinct mRNAs.

Future Research Plans

- Functional Analysis of some of the mRNA targets of newly predicted miRNAs.

Conferences / Workshops

- Presented poster in DST-INSPIRE Meet 2017 at IISER Pune, 3-4 February 2017.
- Presented poster in Young Investigator Meet 2017 (YIM) at Goa, 6-10 March 2017.

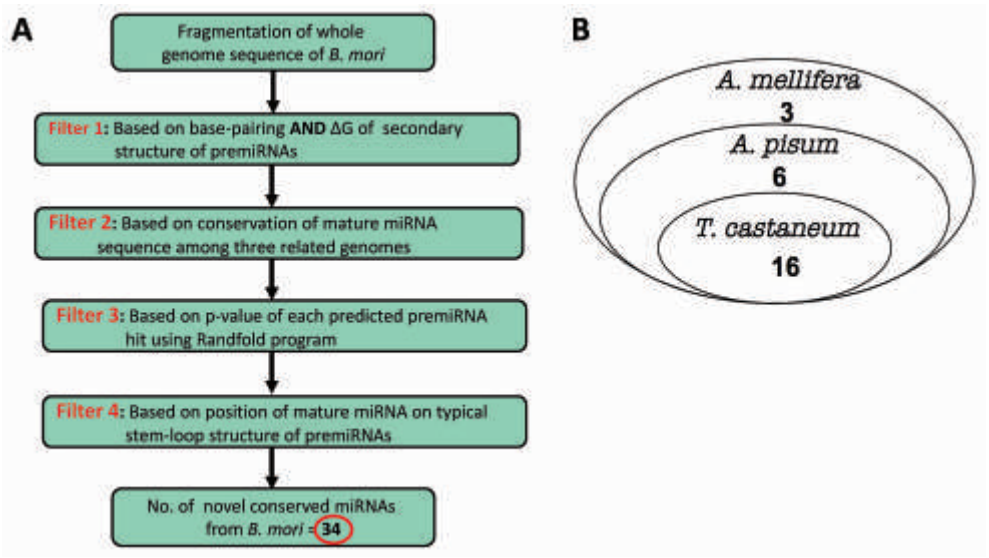


Fig. 1: A) Computational pipeline of different steps involved in genome-wide prediction of conserved miRNAs. B) Conservation of 34 newly predicted B. mori miRNAs in other insect species.

(Wellcome Trust-DBT Indian Alliance Early Career Fellow - Dr. Shekhar Mande's research group)

Md. Zahid Kamal

zahid@nccs.res.in
mdzahidkamal@gmail.com

Project Title: Decoding organism-related evolution of survivin, a hub protein

Background

The relationship between complexity, robustness and evolvability has been the subject of many debates. One can study these issues at the organism or protein level. At the protein level, there's a shortage of clear-cut experimental works. This topic is of central importance, not least because it has biomedical relevance and can potentially influence biotechnological protocols (e.g. directed protein evolution). I focus on a protein with a large number of protein-protein interactions (hub) and ask how a proxy of protein complexity i.e. interactome size affects (a) robustness against point mutations and (b) the capacity to gain/lose interaction partners during evolution (proxy for evolvability).

I have chosen "survivin", a bonafide hub in protein-protein interaction network, as model system. Amongst its various functions, the most conserved function of survivin is to form the chromosomal passenger complex (CPC) and participate in error-free progression of cell division. It is ubiquitously expressed in most cancers but absent in adult tissues; hence, is amongst the most potent targets in cancer therapeutics research.

Aims and Objectives

- High-throughput fitness measurement (growth rate) of ~ 3000 point mutations of survivin.
- High-throughput estimation of effect of all these mutations on various interactions of survivin using phage-display.
- Developing the mechanistic insight of the effect of these mutations in a structural framework.

Collaborators

Dr. Chandra Shekar Prabhakar, *CCMB, Hyderabad*

Dr. Csaba Pal, *BRC, Szeged, Hungary*

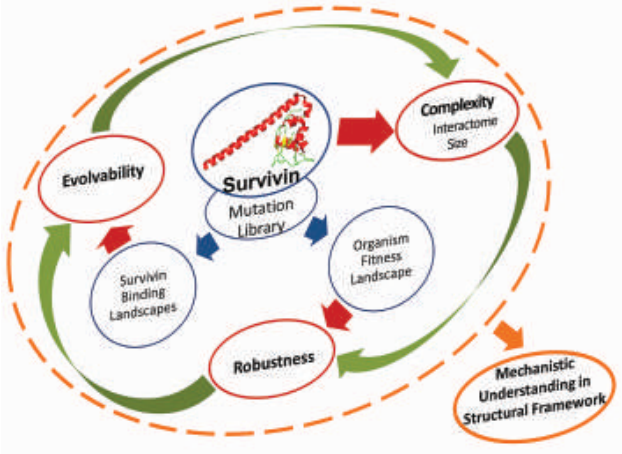


Fig. 1: The scheme depicts the primary goals and approaches of the work. The green arrows indicate the accepted relationship between complexity, robustness and evolvability of biological systems.

Work Achieved

Survivin interacts with ~ 60 proteins. Through computational methods and literature survey, I have downsized this number to 11 direct inter-actors and only 3 interfaces of survivin.

A workflow/pipeline has been established for the phage-display of any desired protein and expression-purification of interacting partner proteins.

Future Research Plans

- Library of survivin point mutations will be created and in a high-throughput manner their effect on various interactions will be estimated.
- System to estimate the effect of survivin mutations on organism growth (fitness) will be established.

Extramural Funding

- Decoding organism-related evolution of survivin, a hub protein. 2015 - 2020. (Wellcome Trust/DBT India Alliance, India)

Talks Delivered

- Unconventional ways to increase enzyme activity; Invited talk; International Conference on Free Radical Biology; Lucknow, India, January 2017.
- Evolution of interactome complexity of survivin, a hub in protein interaction network; Invited talk; Discussionn Meeting on Emergence and Evolution of Biological Complexity; Bengaluru, India, February 2017.

Debashis Mitra

dmitra@nccs.res.in

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

Participants

Surya Srivastava, *SRF*
Tracy Augustine, *SRF*
Jay Trivedi, *SRF*
Kailash Chand, *SRF*
Kruthika Iyer, *SRF*
Muneesh Barman, *JRF*
Sukhadev Kore, *JRF*
Anjali Tripathy, *JRF*
Anindita Dasgupta, *JRF*
Alapani Mitra, *JRF*
Sujata Bhade Kulkarni, *Technical Officer*

Collaborators

Dr. Ashoke Sharon, *BITS, Mesra*
Dr. Manas Kumar Santra, *NCCS, Pune*
Dr. Shekhar C. Mande, *NCCS, Pune*

Background

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS), the incidence of which has reached pandemic levels worldwide. The hallmark of the disease is gradual depletion in the number of CD4+ T cells leading to the onset of opportunistic infections. The therapeutic regimen being used at present can reduce the viral load remarkably but are not the ultimate answer to AIDS patients as a treatment for cure from HIV is yet to be identified. 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 study from our lab has suggested that DNA binding activity of Tat could be one of the potential mechanisms of TAR independent Tat mediated regulation of viral and cellular gene expression. In this context, we have later shown that Tat acts as a repressor of *c-Rel* expression in HIV-1 infected cells. We have shown that Tat down regulates c-Rel promoter activity by interacting with its specific NFκB sites on the c-Rel promoter. Down regulation of NFκB family transcription factor C-Rel by Tat could also be a viral strategy to induce persistent infection in T cells. Further elucidation of Tat's role in regulation of cellular gene expression is currently in progress.

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. In a recent study, we have compared the gene expression profile of wild type and Nef deleted HIV-1 infected T-cells. Our preliminary analysis of

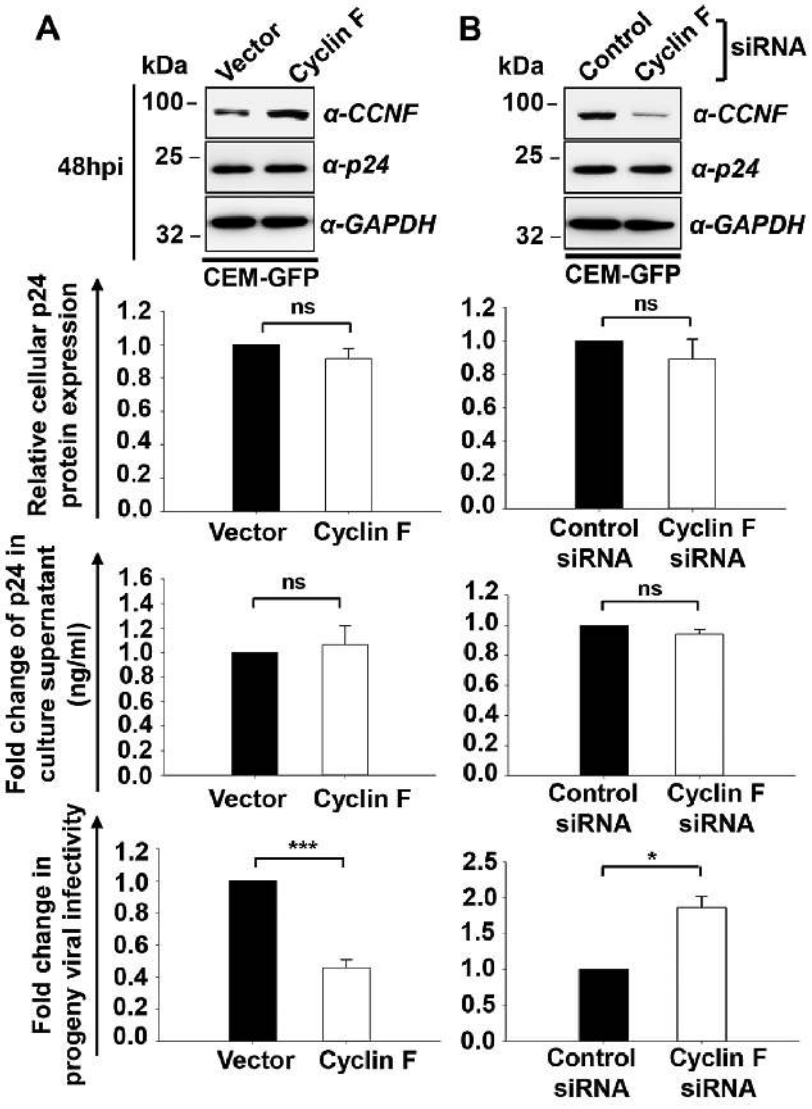


Fig. 1: Cyclin F interferes with infectivity of progeny virions in HIV-1 NL4-3 infected CEM-GFP T cells (A) Cyclin F overexpression does not influence p24 gene expression or viral production, but reduces infectivity of progeny virions. Cyclin F was overexpressed in CEM-GFP T cells and infected with HIV-1 NL4-3 virus (0.5 MOI) 24hrs post transfection. Virus production was compared using p24 ELISA of culture supernatants, 48hpi and these supernatants were used for comparison of progeny virion infectivity using TZM-bl reporter cells by β-gal staining. (B) Silencing of Cyclin F expression in CEM-GFP cells enhances progeny viral infectivity. Cyclin F was silenced in CEM-GFP cells using Cyclin F siRNA (200nM) and cells were infected (0.5 MOI) 24hrs post transfection. Data represent mean ± SEM.

the expression profile indicates that Nef may be responsible for deregulation of lipid metabolism in infected cells. We have also shown earlier that HSP40 and HSF-1 interacts with Nef protein and positively regulate HIV-1 replication. Although involvement of different heat shock protein family members in viral pathogenesis has been reported earlier, a clear understanding of their role in viral replication and infectivity remains to be elucidated. We have initiated a comprehensive study of all the HSP protein family members during HIV infection. Our expression profiling results targeting HSP family members and their isoforms indicate that a significant number of genes belonging to HSP40 and HSP70 family are differentially expressed during infection. Further characterization of the individual role of these isoforms in HIV-1 infection is currently in progress by overexpression and knockdown of the individual isoforms. We are also looking at the role of different HSPs in viral replication and infectivity by knocking down individual HSP family members. Our initial 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. Our studies have clearly shown that HspBP1 inhibits HIV-1 LTR mediated gene expression and viral replication. Furthermore, we have shown that HspBP1 levels are increased in latently infected cells and upon activation, the levels of HspBP1 goes down. Results obtained with over-expression and silencing experiments show that HspBP1 inhibits activation of latently infected cells. Our results finally suggest that HspBP1 inhibits HIV-1 gene expression and replication by restricting p65 from binding to NF-κB enhancer sequence on the viral promoter. Studies on Nef's role in transcription were

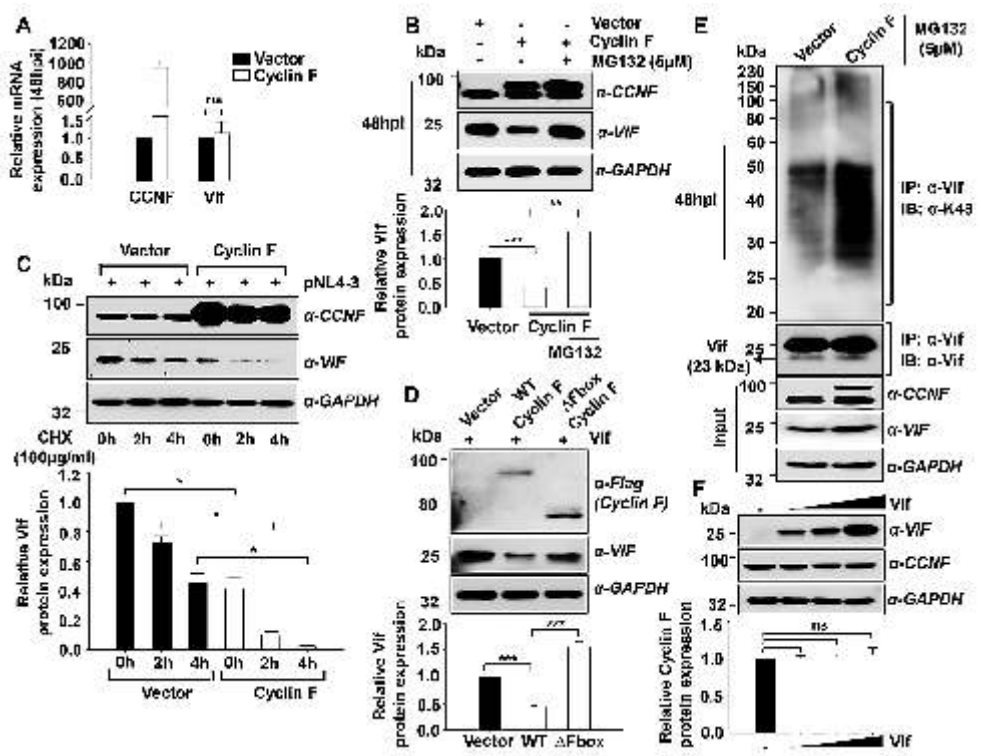


Fig. 2: Cyclin F ubiquitinates and proteasomally degrades Vif during HIV-1 infection. (A) Cyclin F overexpression does not affect Vif mRNA expression during HIV-1 infection. Cyclin F was over expressed in TZM-bl and cells were infected (0.5 MOI) 24h post transfection to analyze Vif expression at mRNA level 48 hours post-infection. (B) MG132 rescues proteasomal degradation of Vif in Cyclin F overexpressed HIV-1 infected cells. Cyclin F was overexpressed in TZM-bl cells and was infected (0.5 MOI) 24hrs post transfection. The cells show restoration of Vif expression with the addition of MG132 (above) after 48 hrs post infection. Densitometric analysis for the same is shown in B (below). (C) Cycloheximide (100μg/ml) pulse chase also revealed accelerated turnover kinetics of Vif with Cyclin F overexpression (above). Densitometric analysis for the same is shown (below). (D) Cyclin F deletion mutant, ΔFbox Cyclin F, is unable to degrade Vif (above). Densitometric analysis for the same is shown below. (E), Cyclin F polyubiquitinates Vif during HIV-1 infection. TZM-bl cells overexpressed with Cyclin F and subsequently infected with NL4-3 virus showed enhanced laddering compared to vector control, when immunoprecipitated with Vif antibody and probed using K48 ubiquitin linkage specific antibody. MG132 was added to vector control as well as Cyclin F overexpressed cells 12 hrs prior to harvesting. (F) Vif over-expression does not alter Cyclin F expression indicating that Vif may not have direct impact on Cyclin F down-regulation during HIV-1 infection. Data represent mean ± SEM.

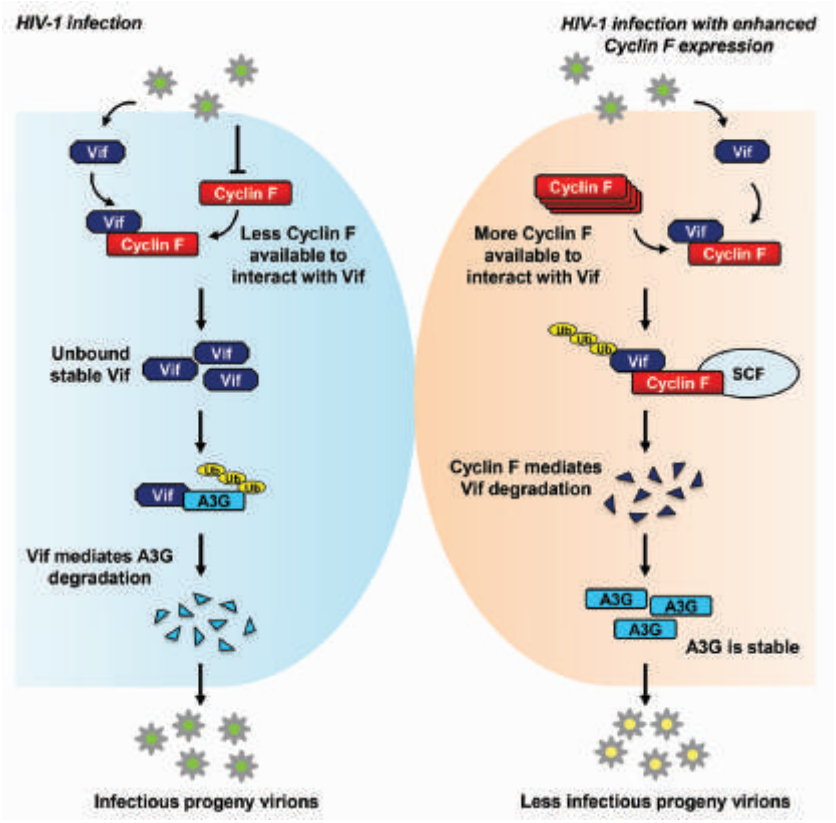


Fig. 3: SCF^{Cyclin} F restricts HIV-1 virion infectivity by ubiquitination and proteasomal degradation of Vif and restoration of A3G levels: a proposed model Left: During HIV-1 infection, Cyclin F undergoes down-regulation. The available Cyclin F still interacts with its substrate Vif during infection. The unbound stable Vif, however mediates degradation of APOBEC3G, leading to production of infectious virions for the next round of replication. Right: In case of Cyclin F overexpression and subsequent HIV-1 infection, more Cyclin F is available to interact with Vif leading to enhanced ubiquitination and proteasomal degradation of Vif through the SCF^{Cyclin} F E3 ligase. This results in the stabilization of APOBEC3G leading to release of less infectious progeny virions.

extended further through transcription factor target search of the differentially expressed genes in the dataset obtained from microarray analysis mentioned above. YY1 has been selected to work further from the obtained transcription factor list as this has been reported to be an important transcription factor suppressing viral transcription by being recruited on the LTR promoter. YY1 over expression leads to dose dependent decline in transcriptional activity from the LTR promoter. Further elucidation of the role of YY1 and Nef 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. We have initiated studies intending to look into the role of cell-cycle associated proteins in HIV-1 pathogenesis. In this context, 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, has been studied recently to identify its role in HIV infection. Cyclin F, also known as FBXO1, is the largest among all cyclins and functions as the substrate binding subunit of SCF^{CyclinF} E3 ubiquitin ligase. Using over expression and knockdown studies, we have identified that Cyclin F negatively influences HIV-1 viral infectivity without any significant impact on virus production

(Fig-1). Subsequently, we have observed that Cyclin F negatively regulates the expression of viral protein, Vif (Viral infectivity factor), at the protein level. We have also identified a novel host-pathogen interaction between Cyclin F and Vif protein in T cells during HIV-1 infection. Subsequently, our results show that Vif is a novel substrate of the SCF^{Cyclin} F E3 ligase, where Cyclin F mediates ubiquitination and proteasomal degradation of Vif through physical interaction (Fig-2). Finally, we have shown that Cyclin F augments APOBEC3G expression through degradation of Vif to regulate infectivity of progeny virions. Taken together, our results demonstrate Cyclin F as a novel F-box protein which functions as an intrinsic cellular regulator of HIV-1 Vif and imparts a negative regulatory effect on maintenance of viral infectivity by restoring APOBEC3G expression (Fig-3).

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 ant-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 screened 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 this library and further characterization of few 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 are currently trying 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 few novel 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. We have also initiated studies looking in to the role of microRNAs and ER stress in HIV pathogenesis. 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.

Milind S. Patole

patole@nccs.res.in

Tight Junction Proteins in Epithelial Cell Invasion by Candida albicans

Background

Although *Candida albicans* is a part of the common fungal flora on human skin and mucosal surfaces, it is known to cause superficial infections by invading and damaging the epithelial cells. Its hyphal stage causes superficial and invasive systemic infections and can invade non-phagocytic epithelial or endothelial cells by either induced endocytosis or by active penetration. Binding of hyphal adhesins to host membrane protein like E-cadherin was observed in the oral epithelial and the endothelial cells. During invasion, many pathogens are known to secrete proteolytic enzymes that degrade cell junctional protein such as E-cadherin. *In vitro* studies using different epithelial and endothelial cell lines have shown that the tip of hyphae stimulate formation of a pseudopodia-like outgrowth from the host cell and the hyphal tip penetrates at the apical end of pseudopodial structure. At the site of invasion local actin polymerization was observed adjoining the hyphae in the pseudopodia region. Extensive actin remodeling leads to formation of new filaments which formed a ring-like rigid arrangement through which the hyphae invade the host cell and this recruitment and remodeling of actin required participation of small Rho-GTPases. Involvement of one of the important TJ protein ZO-1 was observed during the process of actin-mediated endocytosis of *C. albicans*. *C. albicans* infection of epithelial cells led to a reduction of TEER with disruption of barrier, accompanied by loss of junctional proteins.

Aims and Objectives

- To find out role of different TJ proteins, that are known to interact with actin, during *C. albicans* invasion process using *in vitro* cell line model.
- To reveal the fate of TJ during the *C. albicans* mediated invasion of the host epithelial cells.

Participants

Ashwini N. Atre, *Technical Officer*

Work Achieved

In culture media having monolayer of HEK-293T cells expressing ZO-1-GFP at 37°C, allow *C. albicans* cells to germinate and form hyphae, which subsequently invade the mammalian cells in an actin-dependent manner. Upon infection, the peripheral localization of ZO-1 was altered and actin and ZO-1 both were recruited at the site of hyphal invasion. The ZO-1-GFP reporter protein ring formed at the hyphal invasion site overlapped exactly with the circular loop like structure formed by actin as seen by yellow colored hollow circle in overlay of GFP-ZO-1 and TRITC-phalloidin stained actin. To confirm this involvement of ZO-1 in cell invasion process, immuno-staining were performed using anti-ZO-1 antibody. When pseudohyphae which immerge from the yeast form of *C. albicans*, invade the host cells, distinct loss of ZO-1 was seen at the site of ingress of hyphal tip in host cell membrane.

Immuno-staining of other major tight junction proteins was performed in another epithelial cell type after the invasion by *C. albicans*. Immuno-stained preparations using infected rabbit corneal epithelial cell line, SIRC, showed that along with re-localization of ZO-1 other important TJ proteins such as claudin-1, claudin-4, and JAM-A were co-localized with newly formed actin polymers at the site of *C. albicans* invasion.

It was crucial to check the effect of secreted products of hyphal stage, typically found in spent culture medium, on barrier function of epithelial cells. When fully confluent and polarized MDCK cells were grown on trans-well inserts with 0.4µm pore size membrane and incubated for 24 h with the spent culture medium collected from yeast phase of *C. albicans*, loss in TEER was not seen but it was slightly increased as compared to untreated control MDCK monolayers, whereas MDCK cells exposed to spent culture medium from hyphal phase of *C. albicans*, showed time-dependent reduction in TEER indicating loss of tight junction. This decrease in TEER was significant and approximately 70% reduction in TEER was found after 24 hour treatment of MDCK monolayers with the hyphal spent media compared to untreated monolayers. This decrease in TEER can be confirmed by another parameter like dextran flux which indicates paracellular permeability of monolayers. Dextran flux from *C. albicans* hyphal spent medium treated monolayers was also found to increase significantly in comparison to untreated monolayers.

TJs are involved in paracellular transport of molecules and movement across the cell membrane. TJs are composed of transmembrane hydrophobic and soluble cytoplasmic proteins. Soluble proteins namely zonula occludin-1, -2 and -3 anchor actin filaments to TJs. While transmembrane proteins like occludins, claudins, and junctional adhesion molecules play roles in adhesion, permeability, and signaling. Involvement of GTPase belonging to category, RhoA and Cdc42 in epithelial junction formation is documented. These GTPases are known to play an important role in formation of new actin filaments and pseudopodial structures in non-phagocytic epithelial cells. Involvement of GTPase in actin remodeling is demonstrated *in vitro* during invasion of host cell by *C. albicans* [Atre AN, Surve SV, Shouche YS, et al., 2009. Association of small Rho GTPases and actin ring formation in epithelial cells during the invasion by *Candida albicans*. FEMS Immunol Med Microbiol 55:74-84]. Therefore crucial role of tight junction proteins in involvement during *C. albicans* invasion is predictable and is proved in this study.

D. Prakash

dprakash@nccs.res.in

Interactions of *Plasmodium falciparum* with monocytes / macrophages

Background

Plasmodium falciparum can cause malaria at different levels of severity, from asymptomatic, mild-uncomplicated, and severe-complicated malaria, to cerebral malaria (CM). Monocytes and macrophages, which are very important constituents of the innate immune system, are involved in control of infectious microorganisms. The involvement of these cells in malaria has been extensively reported. Studies have demonstrated that monocytes / macrophages (MO/MΦ) are involved in both protection, as well as immunopathology, during malarial infection. The level of protection afforded by these cells, which is through phagocytosis, antibody-dependent cellular inhibition (ADCI), cytokines production, etc., depends upon various components of the parasite. The parasite has a complex life cycle with different stages, at which different proteins are expressed. Due to the heterogeneous nature of MO/MΦ, they can mature into inflammatory or anti-inflammatory type in response to exposure to micro environmental signals, which can vary with respect to receptor expression, cytokine production, effector function and chemokine repertoires. The switching of phenotypes involves expression and secretion of different cytokines, chemokines and other molecules. The switching of phenotypes is reflected in the Th1/ Th2 immune responses which control the infection. It has been suggested that the phenotypic switching of monocytes and macrophages in response to infection is different in humans and mice. The macrophages themselves can produce several factors that influence their own physiology. The term, "classically activated", has been used to designate the effector macrophages that are produced during cell-mediated immune responses. Macrophages can also respond to signals that are produced by antigen-specific immune cells. These signals are more focused and prolonged than innate immune stimuli, and generally give rise to longer-term alterations in

Participants

Deepali B., *SRF*

Ashwin K., *SRF*

Mengesh Deval, *Technician*

Collaborator

Dr. Padma Shastry, *Principal Scientist (Cipla Project), NCCS*

macrophages. Nonetheless, the knowledge of the role of MO/MΦ in malaria is far from complete, requiring further studies on the biology of MO/MΦ, to address a series of important questions in basic MO/MΦ interactions. These studies could lead to novel therapeutic approaches for mitigating the devastating consequences of this infection. Knowledge of the factors influencing the balance between protection and pathology could assist in the design of therapeutics aimed at modulating monocyte and macrophage functions, to improve outcomes. Further, understanding macrophage heterogeneity holds enormous potential because these cells can be biomarkers of diseases, and could also be used as surrogate markers of protection following drug treatment or vaccination. Therefore, the present studies focus on identification of definitive biochemical markers of each of the different macrophage populations, so that individual populations can be manipulated, selectively depleted, or targeted by cell-specific therapeutics.

Aims and Objectives

- Immunomodulation of monocyte/macrophages by synthetic β- hematin.
- Studies on hemozoin-mediated cytokine and chemokine production by human monocyte/macrophages.

Work Achieved

Human monocytes were fed with parasite-derived natural hemozoin, synthetic β-hematin and parasite soluble extract. Subsequently, their RNA was isolated and microarray analysis was carried out. Data analysis revealed an up regulation of 2584 genes and down regulation of 2623 genes in hemozoin-fed adherent human monocytes, while synthetic β-hematin, a structural analogue of hemozoin, up regulated 7557 genes and down regulated 4085 genes. Though β-hematin shows basic structural similarities with parasite hemozoin, it is surprising that it had an influence on more genes in human monocytes. We further analyzed the data for phenotypic gene switch in hemozoin- and β-hematin-fed cells. We characterized the alterations in functions of monocyte/macrophage induced by these compounds by assessing important markers at the transcript level by RT-PCR, secreted cytokine levels by ELISA, and expression of surface molecules by FACS. The initial results of RT-PCR showed up regulation of IL-10 and down regulation of IL-12p70 and CD80, which is characteristic of anti-inflammatory phenotypes, in hemozoin-fed adherent monocytes. β-hematin showed up regulation mRNA of IL-10 and IL12p70. Hemozoin increased transcript levels of CCL1, but not those of CXCL13 and CD206, suggesting a change in phenotype. The levels of cytokines were estimated by ELISA in cell-free culture supernatants collected from monocytes fed with parasite hemozoin and β-hematin. The culture supernatants of hemozoin-fed adherent monocytes showed higher levels of IL-10, IL-6 IL-1β, TNF-α and lower levels of IL12p40, IL12p70, as compared to controls. This is characteristic of the anti-inflammatory phenotype, where high production of IL-10 is seen along with inflammatory cytokines, except IL12. Unlike hemozoin, synthetic β-hematin resulted in production of IL-12p40 and IL-12p70, along with other inflammatory cytokines.

Future Research Plans

- Studies on the signaling pathway involved in β-hematin-mediated immunomodulation.
- Studies on the molecular mechanism of hemozoin-induced cytokine production.

Srikanth Rapole

rsrikanth@nccs.res.in

Quantitative proteomics and metabolomics of breast cancer towards potential biomarkers for early diagnosis and prognosis

Background

Globally, breast cancer (BC) is the most prevalent and highly incident oncological disease among women and one of the leading causes of cancer related deaths in Asia. An estimated incidence of around 15 million breast cancer related cases and around 8 million breast cancer mortalities were reported globally for the year 2013. Even though decades of research have been performed towards the discovery of potential biomarkers, high number of new cases endures due to lack of early diagnosis and clinical screening methods. Breast cancer is characterized as heterogeneous in nature and has four intrinsic subtypes viz. luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) enriched, triple negative breast cancer. In the current cancer treatment practice, tumor histological factors and subtypes are mainly used to determine the therapy for the patient. The foremost screening test for breast cancer is mammography but it very often gives false negative results in different age groups, not able to detect breast cancer and most importantly fails to detect the tumor in young women as dense breast tissue decreases mammographic sensitivity. Hence, there is an urgent need to come up with potential biomarkers which can support the clinicians in early diagnosis as well as differentiating and identifying the breast cancer subtypes.

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, precise subtype identification, assessment of prognosis, prediction of therapeutic effect and treatment monitoring. In this work, we plan to identify potential biomarkers for breast

Participants

Akshada Gajbhiye, *SRF*
Tushar More, *SRF*
Venkatesh Chanukuppa, *SRF*
Khushman Taunk, *Project SRF*
Raju Dhabi, *Project JRF*
Ravindra Taware, *Research associate*
Osheen, *JRF*
Pankaj Birari, *JRF*
Mahendra Jamdhad, *Project SRF*

Collaborators

Dr. Manas Santra, *NCCS*
Dr. Sanjeeva Srivastava, *IIT Bombay*
Dr. Prasad Kulkarni, *ARI Pune*
Dr. Koel Chaudhury, *IIT Kharagpur*

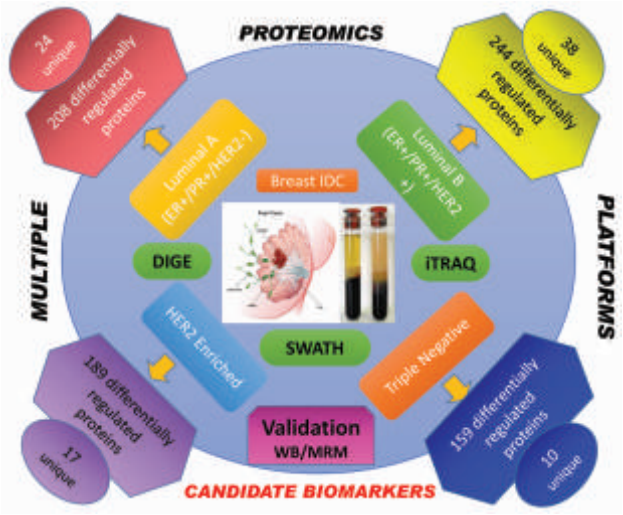


Fig. 1: A representative multipronged quantitative serum proteomics study design showing differentially regulated as well as unique proteins for intrinsic subtypes of breast cancer.

cancer using high throughput mass spectrometry based proteomic, metabolomic and lipidomic approaches in Indian scenario.

Aims and Objectives

- Investigation of serum proteome alterations in breast cancer intrinsic subtypes using multipronged quantitative proteomics.
- Identification of metabolomic alterations in breast cancer using targeted and untargeted metabolomic approaches.
- Elucidation of serum phospholipid alterations in breast cancer using comprehensive quantitative lipidomics approach

Work Achieved

Investigation of serum proteome alterations in breast cancer intrinsic subtypes using multipronged quantitative proteomics

Worldwide, breast cancer continues to be one of the leading causes of cancer related deaths in women. Breast cancer encompasses four major molecular subtypes. As breast cancer treatment majorly depends on the identification of specific subtype, it is important to diagnosis the disease at subtype level. We employed quantitative proteomic approaches viz. 2D-DIGE, iTRAQ and SWATH-MS and identified 307 differentially regulated proteins in which 24 were specific for Luminal A, 38 for Luminal B, 17 for HER2 enriched and 10 proteins were specific for TN subtype (Fig. 1). The differentially expressed subtype specific serum proteins were subjected to pathway analysis using multiple software which revealed the involvement in platelet degranulation, fibrinolysis, lipid metabolism, immune response, complement activation, blood coagulation, immune cell activation, glycolysis, amino acid biosynthesis and cancer signaling pathways in the subtypes of the breast cancer. The

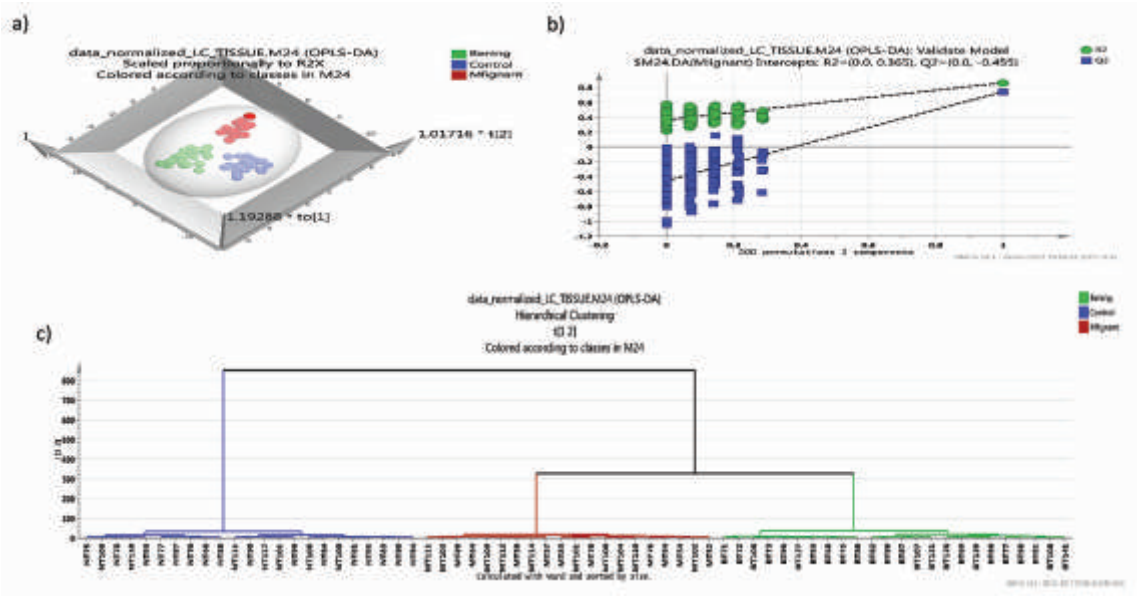


Fig. 2: Multivariate analysis of breast cancer tissue metabolomics LC-MRM/MS. a) OPLS-DA score plot of LC-MRM/MS data for breast cancer subjects (n = 24, red), benign breast patients (n = 24, green) and healthy controls (n = 24, blue), b) plot obtained after performing a random permutation test with 200 permutations on OPLS-DA model (R2Y = 0.86, Q2 = 0.77), R2 is the explained variance and Q2 is the predictive ability of the model. Low value of R2Y and Q2-intercepts at 0.36 and -0.45 depicts the high predictability of the model, c) hierarchical clustering analysis showing clustering of malignant (red), benign (green) and controls (blue).

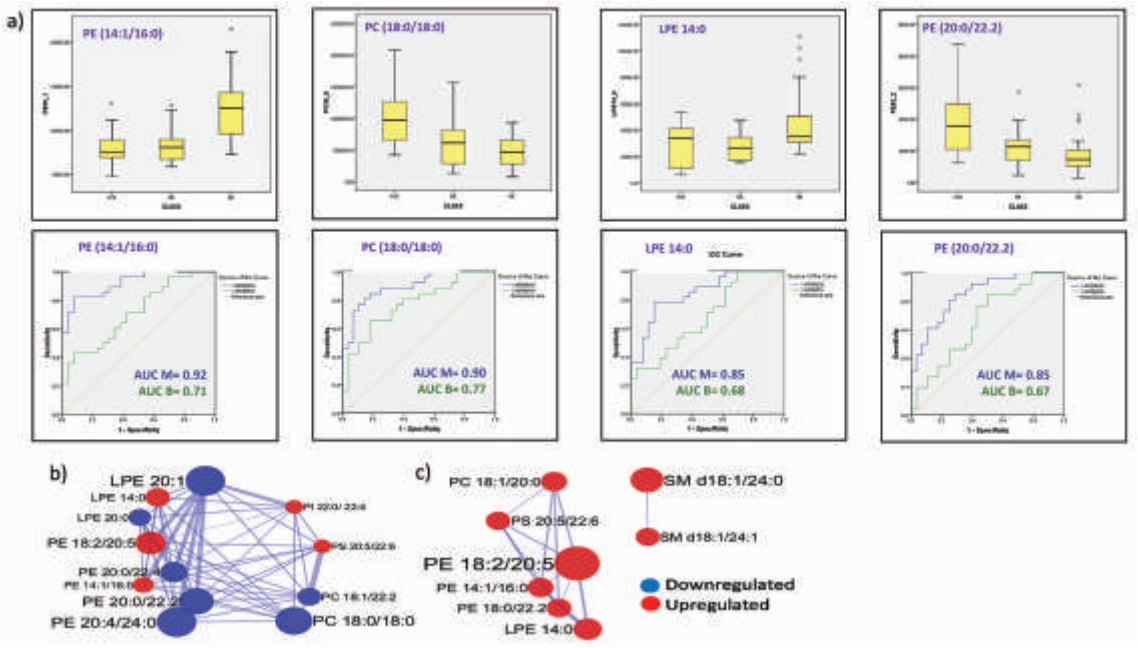


Fig. 3: a) Box-and-whisker plots and ROC curve analysis illustrating higher discriminative ability of phospholipids for malignant samples than benign and healthy controls, b) Network analysis of Malignant vs Healthy control, c) Network analysis of Malignant vs Benign using MetaMapp and Cytoscape.

significant discrimination efficiency of the models generated using multivariate statistical analysis was decent to distinguish each of the four subtypes from controls. Further, some of the statistically significant differentially regulated proteins were validated by western blot and multiple reaction monitoring (MRM) assays. This is the first comprehensive proteomic study on serum proteomic alterations revealed panel of serum proteins specifically altered for individual subtypes of breast cancer.

Identification of metabolomic alterations in breast cancer using targeted and untargeted metabolomic approaches

Although there is a lot of research done in the field of genomics and proteomics in breast cancer, metabolomics is least explored. In this study, we have performed a quantitative metabolomic analysis of normal, benign and malignant tissue and serum samples for the understanding of the molecular events involved in tumor development that are essential for early detection and diagnosis. A study population of 72 subjects comprising of 24 breast cancer, 24 benign and 24 healthy subjects for tissue metabolomic analysis and 142 subjects including 76 breast cancer, 33 benign and 33 healthy subjects for serum metabolomic analysis were recruited. Samples were analyzed using targeted approach by LC-MRM/MS and untargeted approach by GC-MS. Using univariate and multivariate statistical analysis, 42 significant metabolites in tissue samples and 32 significant metabolites in serum samples were identified in breast cancer when compared to control. Similarly, 37 significant metabolites in tissue samples and 35 significant metabolites in serum samples were identified in breast cancer when compared to benign. A decent correlation was observed with metabolomic changes in tissue with that of serum, indicating a bi-directional interaction of metabolites in breast cancer. A representative multivariate analysis of breast cancer tissue samples by LC-MRM/MS is shown in Fig. 2. A panel of three metabolites viz. Tryptophan, Tyrosine, and Creatine present in tissue as well serum indicating as a potential signature for the discrimination of the breast cancer from both control and benign subjects. The altered metabolites identified in breast cancer compared with control were involved in Nitrogen metabolism, Pyrimidine metabolism, Aminoacyl-tRNA biosynthesis, Fatty acid biosynthesis and D-Glutamine and D-glutamate metabolism. This study provides valuable insights into metabolomic alterations which can help to identify potential markers as well as novel therapeutic targets for breast cancer.

Elucidation of serum phospholipid alterations in breast cancer using comprehensive quantitative lipidomics approach

Although efforts are made by researchers in molecular characterization of breast cancer using "-OMIC'S" approaches,

limited work has explored to understand the phospholipid alterations in breast cancer. In this study, we intend to examine five classes of serum phospholipid alterations in breast cancer towards discrimination of breast cancer from benign and healthy controls. Twenty eight each of breast cancer patients and age-matched benign and healthy control serum samples were used to identify alterations of phospholipids using liquid chromatography mass spectrometry (LC-MS). Among the identified and quantified 200 phospholipids, 25 phospholipids were found to be statistically significant in the serum of women with breast cancer when compared with benign and healthy controls. Comparison of serum phospholipids of breast cancer patients and healthy controls revealed 12 phospholipids were found to be differentially expressed in which 6 were up-regulated and 6 were down-regulated. Further, differentially expressed phospholipids were structurally characterized by tandem mass spectrometry. This study manifest that some of the differentially regulated phospholipids identified in this study viz. PE (14:1/16:0), PC (18:0/18:0), LPE 14:0, PE (20:0/22:2) are a panel of potential signature which can discriminate breast cancer from benign and healthy controls. Box-and-whisker plots and ROC curve analysis depict higher discriminative ability of phospholipids for malignant samples than benign and healthy controls (Fig. 3a). Network analysis of Malignant against Healthy control and Malignant against Benign using MetaMapp and Cytoscape are shown in Figs. 3b and 3c. These findings also provide insight into lipidomic information that can be used not only for novel diagnostic solutions but also enhance the understanding of underlying molecular mechanism of breast cancer progression.

Future Research Plans

- Investigation of tissue proteome alterations in breast cancer intrinsic subtypes using label free quantitative approach.
- Investigation of phosphoproteome alterations in breast cancer using quantitative phosphoproteomics approach.
- Identification of urinary metabolites alteration in breast cancer towards early diagnosis.

Bhaskar Saha

sahab@nccs.res.in

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. Many natural compounds help boost the immune reaction against intracellular parasites. We have studied the effect of the natural plant derived compound Kalmegha against *Leishmania* infection by affecting the immune reaction against them.

Aims and Objectives

- The aim of our project was to examine the role of the natural compound Kalmegha in macrophage and T-cell response against *Leishmania* infection.

Work Achieved

We studied the anti-Leishmanial activity of Kalmegha by direct parasite load and cytokine ELISA. We used known anti-Leishmanial drug miltefosine (HPC) as a positive control. The role of the compound in T-cell mediated anti-Leishmanial immunotherapy is being examined in detail.

Participants

Ankita Srivastava, *SRF*
Mukesh Kumar Jha, *SRF*
Aditya Sarode, *SRF*
Divansu Shukla, *SRF*
Sagar Bhadange, *SRF*

Future Research Plans

- We will continue to explore the role of Kalmegha in boosting macrophage and T-cell response against *Leishmania* infection with the aim of using it for immunotherapy against the disease in future.

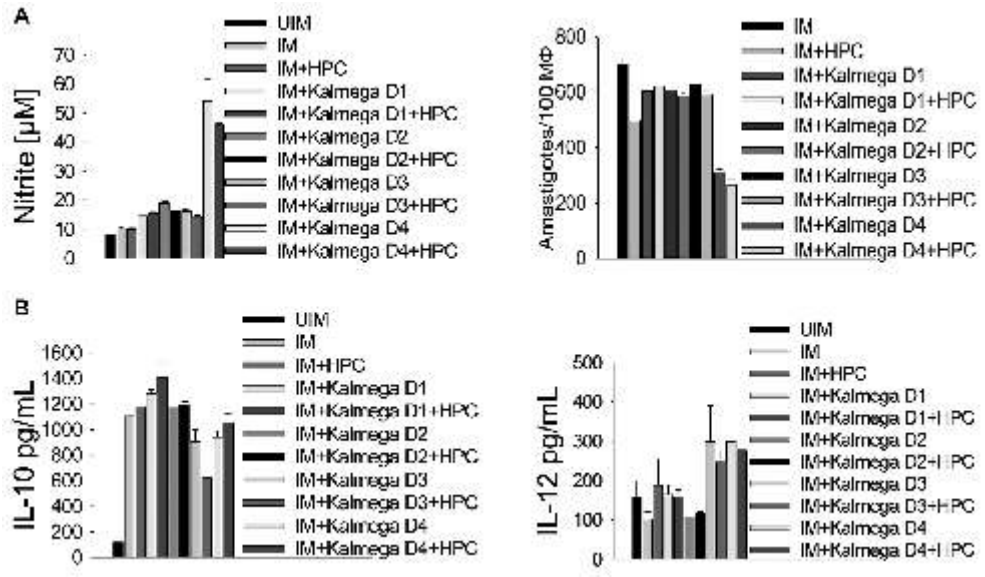


Fig. 1A: BALB/c peritoneal macrophages infected with *Leishmania donovani* (AG83) and treated with HPC and different dilution (D1-1:100, D2-1:50, D3-1:25, D4-1:12.5) of kalmega for 72h, and fixed for amastigotes count and nitric oxide production.
Fig. 1B: BALB/c peritoneal macrophages infected with *Leishmania donovani* (AG83) and treated with HPC and different dilution (D1-1:100, D2-1:50, D3-1:25, D4-1:12.5) of kalmega for 48h, and check the production of cytokine by ELISA method.

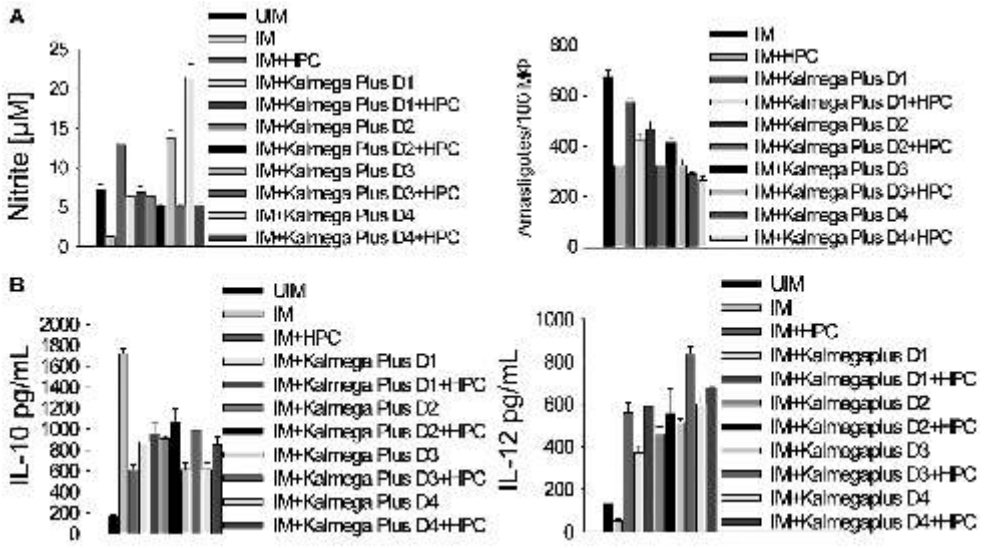


Fig. 2A: BALB/c peritoneal macrophages infected with *Leishmania donovani* (AG83) and treated with HPC and different dilution (D1-1:100, D2-1:50, D3-1:25, D4-1:12.5) of kalmega plus for 72h, and fixed for amastigotes count and nitric oxide production.
Fig. 2B: BALB/c peritoneal macrophages infected with *Leishmania donovani* (AG83) and treated with HPC and different dilution (D1-1:100, D2-1:50, D3-1:25, D4-1:12.5) of kalmega plus for 48h, and check the productionof cytokine by ELISA method.

(DST-INSPIRE Faculty -
Dr. Bhaskar Saha's research group)

Debasri Mukherjee

(Ph.D)

debasri@nccs.res.in
mukherjee.debasri@gmail.com

Project Title: Analysis of the CD40 signalosome complex built-up in macrophages

Background

Cellular response to any external stimulus is modulated by dose and duration of the stimulation. Perturbations to signaling pathways brought about by alterations to the receptor-ligand interactions at the cell membrane bring about distinct effector functions. In our lab we are studying dose duration encoding and its effect on the building up of a signalosome complex using CD40-CD40 ligand as a model receptor-ligand pair. We are also studying the effect of sunch encoding on the reciprocal effects of IL10 and IFNγ receptors.

Aims and Objectives

- To study dose-duration encoding on the built-up kinetics of CD40 signalosome kinase and reciprocal effects of IL10 and IFNγ receptors.

Work Achieved

Identification of signaling intermediates for CD40 signalosome built-up using LCQ-Orbitrap system is almost complete. Validation of the identified intermediates in in progress, while work on IL10-IFNγ reciprocity has been completed.

Future Research Plans

- To complete of identification and validation of intermediates and analyzing their function expression profile as a function of dose and time.

Collaborators

Dr. Debasish Bandyopadhyay, *Professor, Dept.of Physiology, University of Calcutta, Kolkata, India*

Dr. Bhaskar Saha, *Scientist G, Pathogenesis and Cellular Response, Lab 5, NCCS, Pune, India*

Arvind Sahu

arvindsahu@nccs.res.in

Understanding the molecular basis of viral complement regulation

Background

Being a major first line of immune defense, the complement system keeps a constant vigil against viruses. Its ability to recognize a large panoply of viruses and virus-infected cells, and trigger the effector pathways, results in neutralization of viruses and killing of the infected cells. This selection pressure exerted by the complement system on viruses has made them evolve a multitude of countermeasures. These include targeting the recognition molecules for avoidance of detection, targeting key enzymes and complexes of the complement pathways like C3 convertases and C5b-9 formation – either by encoding complement regulators or by recruiting membrane-bound and soluble host complement regulators, cleaving complement proteins by encoding proteases, and inhibiting the synthesis of complement proteins. Additionally, viruses also exploit the complement system for their own benefit. For example, they use complement receptors as well as membrane regulators for cellular entry as well as their spread. Our laboratory focuses on complement regulators encoded by viruses, particularly those encoded by pox and herpes viruses. Our current emphasis is on understanding the molecular basis of complement regulation by pox and herpesviral complement regulators and their role in viral pathogenesis.

Aims and Objectives

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

Participants

Jitendra Kumar, *SRF*
Avneesh Kumar Gautam, *SRF*
Ashish Kamble, *SRF*
Hemendra Singh Panwar, *SRF*
Hina Ojha, *SRF*
Arya Ghate, *SRF*
Renuka Nawadkar, *JRF*
Palak Agrawal, *JRF*
Rajashri Shende, *Project student*
Sweta Khobragade, *Project student*
Yogesh Panse, *Technical Officer A*

Collaborators

Payel Ghosh, *S. P. Pune University, Pune*
Shekhar Mande, *NCCS*
Girdhari Lal, *NCCS*

Work Achieved

Poxviruses are one of the most successful pathogens. It is believed that one of the key reasons for their success is their ability to subvert both innate as well as adaptive immune barriers of the host. The most notable member of this family is variola virus, the causative agent of smallpox, which killed hundreds of millions of people before its successful eradication in 1977 owing to mass vaccination by vaccinia virus under the aegis of World Health Organization. Intriguingly, though variola and vaccinia viruses are closely related, they differ in their host tropism – variola virus is a human-specific virus, while vaccinia virus causes repeated outbreaks in dairy cattle in India and Brazil.

Consistent with their species tropism, earlier we have shown that variola virus complement regulator SPICE exhibits selectivity in inhibiting the human alternative complement pathway and vaccinia virus complement regulator VCP display selectivity in inhibiting the bovine alternative complement pathway. While probing the role of the alternative pathway in neutralizing vaccinia virus, we observed that the virus is resistant to the alternative complement pathway-mediated neutralization, but susceptible to the classical complement pathway (CP)-mediated neutralization. We thus examined whether VCP exhibits specificity in regulating bovine CP and if yes how it subverts this pathway.

VCP displays selectivity in inhibition of bovine classical complement pathway

Examination of regulatory potential of VCP against the CP of various animal species showed that it is more potent against CP of calf, buffalo, goat and cat compared to the CP of human (Fig. 1). Intriguingly, this is consistent with species tropism of vaccinia virus. On the other hand, SPICE displays better effect against the CP of monkey and human compared to the CP of calf and buffalo. Consequently, the maximum relative difference in the inhibitory activity of VCP and SPICE against calf and buffalo CP was 43-fold and 45-fold, respectively; there was however little or no difference (< 2-fold) in the inhibitory activities of VCP and SPICE against monkey and human CP. These results clearly suggest that VCP exhibits selectivity in inhibiting the bovine CP compared to SPICE.

VCP and SPICE differ in only 11 amino acids. We thus next determined which of the variant residues of VCP contribute to its enhanced activity against the bovine CP. For this, we generated 11 single amino acid mutants of SPICE wherein each of the variant amino acids of VCP was substituted at the corresponding position in SPICE and examined their ability to inhibit the CP activity of calf sera. A significant increase in the inhibitory activity was observed in 5 of the 11 mutants namely K108E, K120E, N178D, N144E and T214K compared to SPICE. These results thus indicated that primarily acidic residues located in the CCP domains 2 and 3 are responsible for the selectivity of VCP towards bovine CP.

Since VCP is a member of the protein family termed 'regulators of complement activation (RCA)', it is expected to target C3-convertases. We thus next sought to dissect the mechanism by which VCP targets the bovine CP C3-convertase and identify the variant residues of VCP that contribute to its enhanced activity against the bovine CP. We observed that it inactivates

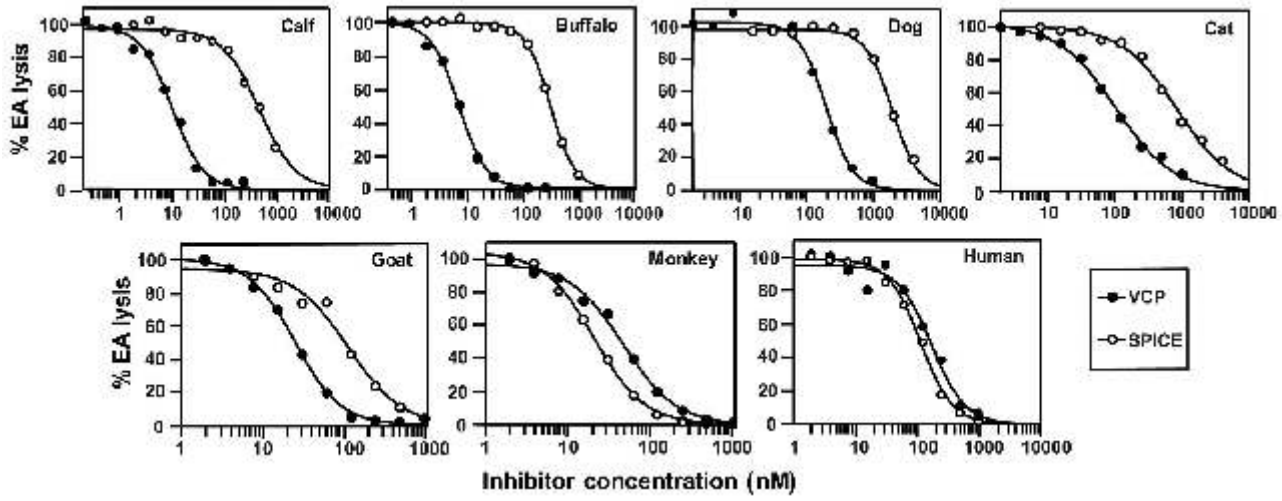


Fig. 1: Relative inhibition of the classical complement pathway of various species by VCP and SPICE. Inhibition of the classical complement pathway in non-primate and primate species by VCP (filled circle) and SPICE (open circle) was assessed by the hemolytic assay.

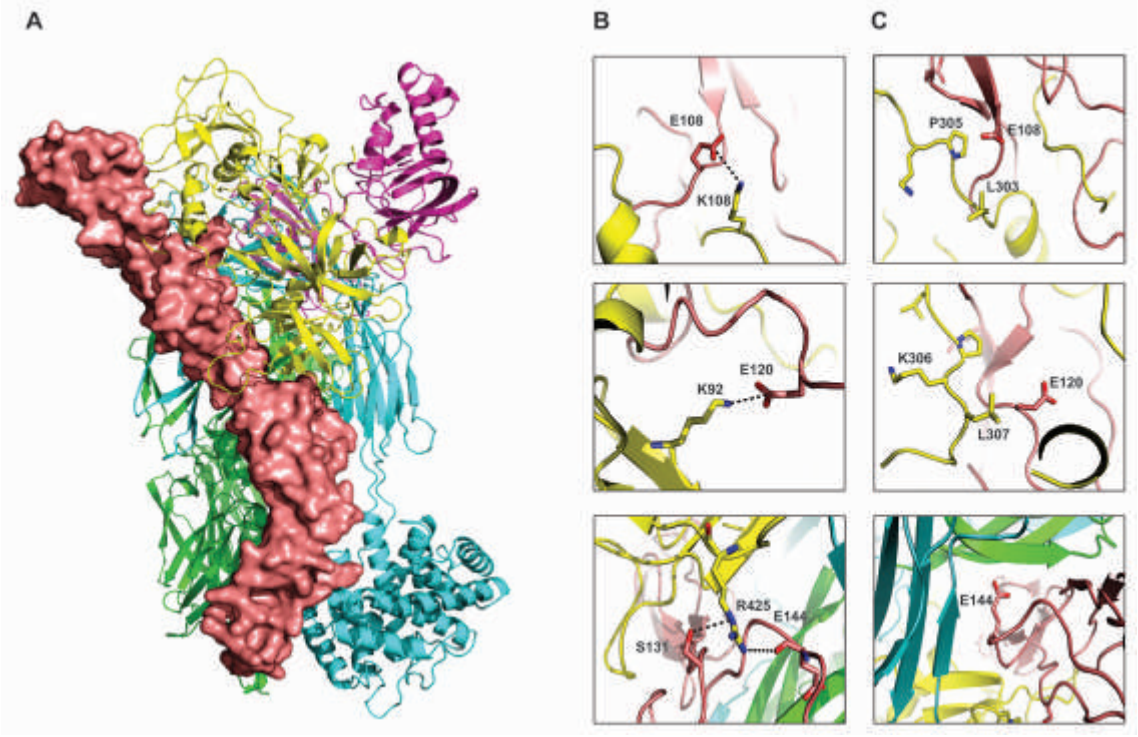


Fig. 2: Mapping of high propensity interactions of functionally important residues of VCP onto the modelled structure of C4b:VCP:factor I complex. (A) The model of the trimolecular complex of bovine C4b:VCP:bovine factor I. Individual chains of C4b have been coloured differently and depicted as cartoon. The colours green, cyan and pink represent the beta, alpha and gamma chains of bovine C4b. The model of bovine factor I is also shown as cartoon (yellow), while the model of VCP is shown in surface representation. (B and C) Potential interactions (present within 5Å radius) of the functionally important residues of VCP (in cartoon; salmon) with bovine and human factor I (in cartoon; yellow). The functionally important residues are shown as sticks and coloured by atom type in the theme of the original protein colour. The left panels (B) depict the interaction of VCP with bovine factor I, while the right panels (C) depict the interaction of VCP with human factor I.

the bovine CP C3-convertase by supporting the inactivation of one of its subunits C4b by protease factor I and five substitutions (H77Q, K108E, K120E, L131S, and N144E) contributed to this increase. These data yet again supported the premise that primarily acidic residues of VCP dictate its specificity towards the bovine CP.

Next, in order to identify the major determinants capable of directing the specificity of VCP towards the bovine CP, we generated and tested the inhibitory activities of multi-residue mutants of SPICE. Hence, we constructed double (K108E/K120E), triple (K108E/K120E/N144E & K108E/K120E/N178D), tetra (K108E/K120E/N144E/L131S) and penta (K108E/K120E/N144E/L131S/H77Q) substitution mutants and measured their ability to inactivate C4b. The major shift in activities was achieved by changing lysines to glutamates at position 108 and 120 (K108E/K120E) and further substitution of a glutamate at position 144 generated a mutant (K108E/K120E/N144E) capable of inhibiting the bovine CP equal to that of VCP. Together these data indicated that primarily the glutamates at positions 108, 120 and 144 skew the specificity of VCP towards the bovine CP.

Species selective inactivation of bovine C4b by VCP is primarily dictated by its interaction with factor I

Inactivation of bovine C4b by VCP requires docking of the protease factor I onto the C4b-VCP complex, which then cleaves C4b into its inactive form. Thus, specificity of VCP for bovine C4b can be influenced by its interaction with bovine C4b, bovine factor I or both. Measurement of binding of the double (K108E/K120E) and triple mutant (K108E/K120E/N144E) to bovine C4b using surface plasmon resonance (SPR) assay showed that if anything, binding of these mutants is less than that of SPICE suggesting E108, E120 and E144 do not interact with C4b and therefore are likely to interact with factor I.

To resolve whether these residues of VCP indeed interact with bovine factor I, we performed C4b inactivation assay using

bovine C4b in combination with bovine or human factor I. Incubation of bovine C4b and bovine factor I with VCP, SPICE or the mutants demonstrated that VCP is highly efficient in supporting the inactivation of C4b compared to SPICE and that substitution of three glutamates at positions 108, 120 and 144 are responsible for this increased specificity of VCP. Importantly, replacement of bovine factor I with human factor I in the above assay resulted in drastic abrogation of C4b inactivation ability of VCP as well as the mutants. Specifically, the activity of VCP as well as that of the three mutants decreased more than 40-folds. Thus, it can be envisaged that it is the interaction of VCP with factor I that determines species selective inactivation of C4b by VCP.

Next, to get a detailed view of the interactions of these residues with bovine factor I, and explain why human factor I does not interact with VCP, we generated models of the bovine C4b:VCP:bovine factor I and bovine C4b:VCP:human factor I complexes. Our models indicated that specific interactions are responsible for the experimentally observed behaviours. The negatively charged glutamates at positions 108, 120 and 144 in VCP were found to directly interact with the positively charged residues of bovine factor I, but such interactions were absent in human factor I: i) E108 of VCP was found to make ionic interaction with K108 of bovine factor I, but such interaction was missing with human factor I, ii) E120 of VCP showed ionic interaction with K92 of bovine factor I. At a similar position, human factor I also had K306 albeit with its side chain pointing in the opposite direction. Another residue seen in the vicinity of E120 was L307 which again does not seem to be involved in any favourable interactions with VCP, and iii) E144 of VCP showed direct interaction with the oppositely charged R425 of bovine factor I. In addition, R425 of bovine factor I also showed weak interactions with S131 of VCP. In human factor I, however no such interactions were observed (Fig. 2).

In summary, our results indicate that VCP's specificity towards the bovine CP is owing to its enhanced C4b inactivation ability, which is shaped by the presence of three acidic residues of domains 2 and 3 that make direct interaction with bovine factor I. Importantly, these acidic residues are conserved in VCP-like molecules of various poxviruses that infect domestic animals. We therefore suggest that poxviral complement regulators are one of the mediators of poxvirus tropism. These results also add to our knowledge on how poxviruses overcome the host complement system to induce a state of viral pathogenesis.

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 locally produced C3a, C4a and C5a during viral infections.

Manas Kumar Santra

manas@nccs.res.in

Ubiquitin Ligase/microRNA feedback loop regulates Slug-mediated invasion in breast cancer

Participants

Dr. Sweta Mishra, *Postdoctoral Fellow*
Debasish Paul, *SRF*
Neha Gupta, *SRF*
Parul Dutta, *SRF*
Rajesh Kumar Manne, *SRF*
Sachin Meshram, *SRF*
Sehbanul Islam, *SRF*
Srinadh Choppara, *SRF*
Yashika Agrawal, *SRF*
Ganesh Kumar Barik, *JRF*
Ganga Shankaran, *Project Asistant*

Collaborators

Dr. Debasis Manna, *IIT Guwahati, Assam*
Dr. Shantau Pal, *IIT Bhubaneswar, Bhubaneswar*
Dr. Srikanth Mahapatra, *IIT Bhubaneswar, Bhubaneswar*
Dr. Madhulika Dixit, *IIT Chennai, Chennai*
Prof. Michael R Green, *Umass Medical School, MA, USA*
Dr. Samit Chattopadhyay, *NCCS*
Dr. Gopal Kundu, *NCCS*
Dr. Debashis Mitra, *NCCS*
Dr. Srikanth Rapole, NCCS

Background

Breast cancer is one of the leading causes of death worldwide and is the second most common cancer in women. Over 1.3 million cases of invasive breast cancer are diagnosed worldwide and more than 450,000 women die of this disease each year. While significant progress has been made in understanding the pathology of breast cancer, to further improve therapy, it is important to identify new therapeutic targets to minimize undesired side effects. Understanding the molecular players involved in the regulation of breast cancer progression and metastasis is the key to developing improved treatment strategies. The ubiquitin-proteasome machinery is involved in many diseases, including cancer. Inhibitors and activators of E3 ubiquitin ligases are therefore promising targets for therapy, since they dictate the proteins to be ubiquitylated and the manner of their ubiquitylation. Therefore, an in-depth understanding of this class of genes is important.

FBXO31 is a member of the F-box protein family and a component of the SCF E3 ubiquitin ligase. It plays an important role in cell cycle progression, DNA damage response, tumorigenesis, and neuronal development. It targets multiple cellular substrates, such as cyclin D1, MDM2, p38, Ctd1, FOXM1 and Par6c, and promotes their polyubiquitylation-mediated proteasomal degradation. More recently, it has been shown that FBXO31 functions as a dedicated DNA damage checkpoint protein by arresting cells at the G1 phase of the cell cycle through proteasomal degradation of cyclin D1, and by preventing MDM2-mediated proteasomal degradation of p53.

Cellular proteins are maintained at basal levels either through transcriptional, post-transcriptional or post-translational regulation. Post-transcriptional

regulation has emerged as one of the major players in malignancy. Small non-coding RNAs, microRNAs (miRNAs), have emerged as crucial gene regulators at the post-transcriptional level and the levels of their expression are frequently altered in cancer and other diseases. miRNAs are transcribed as a primary miRNA (pri-miRNA) by RNA polymerase II or III. This pri-miRNA is processed by Drosha and Pasha to generate a precursor miRNA (pre-miRNA), which is further processed by Dicer to form the mature miRNA in the cytoplasm. The mature miRNA then associates with the RISC complex to function as a post-transcriptional or translational regulator. It is estimated that more than 60% of human protein-coding genes are regulated by miRNAs.

Given the predominant role of FBXO31 in growth arrest and DNA damage checkpoint activation, it becomes important to understand its comprehensive regulation. In this study, we identified miR-93 and miR-106a as negative regulators of FBXO31 during normal cellular growth but they fail to play this role under genotoxic stress. Further, we showed that these miRNAs promote scratch wound healing and cellular invasion through stabilization of Slug, which in turn is proteasomally degraded by FBXO31. Most interestingly, we unraveled that Slug directly drives the expression of miR-93 and miR-106a by binding to their promoter. Our study thus expands the understanding of the molecular mechanisms involved in the onset and progression of cancer, by highlighting for the first time the presence of a feedback loop involving miR-93, miR-106a, FBXO31 and Slug.

Aims and Objectives

- To determine how FBXO31 is maintained at low levels in unstressed cells.
- To study the molecular mechanism of regulation of FBXO31 expression.

Work Achieved

How is FBXO31 maintained at low levels in unstressed cells?

The expression level of any cellular protein is maintained either at the transcriptional, or translational, or post-translational level. FBXO31 levels are reported to oscilate during cell cycle progression, being maximum at the G2 phase of the cell cycle. However, the mechanism behind these variations in expression levels of FBXO31 at different phases is not well understood. We observed that FBXO31 is not regulated at the transcriptional level in different phases of cell cycle. Instead, we found that it is regulated at the proteasomal level.

We then checked the possibility of the involvement of miRNAs in the regulation of FBXO31 expression. Our in silico analysis predicted that the the 3'UTR of FBXO31 has putative binding sites for 6 potential microRNAs. These microRNAs have two similar putative binding sites on the 3'UTR of FBXO31 which are conserved among mammals, a 7-mer-m8 seed sequence AGCACTTTA (75–81 bp from the start of 3'UTR), and an 8-mer seed sequence ATGCACTTTG (145–152 bp from the start of 3'UTR).

MiR-93 and miR-106a maintain the expression levels of FBXO31

We evaluated the effect of the in silico-predicted miRNAs on the expression of FBXO31 using a luciferase reporter assay. The activity of the luciferase reporter containing the FBXO31 3'UTR was suppressed to different extents in the presence of miRNAs -20b, -93, -106a and -106b (Figure 1A). Immunoblotting data further revealed that these miRNAs also suppressed the levels of FBXO31 protein (Figure 1B). Among these miRNAs, miR-93 and miR-106a showed potent effects on FBXO31 expression without affecting mRNA levels (Figure 1B). Based on above results, we selected miR-93 and miR-106a to understand the molecular mechanism of FBXO31 regulation.

We then generated mutants by altering the seed sequence of miR-93 and miR-106a in the 3' UTR of FBXO31 luciferase reporter, to confirm the specificity of miR-93 and miR-106a-mediated regulation of FBXO31. The results revealed that the luciferase activity was significantly increased upon mutation of either of the seed sequences (Figure 1C). Analysis of the results demonstrated that miR-106a requires both seed matches, while miR-93 preferentially targets the first seed sequence for regulating FBXO31.

What is the molecular mechanism of regulation of FBXO31 expression?

MicroRNAs-93 and 106a fail to regulate FBXO31 in response to genotoxic stress

Previous studies showed that FBXO31 is stabilized under various genotoxic stresses, suggesting that regulation of FBXO31 may be different under these conditions. Hence, the involvement of miR-93 and miR-106a in the regulation of FBXO31 under genotoxic stress was examined. In agreement with the previous report, luciferase activity of FBXO31 3'UTR as well as endogenous FBXO31 levels were significantly increased upon exposure to ionizing radiation (IR) (Figure 2A). Interestingly,

ectopic expression of miR-93 and miR-106a did not affect the stabilization of FBXO31 in response to IR (Figure 2B and 2C) and etoposide treatment (Figure 2D and 2E). This effect was further evident from our cell cycle data wherein overexpression of these miRNAs could not prevent the FBXO31-mediated G1 arrest following genotoxic stress. These results collectively suggest that miRNAs could not suppress FBXO31 expression either due to their inability to bind the FBXO31 3'UTR or due to an alteration of their expression levels. Interestingly, the levels of miR-93 and miR-106a were found to be significantly decreased upon IR (Figure 2F) and etoposide treatment (Figure 2G). Further, RNA immunoprecipitation data demonstrated that these miRNAs are incapable of binding to the 3'UTR of FBXO31 under genotoxic stress. Collectively, these results suggest that while miR-93 and miR-106a repress FBXO31 in unstressed cells, they were unable to do so under genotoxic stress due to their reduced expression as well as their reduced binding at the 3'UTR of FBXO31.

FBXO31 suppresses cell invasion and migration by downregulating the EMT regulator, Slug

Owing to its growth suppressive effect, expression of FBXO31 is maintained at low levels under unstress conditions. Examination of the levels of miRNAs in different breast cancer cell lines revealed an increase in the levels of both miR-93 and miR-106a in most of the cell lines, indicating that miRNAs suppress FBXO31 expression in cancer. We further found that ectopic expression of miR-93 and miR-106a led to a significant increase in the number of colonies formed in the long term survival assay, which may be partly due to inactivation of FBXO31 (Figure 3A). This is in agreement with previous reports showing the oncogenic function of miR-93 and miR-106a. Further, most of the oncogenes promote cellular transformation through the activation of EMT. We therefore tested the effect of miR-93 and miR-106a on scratch wound healing and invasion. Our results demonstrated that ectopic expression of miR-93 and miR-106a promotes scratch wound healing as well as invasion, suggesting that these miRNAs may regulate EMT promoters (Figure 3B, 3C). Slug is among the EMT promoters with a well-established role in the enhancement of wound healing. We therefore checked whether these miRNAs have any role in Slug stability and found that mRNA as well as protein levels of Slug were significantly increased upon ectopic expression of miR-93 and 106a, with concomitant suppression of E-cadherin, indicating that stabilization of Slug is responsible for miR-93 and miR-106a-mediated EMT (Figure 3D and 3E). Further, ectopic expression of FBXO31 suppressed the miR-93 and miR-106- mediated scratch wound healing and invasion, suggesting that FBXO31 may affect the expression level of Slug (Figure 3B and 3C).

Collectively, our results suggest that there could be a cross talk between miRNAs, FBXO31 and Slug. We then checked the effect of FBXO31 on Slug and found that overexpression of FBXO31 significantly decreased the levels of Slug in a dose-dependent manner and had minimal effect on other EMT regulators, Twist and Snail. FBXO31-mediated decrease of Slug is further evident by the scratch wound healing and invasion assay, wherein ectopic expression of FBXO31 inhibited Slug-mediated invasion and wound healing (Figure 3B and 3C). Collectively, these results suggest that miR-93 and miR-106a promote cell survival, and accelerate wound healing as well as invasion by stabilization of Slug through the suppression of FBXO31.

FBXO31 promotes the ubiquitination and proteasomal degradation of Slug

The above results suggest that FBXO31 antagonizes the function of Slug presumably by negatively regulating its levels. Since FBXO31 is part of an E3 ubiquitin ligase, we sought to ask whether it is directly involved in the proteasomal degradation of Slug. FBXO31 failed to cause degradation of Slug in the presence of proteasome inhibitor, MG132, suggesting that FBXO31 regulates Slug in a proteasome-dependent manner. Further, we found that ectopic expression of a mutant form of FBXO31 lacking the F-box motif (ΔF-FBXO31) failed to downregulate Slug, indicating that FBXO31 regulates Slug through the SCF E3 ubiquitin ligase complex. Furthermore, co-immunoprecipitation experiments indicated that FBXO31 physically interacts with Slug, and facilitates its polyubiquitination.

We then sought to determine whether FBXO31 regulates Slug at the physiological level and found that knockdown of FBXO31 resulted in a significant stabilization of Slug (Figure 3F). In addition, cycloheximide pulse chase studies also revealed an enhanced stability of Slug in FBXO31 knockdown cells. Knockdown of FBXO31 also resulted in decreased levels of polyubiquitylated Slug. Collectively, our results demonstrated that FBXO31 maintains the cellular levels of Slug (Figure 3G).

Our initial observations have indicated that miR-93 and miR-106a are suppressed and FBXO31 is stabilized upon exposure to genotoxic stress. Hence, we further sought to understand the expression levels of Slug under genotoxic stress. Interestingly,

we found that Slug is decreased under genotoxic stress conditions. To check whether FBXO31 has any role in Slug regulation under genotoxic stress, FBXO31 knockdown and control cells were exposed to genotoxic agents like Etoposide and IR treatment. We observed that FBXO31 destabilizes Slug under genotoxic stress.

Reciprocal expression pattern of FBXO31 and Slug in breast cancer progression

Our in vitro data suggested that FBXO31 negatively regulates Slug, prompting us to check their levels in different breast cancer cell lines as well as patient samples. Our results revealed the existence of an inverse correlation between Slug and FBXO31 levels in most of the breast cancer cell lines. To confirm this converse relationship between FBXO31 and Slug, samples from breast cancer patients (patient tissue with matched normal breast tissue) were immunohistochemically stained. Our data indicate that FBXO31 is expressed in all non-cancerous tissues (Figure 3H). However, as cancer progressed from grade I to grade III, FBXO31 staining intensity decreased and high grade breast cancer tissue samples exhibited negative staining. On the other hand, Slug expression was low/null in normal tissue and was significantly increased in grade I-III in infiltrating ductal carcinoma. The expression pattern of Slug significantly correlated with tumor size, stage of the cancer and the tumor grade as shown in the figure (Figure 3H).

FBXO31 regulates a feedback loop wherein Slug drives miR-93 and miR106a expression

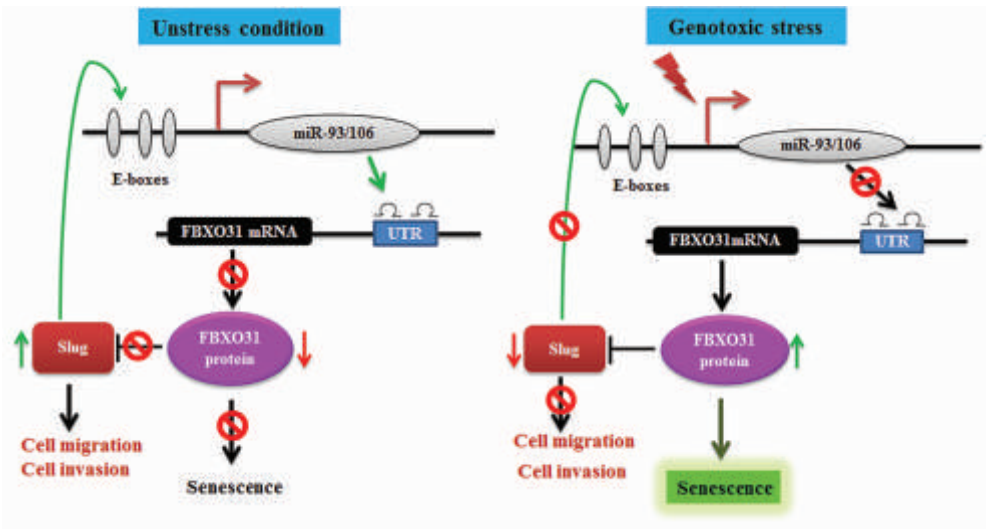
Previous reports have demonstrated that Slug regulates microRNAs at the transcriptional level by binding to the E-box motif in the promoter region. Our results also hint at the existence of a cross-talk between miRNAs, FBXO31 and Slug. Therefore, we went on to check if Slug regulates miR-93 and miR-106a as well, and found that it regulates these miRNAs at the transcriptional level. We further checked the effect of FBXO31 on the levels of miRNAs, since it degrades Slug. Interestingly, levels of both the miRNAs were decreased upon ectopic expression of FBXO31 and their levels were elevated upon depletion of FBXO31.

Since FBXO31 is a known inducer of senescence, we investigated the effect of miR-93 and miR-106a on FBXO31-mediated senescence. Senescence associated β-galactosidase staining revealed that overexpression of miR-93 and miR-106a inhibits senescence that is induced by FBXO31. Collectively, our studis demonstrate that miR-93 and miR-106a act as oncogenes by interfering with the tumor suppressive function of FBXO31, and provide evidence for the existence of a feedback mechanism between FBXO31, Slug, miR-93 and miR-106a.

Future Research Plans

In the coming year we would like to investigate whether FBXO31 plays any role in other phases of the cell cycle, as well as in signaling pathways associated with cancer. We would also like to determine how FBXO31 is regulated at different phases of the cell cycle.

Summary



Vasudevan Seshadri

seshadriv@nccs.res.in

Insulin translation regulation by 5'UTR binding protein: pancreatic α-amylase

Background

Insulin regulates glucose homeostasis in mammals and its biosynthesis is regulated by glucose. In the initial phase of glucose stimulation, insulin biosynthesis is regulated mainly at the translational level. Untranslated (UTR) region of the insulin mRNA is crucial for insulin translation regulation, where 5' and 3'UTR synergistically work to enhance insulin biosynthesis. We previously identified PDI and PABP as the proteins that associate with insulin 5'UTR and play an important role in insulin translation activation. We now report that pancreatic α-amylase can also bind to insulin 5'UTR and results in reduced insulin biosynthesis. We identified α-amylase in the biotin 5'UTR RNA pull down from total pancreatic tissue extract of rat by mass spectrometry. The interaction was confirmed by competitive RNA mobility shift assay and yeast 3 hybrid assay with insulin 5'UTR RNA and amylase protein. We showed that purified recombinant amylase protein can associate with insulin 5'UTR specifically. Luciferase reporter assay with insulin 5'UTR suggests that amylase association to the insulin 5'UTR leads to reduced translation. The physiological relevance of these observations was not clear as pancreatic α-amylase primarily is expressed in acinar cells (exocrine) of the pancreas, whereas insulin is produced by the beta cells. However we show that treatment of mouse insulinoma cells with TNF-α/IFN-Δ, led to the production of amylase in these cells along with the decreased production of insulin. These findings suggest an intricate regulation of insulin production during certain pathological conditions like pancreatitis.

Aims and Objectives

- To study the role of alfa amylase in insulin regulation and diabetes.
- To study the role of cytokine induced amylase and its role in auto immune pancreatitis.

Participants

Rucha Sarwade, *SRF*
Naina Gaikwad, *JRF*
Vishal Dandewad, *SRF*
Pranita Borkar, *SRF*
Jatin Behari, *JRF*
Shehnaz Bano, *JRF*
Shilpa Kamble, *Project Assistant*
Sarika Srivastav, *DST-SERB NPDF*
Dileep Moundekar, *Technician*

Work Achieved

It has been previously reported that specific cytoplasmic *trans acting* factors interact to insulin 5'UTR RNA to regulate insulin translation. One of the trans acting protein was identified as PDI (Protein disulfide isomerase) which positively regulate insulin translation in glucose dependent manner whereas Hud was shown to down regulate insulin translation during glucose starvation. However, the presence of multiple insulin 5'UTR binding complex and high total molecular weight of insulin 5'UTR binding complex suggested the presence of other factors which can bind to insulin 5'UTR and regulate insulin translation. In order to further characterize the insulin 5'UTR binding proteins, we performed biotin affinity pull down assay with rat total cytoplasmic pancreatic tissue extract and biotin labelled insulin 5'UTR RNA of rat insulin gene1 followed by mass spectrometry of eluted proteins. For negative control biotin labeled deletion mutant of insulin 5'UTR RNA was incubated with total pancreatic extract. Pancreatic alfa-amylase was identified one of the insulin 5'UTR binding protein.

Pancreatic α-amylase is present in Insulin 5'UTR binding complex

Pancreatic alfa amylase is a digestive enzyme which catalyzes the starch into disaccharides since we have found this protein in insulin 5'UTR binding complex, we wanted to confirm the interaction of pancreatic alfa amylase to insulin 5'UTR RNA. We performed RNA-EMSA where radiolabelled rat insulin 5'UTR RNA was incubated with glucose treated total pancreatic

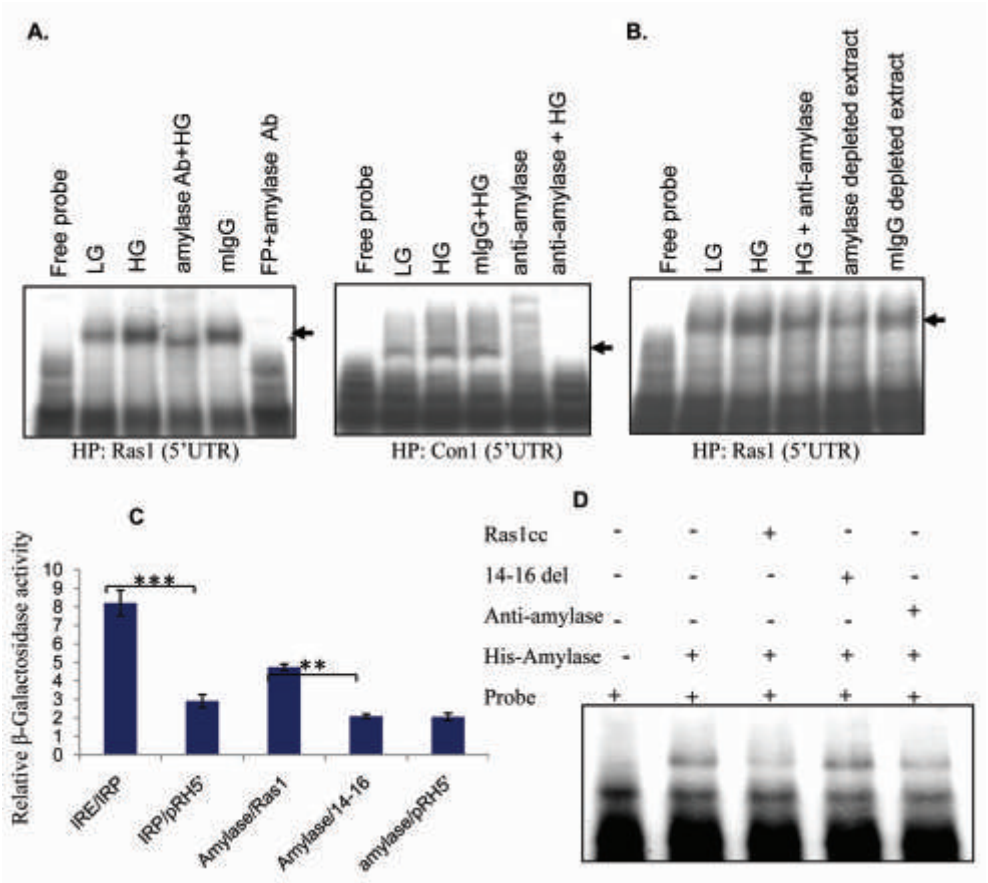


Fig.1: Presence of pancreatic α-amylase in insulin 5'UTR binding complex. (A) Supershift RNA mobility shift assay was performed with radiolabelled (αP^{32}) full length insulin 5'UTR (Ras1) and minimal element of insulin 5'UTR probe (Con1). Glucose treated cytoplasmic extract of rat pancreatic tissue was incubated with radiolabelled probe in presence or absence of amylase antibody or control IgG antibody. (B) RNA EMSA with amylase depleted pancreatic tissue extract and insulin 5'UTR. For control depletion IgG antibody was used.. (C) Yeast 3 hybrid assay, where all pairs of α-amylase and insulin 5'UTR were co-transformed into L40 ura-strain of saccharomyces cerevisiae (yeast) and relative β-galactosidase (reporter enzyme) activity was measured. IRE-IRP interaction was used as a positive control. (D) Competitive RNA-EMSA was performed with full length insulin 5'UTR probe and recombinant amylase protein in presence or absence of cold competitor (Unlabeled insulin 5'UTR) and amylase antibody. 14-16, 41-43 deletion mutant of insulin 5'UTR cold competitor was used as a non specific binding competitor. (E) Competitive RNA-EMSA with insulin 5'UTR and recombinant amylase in presence or absence of self and non self cold competitor.

cytoplasmic tissue extract and anti-amylase antibody. Presence of amylase antibody inhibited the insulin RNA-protein complex formation whereas addition of the control antibody (mouse IgG) had no significant effect on the complex formation. The RNA-protein complex mobility was super shifted when shorter fragment of insulin 5'UTR (Con1) was used in the presence of amylase antibody (Fig 1A). Furthermore, Immunodepletion of pancreatic alfa amylase from total pancreatic cytoplasmic tissue extract using amylase antibody followed by gel shift assay with amylase depleted extract showed reduced RNA-protein complex formation whereas there was no significant change with control antibody depleted extract (Fig 1B). These results suggest that pancreatic α-amylase is present in the insulin 5UTR binding complex.

Pancreatic α-amylase can associate with 5'UTR of insulin mRNA

Protein sequence analysis of pancreatic α-amylase suggest that there is not well defined RNA binding motif present in the sequence which led us to check direct RNA binding activity of

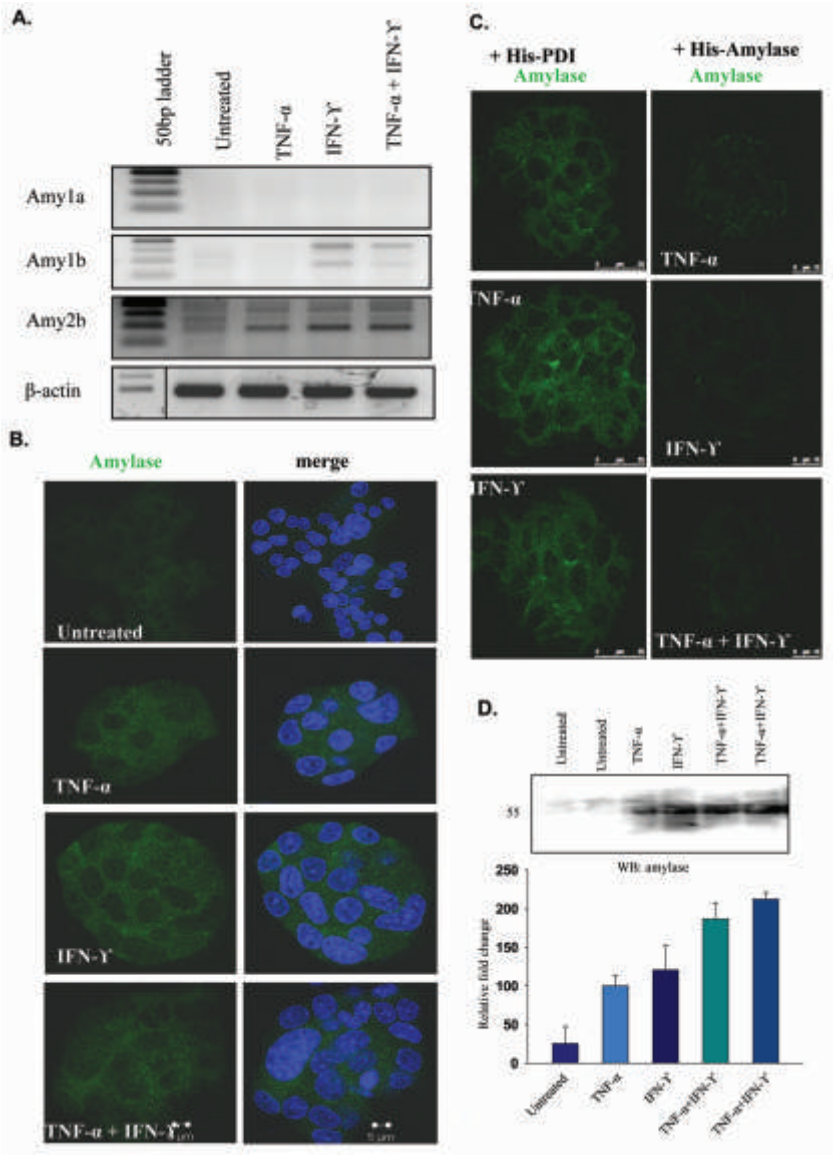


Fig.2: Pancreatic alfa amylase expression induces in mouse insulinoma cells (Min6) upon TNF-α and IFN-Ɣ stimulation. (A) Min6 cells was treated with 50ng TNF-α and IFN-Δ for 10hrs, total RNA was isolated and amylase expression was analyzed by RT-PCR using UTR specific primers. (B) Immunostaining of TNF-α and IFN-Δ treated Min6 cells using amylase antibody followed by alexa-488 conjugated secondary antibody. (C) Immunostaining of TNF-α and IFN-Δ treated Min6 cells in presence or absence of recombinant amylase protein with amylase antibody. For negative control recombinant PDI (protein disulfide isomerase) protein was added with amylase antibody. Images were captured under laser scanning confocal microscope (TCS SP5; Leica). (D) Min6 cells were treated with TNF-α and IFN-Δ cytokines, total extract was made and alfa amylase expression was analysed by western blotting using antibody against pancreatic amylase. Untreated cells were used as a control. Band intensity was quantitated by ImageJ software and relative intensity graph was plotted using Sigma Plot.

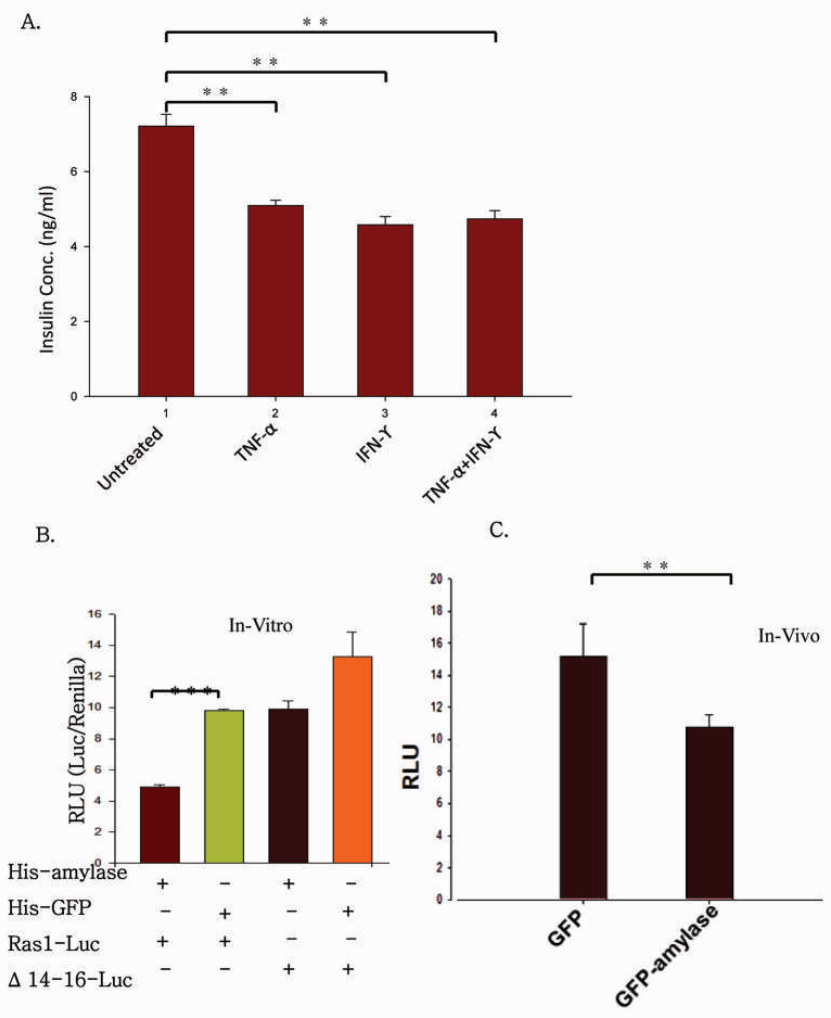


Fig.3: Pancreatic α-amylase is negatively regulates insulin translation. (A) Insulin ELISA to check total insulin secretion in culture supernant of TNF-α and IFN-Δ cytokine treated Min6 cells. (B) In vitro translation assay was performed with recombinant pancreatic alfa amylase and in-vitro transcribed insulin 5'UTR RNA in presence of RRL (rabbit retic lysate). GFP recombinant protein used as a control. To check the specificity of translation 14-16, 41-43 deletion mutant of insulin 5'UTR RNA was used as a negative control. For general translation control renilla luciferase RNA was used. Relative luciferase activity was calculated and graph was plotted. (C) In vivo translation assay where insulin 5'UTR luciferase reporter plasmid constructs was co-transfected along with either pancreatic alfa amylase or GFP construct in HEK cells. For internal translation control renilla luciferase was used. Relative luciferase was calculated using dual luciferase assay kit (Promega).

pancreatic α-amylase in insulin 5'UTR binding complex. Thus, we performed yeast 3 hybrid assay, where RNA-protein interaction was measured by LacZ reporter gene expression level. RNA (Insulin 5'UTR) and amylase protein plasmid construct (pRH5'UTR and pYEASTrp2amylase) were generated and co transformed into L40 ura- yeast strain, and reporter gene LacZ (β-galactosidase) expression was assessed. We found increased relative β-galactosidase activity in case of amylase and Insulin 5'UTR compared to control transformation, suggesting specific interaction of amylase with 5'UTR of insulin mRNA (Fig.1C). In addition, insulin 5'UTR interaction with bacterially expressed and affinity purified recombinant alfa amylase was also analyzed by RNA-mobility shift assay where a specific RNA-protein complex was observed. Addition of 70-fold molar excess of unlabeled insulin 5'UTR (Self cold competitor) resulted in complete inhibition of RNA-protein complex formation whereas addition of non specific RNA (Min RNA) or 14-16 deletion mutant of insulin 5'UTR RNA had no significant effect on RNA-protein complex formation (Fig 1D and Fig. 1E respectively). In summary, these data suggest that pancreatic α-amylase specifically interacts with insulin 5'UTR in a sequence specific manner.

Th1 cytokines (TNF-α or IFN-Y) induce amylase expression in mouse insulinoma cell lines

Pancreatic α-amylase is exclusively expresses in acinar cells (exocrine) of the pancreas, secreted to the small intestinal to stomach and function as a hydrolase enzyme to breakdown the

starch into disaccharides. However, during acute or chronic pancreatitis condition Th1 cytokines levels increased from T cells and macrophages in exocrine cells which infiltrate to endocrine cells resulting in β cell dysfunction and diabetes. We hypothesized that high level of Th1 cytokines may induce the amylase expression in β cells leading to reduced insulin production. In order to test this Min6 cells were treated with TNF-α and/or IFN-Δ cytokines and alfa amylase expression was analyzed at RNA level by RT PCR using UTR specific primers as amylase is present in various isoform in the mammalian cells differing in UTR sequence. Pancreatic alfa amylase 2b was shown to express specifically at RNA level in MIN6 cells upon high concentration of TNF-α and IFN-Δ stimulation (Fig 2A). In order to assess amylase expression, immunostaining of MIN6 cells was performed using amylase specific antibody (Fig 2B). Specificity of amylase staining was showed by specific inhibition of signal by recombinant amylase while addition of non specific protein (PDI) during immunostaining had no effect (Fig 2C). Furthermore, amylase expression was analyzed in TNF-α or IFN-Δ treated Min6 cells extract by western blotting. Specific amylase expression was observed that increases when cells was treated with both cytokines (Fig 2D). All together these data suggest that increased level of TNF-α or IFN-Δ cytokines activates α-amylase gene expression both at RNA and protein levels.

Pancreatic α-amylase reduce insulin secretion by suppressing insulin translation

As per previous experiment increased TNF-α and IFN-Ɣ cytokine levels, induces amylase expression in Min6 cells. To test whether this amylase expression affects the insulin secretion or translation, we first performed insulin ELISA with culture supernatant of TNF-α / IFN-Δ cytokine treated MIN6 cells. We observed decreased insulin levels in TNF-α and IFN-Δ treated cells compared to untreated (Fig 3A). In the previous experiments we have shown that α-amylase specifically interacts with insulin 5'UTR, so it's very likely that insulin biosynthesis may be regulated at translational levels in β cells during increased level of TNF-α and IFN-Δ. Here we assesses the role of alfa amylase in insulin translation regulation, in-vitro translation assay was performed where firefly luciferase reporters containing either the insulin 5'UTR or the 14-16 deletion mutant of insulin 5'UTR was in vitro transcribed and incubated with either recombinant His-amylase or control His-GFP protein in presence of rabbit retic lysate (RRL). For general RNA control renilla luciferase was used. In presence of amylase relative luciferase activity was decreased compared to control protein. There was also significant decrease in translation compare to 14-16 deletion mutant RNA (Fig 3B). We co-transfected either insulin 5'UTR luciferase construct along with amylase or GFP in HEK cells and measured the relative luciferase activity. There was about 50% decrease in relative luciferase activity (RLU) upon amylase over expression compared to control protein GFP (Fig 3C). In summary, pancreatic alfa amylase specifically interacts with insulin RNA and negatively regulates its translation.

Future Research Plans

It has been suggested that autoimmune pancreatitis (AIP) associated diabetes mellitus is caused by infiltration of activated CD8+ and CD4+ T cells from exocrine and ductal cells to endocrine β cells followed by increased levels of Th1 cytokines (TNF-α, IFN-Δ and IL-1β) resulting increased expression of autoantibodies against alfa amylase and β cell dysfunction (Endo et al., 2009;Tanaka et al., 2001). This prompted us to check the alfa amylase expression in β cells in response to increased Th1 cytokines (TNF-α and IFN-Δ). We treated mouse insulinoma β cell line (MIN6) with high concentration of TNF-α and IFN-Δ and amylase expression was assessed at RNA and protein level. As expected, we found alfa amylase expression in β cells. This strengthened our hypothesis that during acute or chronic auto immune pancreatitis increased level of TNF-α and IFN-Δ cytokines might induce the amylase expression in pancreatic β-cells which eventually reduce insulin production, leading to β cell dysfunction.

Anjali Shiras

anjali@nccs.res.in
anjalishiras@gmail.com

Understanding the mechanism of transformation elicited by a novel l ong non-coding RNA – Ginir

Participants

Meenakshi Setia, *SRF*
Mohsina Anjum Khan, *SRF*
Gulshan, *SRF*
M S Pavan Kumar, *SRF*
Divya Kumari, *SRF*
Abhishek Teli, *JRF*
Dr. Neelima Dubey, *Research Scientist, Unilever*
Suchismita Panda, *Project Assistant*
Abir Mondal, *Project Assistant*
Prashant Phulpagar, *Project Assistant*
Snigdha Dhali, *Technician C*

Collaborators

Dr. Ravi Sirdeshmukh, *Institute of Bioinformatics, Bangalore*
Dr. Gopi HN, *Indian Institute of Science Education and Research (IISER), Pune*
Dr. Dattatraya Muzumdar, *KEM Hospital, Mumbai*

Background

The recent surge of information regarding evolutionary conservation, functionality and annotation of sequences from mammalian genome has revealed that the major bulk of genome is non-coding and includes small and long noncoding RNAs. Analyses of the FANTOM dataset of the human and mouse transcriptome has publicized that about 63% of mammalian genome is pervasively transcribed, even from retrotransposon elements and amongst them greater than 73% of genes show some form of antisense transcription (Katayama et al, 2005). The advancement of knowledge of various transcriptomes followed by identification of several long noncoding RNAs (lncRNAs) has led to exposition of regulatory roles for them in transcription, post transcriptional gene silencing (PTGS), organogenesis, differentiation, epigenomics and an enlarged role in a variety of physiological disease processes (Quinn & Chang, 2016).

This study describes identification and functional characterization of a novel linc RNA pair Ginir/Giniras that plays a critical role in maintenance of genomic stability by mediating cellular homeostasis. Mechanisms that tilt towards increased expression of one of the transcripts Ginir in cells result in genomic instability and perpetuate cells towards a transformed and metastatic phenotype.

Aims and Objectives

- To determine the specificity of Ginir action by knock-down studies in mouse cells.
- To study contribution of interacting protein partners of Ginir in inducing genomic instability.

- To understand mechanisms through which non-coding RNA Ginir and its cellular targets interact and mediate cellular homeostasis.

Work Achieved

Our earlier studies have elaborated on identification and functional characterization of a novel linc RNA pair Ginir/Giniras in maintenance of genomic stability and thereby mediating cellular homeostasis. Our previous studies have shown that Ginir exerts regulatory roles during cell cycle division and ensures genomic stability. However, in situations where Ginir expression is increased, cells become oncogenic. In an attempt, to understand the mechanisms that cause malignant transformation due to Ginir over-expression, we generated cells that ectopically expressed Ginir in mouse NIH/3T3 cells and used these cells to decipher mechanistic clues for Ginir action

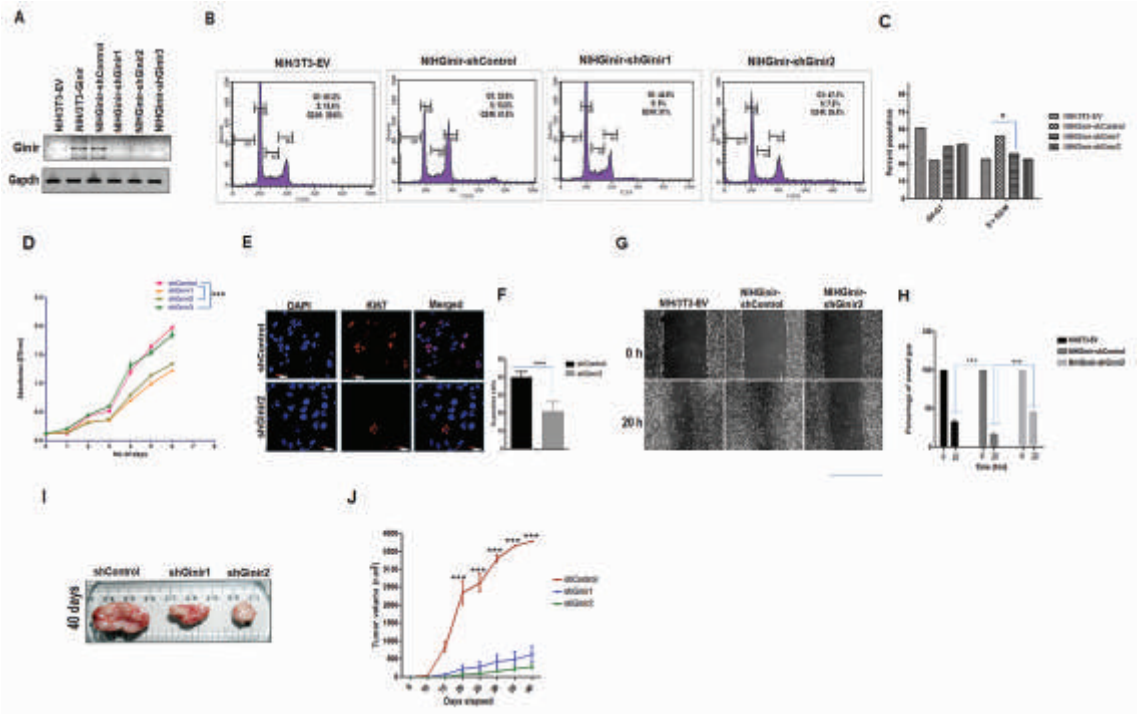


Fig. 1: Ginir knockdown attenuates tumorigenicity of NIH-Ginir cells

A. Representative RT-PCR analysis for determining Ginir levels in NIH-EV, NIH-Ginir and NIH-Ginir cells expressing Ginir-shRNA (1, 2, 3 & scrambled). Gapdh served as loading control. Cells transfected with scrambled shRNA served as control in all experiments.

B & C. Representative cell cycle profiles of Ginir stable k.d. (shRNA1, 2 and 3) cells obtained by propidium iodide (PI) staining and assayed with flow cytometry (B). Quantitative analyses of cells in different phases of cell-cycle. Bars: SEM; *P<0.05, two tailed; by Fisher's Exact test (C).

D. Cell proliferation analysis by MTT assay for the indicated cells. ***P< 0.0001, one tailed, by paired Student's t-test. Data are mean ± S.E.M.

E & F. Immuno-fluorescence confocal microscopy for Ki67 staining in NIH-Ginir, NIH-Control and Ginir knock down (shRNA1, 2 and 3) cells. Scale bars-20μm (E). Quantification of Ki67 immunostaining shown as percentage of positively stained cells compared to total number of cells per field (number of fields = 10) in NIH-Ginir-shGinir2 and Control cells. **P ≤ 0.001. Two tailed, by unpaired Student's T test. Values are mean ± S.E.M (F).

G & H. Wound healing assay with NIH/3T3, NIH-Ginir-shControl and NIHGinir-shGinir2 cells. The wound gaps were observed at two different time points- 0 hour and 20 hours post wound formation (n=3) (G). Statistical analysis of wound closure in mentioned cells wherein the percentage of wound gap measured at different time points was plotted and the values are represented as mean ± S.E.M; ***P < 0.0001 by regular two way ANOVA test (H).

I. Representative tumor pictures demonstrating effect of Ginir-shRNA on tumor induction potential of NIH-Ginir cells assessed using NOD/SCID mice xenograft assay (n=5). Tumor volumes were measured at regular intervals of 5 days and the tumors were dissected after 40 days post injection.

J. Tumor kinetics showing effect of shGinir1 and shGinir2 on NIH/3T3-Ginir induced tumor growth in NOD/SCID mice assayed over a period of 40 days. Data are mean ± S.E.M; ***p value< 0.0001 by regular two way ANOVA test.

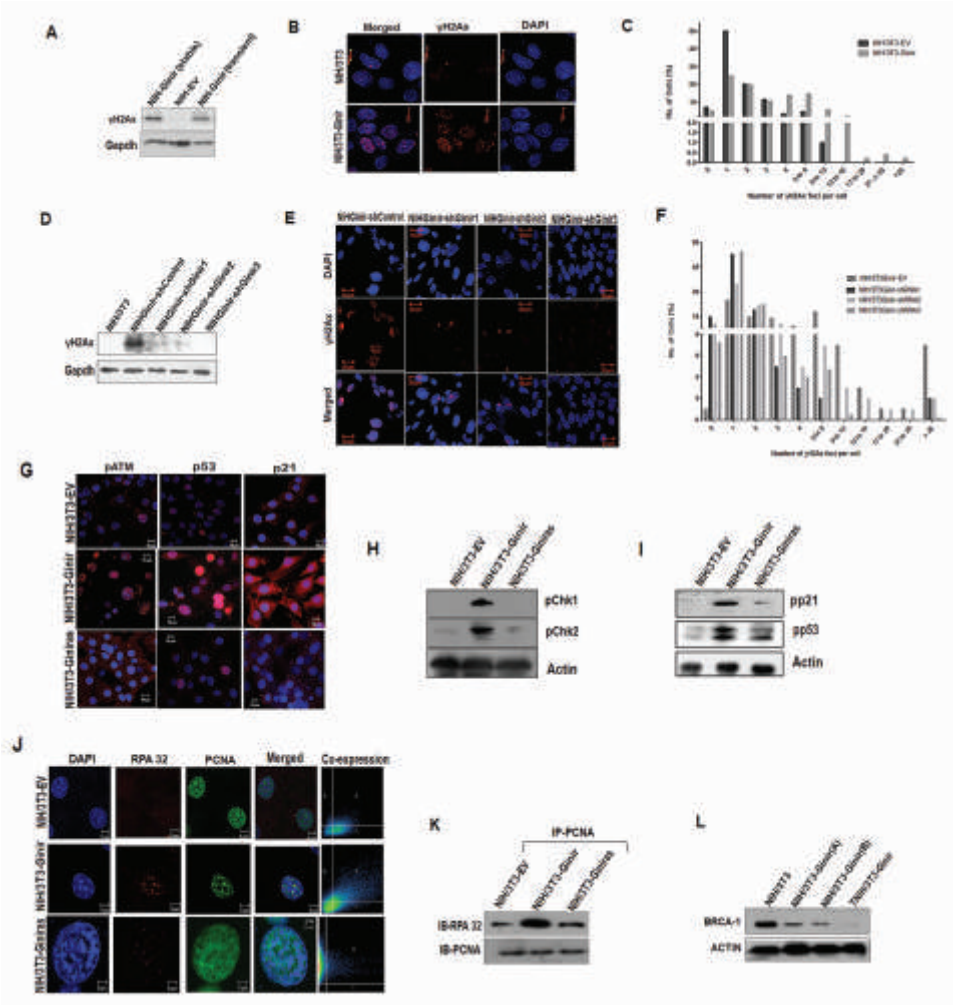


Fig. 2: Ginir RNA induces defects in DDR response and increase in dsDNA breaks

A. Representative blots for γH2Ax (DNA damage marker) protein expression in NIH/3T3, NIH-Ginir (stable cell-line) and NIH-Ginir cells (transiently transfected with Ginir cDNA for 48 hours). 30 µg of whole cell protein lysates of mentioned cell lines were loaded on 16% SDS PAGE. Gapdh served as internal control for equal loading. Data shown are representative of experiments done with three independent transfectants.

B. Confocal images showing immunostaining for γH2Ax in NIH/3T3 and NIH-Ginir (48 hours post transfection) cells. Scale bars- 10µm.

C. Quantification of γH2Ax foci in mentioned cell lines wherein percentages of cells showing a given number of foci were plotted. The number of foci per cell (n=500) were counted using Image J tool; version 1.41.

D. Western blotting for γH2Ax in NIH/3T3, NIH-Ginir-shControl and NIH-Ginir-shRNA1, shRNA2 and shRNA3 cells. 30 µg of whole cell protein lysates of mentioned cell lines were loaded on 16% SDS PAGE. Gapdh served as loading control.

E. Confocal images for γH2Ax staining in NIH-Ginir-shControl and NIH-Ginir-shGinir (shRNA 1, 2 & 3) cells. Scale bars- 20 µm.

F. Quantification of γH2Ax foci in mentioned cell lines using Image J tool; the number of foci per cell were counted (n=500) for each cell-type & percentage of cells showing specific number of foci are plotted.

G. Confocal images showing immunofluorescence for DNA Damage Repair (DDR) proteins like pATM, p53 and p21 in NIH/3T3, NIH-Ginir and NIH-Giniras cells. Nuclei were stained with DAPI (blue). Scale bars- 10µm.

H & I. Western blot analyses with antibodies for pChk1 & pChk2 (H) and pp53 and pp21 (I) in NIH-EV and NIH-Ginir/Giniras cells. Actin served as loading control.

J. Co-localization of RPA32 with PCNA in NIH-EV and NIH-Ginir/Giniras cells by confocal imaging. Scale bars- 5 µm. Images at extreme right show co-localization analysis of RPA32 with PCNA.

K. Co-immunoprecipitation studies were carried out with lysates prepared from NIH/3T3, NIH-Ginir/Giniras cells. Whole cell proteins were immune-precipitated with PCNA antibody and immunoblotted (IB) using RPA32 antibody.

L. Western blotting for Brca1 in NIH/3T3-EV, NIH-Ginir (two independent transfections) and TNIH-Ginir cells (cells derived from NIH-Ginir induced tumors). 50 µg of whole cell protein lysates of mentioned cell lines were loaded on 7% SDS- PAGE. Gapdh served as loading control.

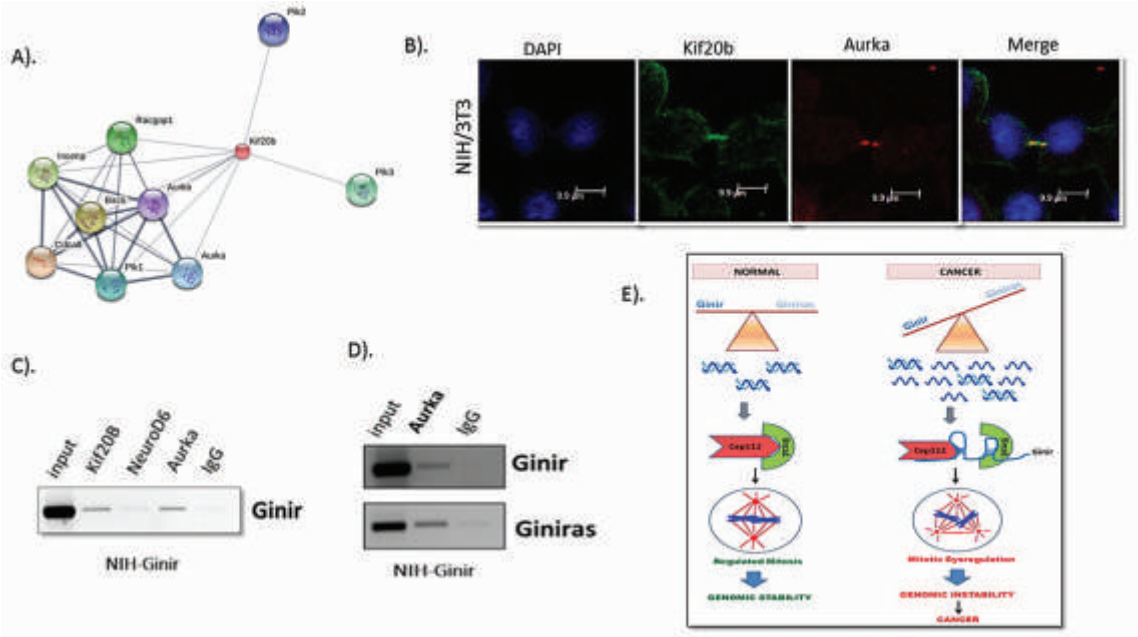


Fig. 3: Aurora kinase A interacts with Ginir along with Kif20b

A. STRING analysis showing interactome of Kif20b in mouse cell system based on the information available through protein-protein interaction databases.

B. Co-localization of Aurora kinase A and Kif20b in NIH/3T3 cells during cytokinesis. The cells were synchronized with Aphidicolin at G1/S phase and immunostaining was done at 18-20 h after release.

C & D. RNA-IP with Aurora kinase A (Aurka) for enrichment of Ginir through RT -PCR with oligo dT/random cDNA primers and (B) for enrichment of Ginir and Giniras through strand specific cDNA (C) in NIH-Ginir cells.

E. Schematic model proposing mechanism of Ginir in inducing cellular transformation.

and function. Since, interaction of noncoding RNA pair –Ginir/Giniras with proteins could be one of the ways through which Ginir could ensure genome stability; we performed biotin pull-down assays followed by Mass-spec analyses. Amongst, the various proteins identified in Mass-Spec, we found that centrosomal protein Cep112 was an interacting protein partner for Ginir but not for its full length natural antisense transcript - Giniras; Next, our localization studies indicated that Ginir RNA targeted centrosomal protein Cep112 by binding to it and thereby altered its sub cellular localization. This binding impaired its interaction with Brca1 protein. Importantly, interference in Cep112-Brca1 interaction due to high Ginir levels caused stress during DNA replication and induced abnormal mitotic spindle dynamics that resulted in genomic instability and propelled cells towards oncogenesis. Next, we asked if high level of Ginir expression was continuously required for the induction and/or maintenance of the transformed state of NIH-Ginir cells. In order to address this, we generated stable clones of NIH-Ginir cells in which Ginir expression was downregulated by superimposing the expression of Ginir-specific shRNAs. Three stable clones from NIH-Ginir cells that expressed short hairpin RNAs with target specificity to three different regions of Ginir RNA sequence (sequences specified in Methods) shGinir1, shGinir2 and shGinir3 were generated. In shGinir1 and shGinir2 cells, the endogenous Ginir RNA levels had decreased to the levels comparable to that of NIH/3T3 cells (Fig 1A). Most of the shRNA expressing cells displayed a reversal of their refractive cell morphology appearing more like normal NIH/3T3 cells, maintained a reduced level of S+G2/M cell fraction (Fig 1B,C), showed reduced proliferation potential (Fig 1D), and had substantially low Ki67 positivity (Fig 1E, F). The NIH-Ginir-shGinir1 and NIH-Ginir-shGinir2 cells also showed impaired 2D cell migration ability in culture (Fig 1G, H) and they displayed reduced potential to form tumours in SCID mice (Fig 1I, J). The Ginir knockdown experiments provided strong evidence that a high level of Ginir RNA expression was continuously required for the maintenance of the transformed state.

Ginir RNA induces dsDNA breaks and activates ATM/Chk1/Chk2/p53 DNA damage response (DDR) pathways

A distinctive feature of NIH-Ginir cells was expression of γH2AX (Fig 2A) seen as high numbers of repair foci in their nuclei (Fig

2B, C). These foci were found decreased considerably in Ginir knock-down (NIH-Ginir-shRNA1/2) cells (Fig 2D, E, F). Similarly, the repair foci were considerably less in NIH-Giniras and NIH-EV cells. Importantly, transient expression of Ginir RNA in NIH/3T3 cells also led to high γH2AX foci demonstrating that this effect was induced by Ginir RNA even on transient overexpression and was not a secondary consequence of cell transformation (Fig 2A, B, C). Consistent with increased level of γH2AX foci, Ginir cells showed activation of members of DNA damage and checkpoint pathways that included, Chk1, Chk2 kinases and pp53, pp21 proteins and yet there was no growth arrest (Fig 2G, H, I). NIH-Ginir cells showed an increased pATM expression as well as enhanced nuclear retention of p53 and p21 (Fig 2G). Due to the activation of Chk1 pathway, NIH-Ginir cells had accumulated high levels of replication protein RPA32 co-localizing with PCNA protein as well as ssDNA resulting from stalling or collapse of replication forks in the replication factories (Fig 2J). This may be an outcome of increase in the firing of dormant replication origins that were induced by Ginir RNA (Fig 2J, K) (Cseresnyes et al, 2009). Prolonged stalling of the replication forks was being sensed as dsDNA breaks activating DNA-damage response. Also, NIH-Ginir cells possessed lower amounts of Brca1 protein as compared to NIH/3T3 cells (Fig 2L) and showed an accumulated level of phosphorylated p53 (pSer20) that worked together to impair repair of DNA damage, compromise tumour suppressor function and thereby promote genomic instability (Fig 2I). It may be noted that the activation of DNA damage response was only manifested by Ginir and not Giniras, indicating that only Ginir RNA had the ability to potentiate this response.

Aurora kinase A interacts with Ginir along with Kif20b

In our previous studies, we showed that Kif20b was an interacting protein partner for Ginir RNA. Kif20b is a member of kinesin 6 superfamily of proteins, also known as M-phase phosphoprotein 1(MPP1 or MPHOSPH1) which is important molecular motor protein required for completion of cytokinesis. There are several reports which show overexpression of this protein in various human cancers such as bladder cancer, hepatocellular carcinoma etc. In case of Ginir over expressing cells, we did not find significant increase in the protein expression levels of Kif20b, however the localization of this protein was changed. The accumulation of the protein was more towards the membrane in Ginir over expressed cells as compared to normal NIH/3T3 cells. To study the effect of interaction of Ginir with Kif20b, we determined further downstream protein interactors of Kif20b to study whether there were any effects on molecular functioning of Kif20b in case of Ginir overexpression. Various bioinformatics tools and databases used for studying protein-protein interactions led us to a list of putative interactors of Kif20b which included Aurora kinase A and B, Plk1 and 2, Cyclin B1, Cdk1 etc. (Fig.3A). We found that the interaction of Kif20b with Aurora kinase A (Aurka) occurred in a stage specific manner. Aurka was found to co-localize with Kif20b at the mid-body of cytokinetic furrow (Fig.3B) and not at any other cellular location or phase of cell cycle. To study the association of Aurka with Ginir RNA, we performed RNA-IP and found that Ginir was enriched in elutes after immunoprecipitation with Aurka (Fig.3C). Semi quantitated PCR with strand specific cDNAs for sense and antisense strands showed that Aurka interacts with both Ginir and its antisense counterpart Giniras (Fig.3D). Our aim is to study the significance of these interactions in regulation of cytokinesis in Ginir over expressed cell system (endogenous and exogenous). Ours is one of the first reports exemplifying as to how interaction of proteins Brca1-Cep112 is impaired due to excess levels of Ginir RNA causing defects in multipolar spindle formation leading to genomic instability and culminating into malignant transformation (Fig. 3E). Our findings introduce an entirely novel mechanism of involvement of noncoding RNAs in oncogenesis.

Future Research Plans

- Unravel interaction of Ginir and Giniras with its target proteins in a stage specific manner.
- Identify the protein interactome for noncoding RNA - Ginir and study its role in maintenance of genomic instability.

Yogesh S. Shouche

yogesh@nccs.res.in

Human Microbiome Indian perspective

Participants

Rahul Bodkhe, *JRF*
Satish Kumar, *Teacher Fellow*
Abhijit Kulkarni, *JRF*
Deepak Khairnar, *SRF*
Sahabram Dewala, *SRF*
Shreyas Kumbhare, *SRF*
Mangesh Suryawashi, *SRF*
Diptaraj Chaudhari, *Project SRF*
Vikas Ghattargi, *Project Assistant*
Kunal Jani, *Project Assistant*
Rakesh Kumar Yadav, *Project Assistant*
Akshay Gaike, *Project Assistant*
Dr.Dhiraj Paul, *DST Fast Track Fellow*
Dr.Kusum Dhakar, *SERB National PDF*

Collaborators

Govind Makharia, *All India Institute of Medical Science (AIIMS), New Delhi*
Sanjay Juvekar, *King Edward Memorial (KEM) Hospital, Pune*
C. S. Yajnik, *King Edward Memorial (KEM) Hospital, Pune*
Ashish Bavdekar, *King Edward Memorial (KEM) Hospital, Pune*
Saroj Ghaskadbi, *Department of Zoology, Savitribai Phule Pune University, Pune*
Sandeep Salvi, *Chest Research Foundation (CRF), Pune*
Dinbandhu Sahoo, *Institute of Bioresources and Sustainable Development (IBSD), Imphal*
Seppo Salminen, *University of Turku, Finland*
E.R.B. Moore, Culture Collection, University of Göthenburg and CCUG, Sweden
Joakim Larsoon, *University of Gothenburg, Sweden*

Background

The human microbiota is the aggregate of microorganisms, which resides on the surface and in deep layers of skin (including in mammary glands), in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts. It is the aggregation of transient and resident micro-organisms including eubacteria, archaea, fungi and viruses. The contribution of these microbes is essential for the innate working of the host physiology. As the gastrointestinal tract harbors the maximum number of these microbes, microbiome studies focus on this unique ecological niche. Although gut microbiome studies are in their preliminary stages, they are establishing some fundamental understanding about the co-relationship shared between the commensal microorganisms and the human host. The gut microbiota alone is responsible for contributing close to 3.3 million genes which are responsible for functions ranging from digesting complex food material, training immune cells, synthesis of essential metabolites for the host, development of bones and regulation of behavior via the gut-brain axis.

Increasing evidence suggests that the human gut microbiota changes according to diet, age, lifestyle, climate and geography, genetic make-up, early microbial exposure and health status. Hence, it becomes increasingly relevant to have a greater understanding of population specific microbiome to devise targeted therapies. Studying the Indian population is relevant given the known dietary and geographical variety, family structure, ethnic diversity and the presence of many endemic tribes.

Aims and Objectives

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

Work Achieved

Pune Microbiome study – molecular analysis of human microbiome

The study was aimed to explore the microbial communities associated at different body locations in healthy human beings and understanding the changes in abundance and diversity of the microbial flora as a function of age.

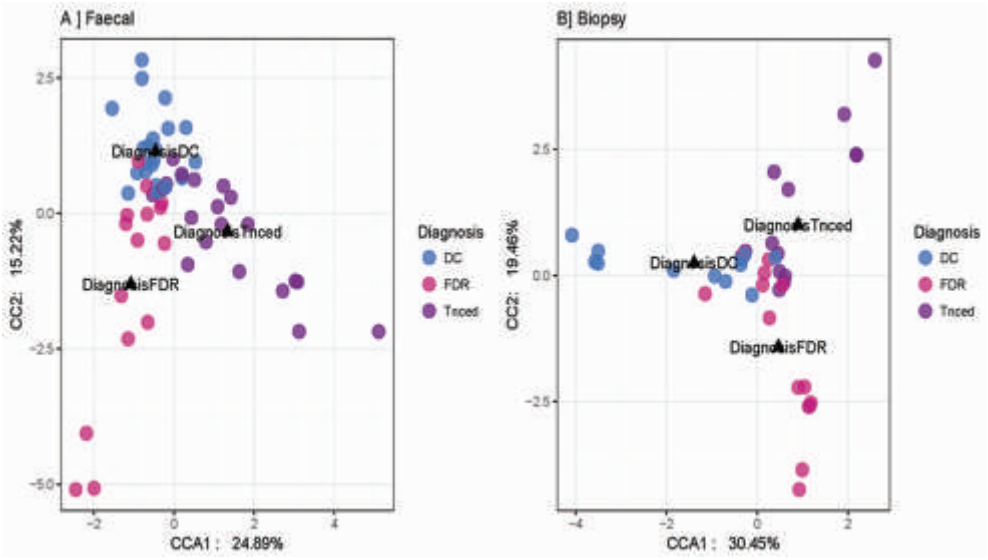


Fig. 1a: Ordination of individuals using CCA with diagnosis as constraint for both sampling sites.

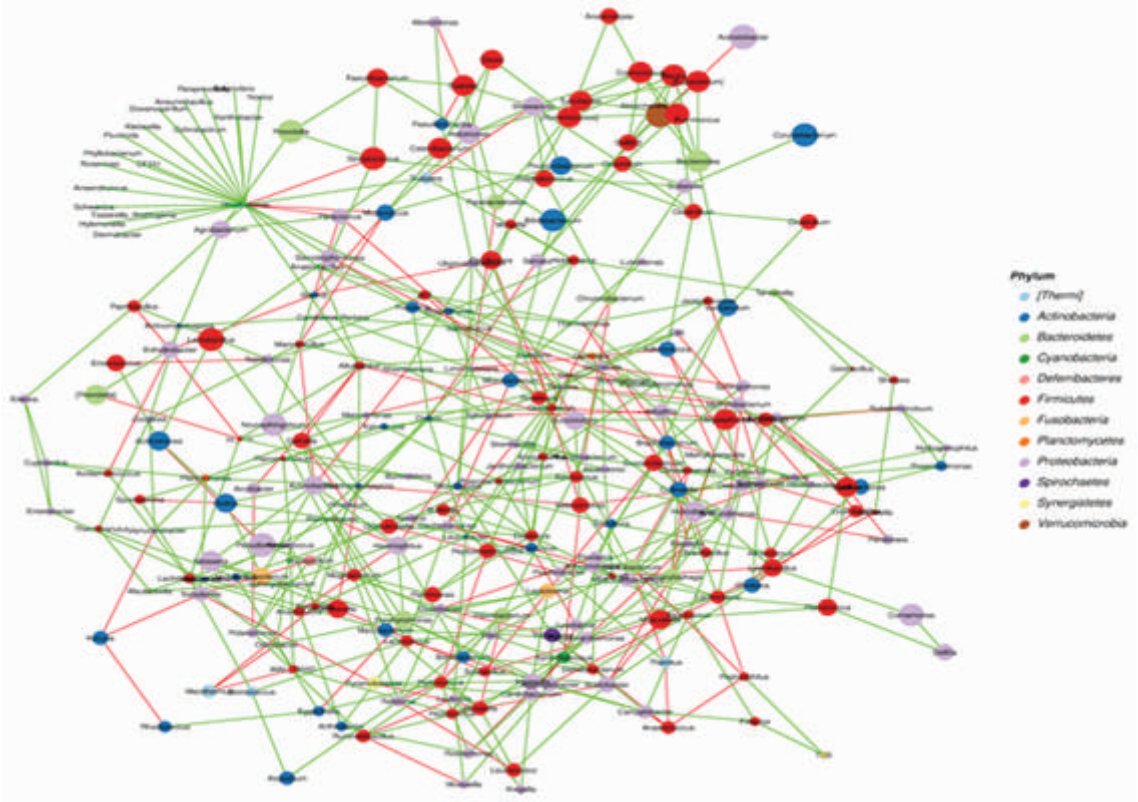


Fig. 1b: Microbial network inference in TnCeD: The OTUs are merged at genus level and nodes are coloured based on their phylum affiliation and size of the node is proportional to the mean abundance of the genera. Green lines represent positive interaction while red lines are indicative of negative interactions.

For devising population specific therapies, it is important to understand the key contributors in the gut microbiota in that population. Indian population is one of the most ethnically, culturally and geographically diverse population but gut microbiome features are not completely known. While comparing gut microbiota of healthy Indian subjects with other populations, certain key features that are highlighted by a recent study by Bhute *et al.* reveals that there exists inter-

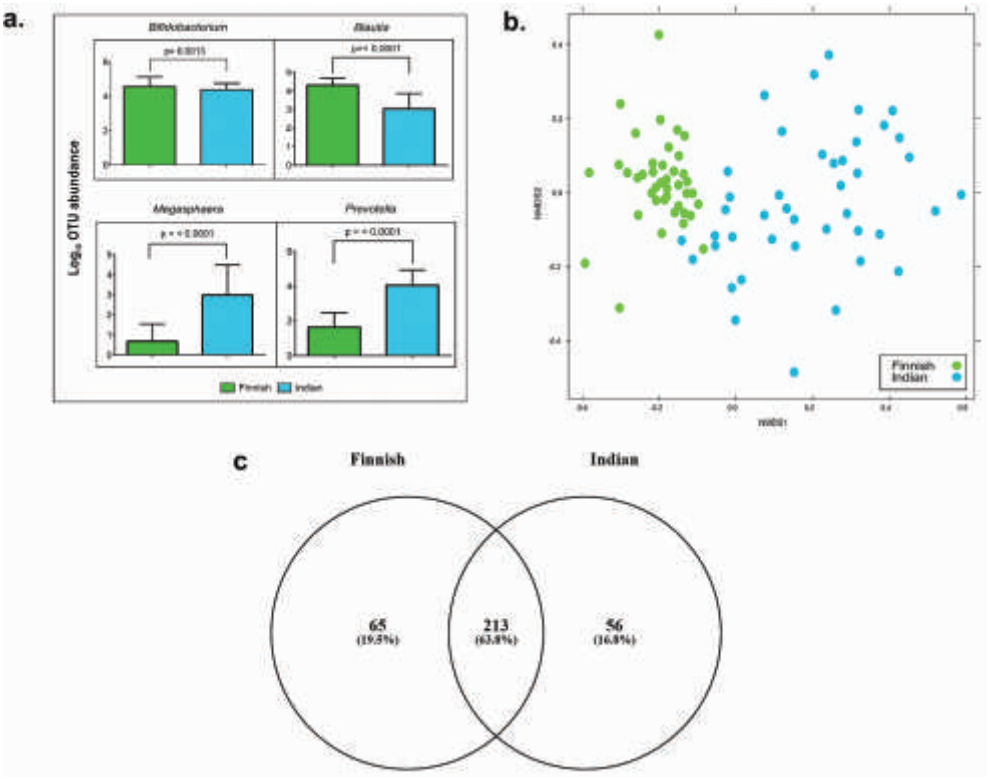


Fig. 2a: Beta diversity analysis. (a) Box plot illustrating differences in relative abundances of key contributors (most dominant) of bacterial community between Indian (blue) and Finnish children (green). (b) Beta diversity analysis using NMDS plot based on OTU level differences between Finnish (green) and Indian (blue) children (c) Venn diagram representing shared and unique bacterial genera between gut bacterial community of Finnish and Indian children

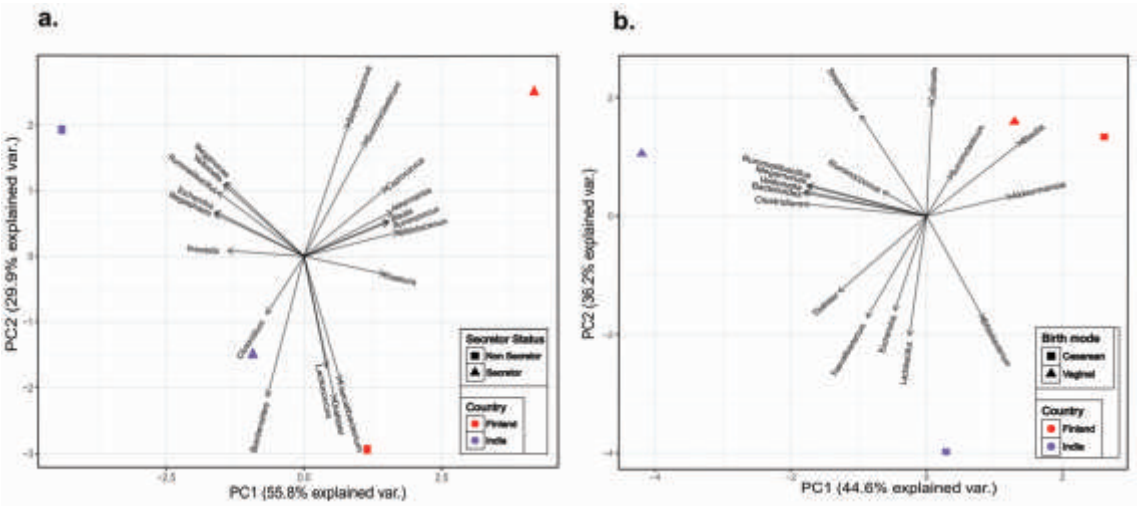


Fig 2b. Principal Component analysis. PCA was performed using the bacterial genera data, relative abundance which were observed to be differing with FUT2 secretor state and birth mode in both the populations. Results were plotted according to the PC1 and PC2 scores with the percent variation explained by respective axis. Specific colors were used to indicate the region and different shapes to indicate either the secretor state (a) or birth mode (b). Bacterial genera with their names were indicated by arrows.

individual variations in the gut microbial communities and although the gut microbiomes of Indians is different from that of the Americans, it shared high similarity with individuals of Indian Subcontinent i.e Bangladeshi. Distinctive feature of the Indian gut is the predominance of genus *Prevotella* and *Megasphaera*. Also, when compared to non-human primates, Indians share more OTUs with omnivorous mammals and exhibit high potential for glycan biosysnthesis and xenobiotic metabolism.

Celiac Disease and gut microbiome

Celiac disease (CeD) is an autoimmune disease characterized by the hypersensitive reaction to gluten in the small intestine of genetically susceptible individuals. Approximately 1% of the world population is affected by CeD. Recently, the potential role of intestinal microbiota as a contributing factor to development of CeD has been hypothesized. While most of the research is focused on paediatric subjects, understating of changes in adult intestinal microbiota before and during active disease is lacking. Also, the studies with microbiome profile of faecal as well as biopsy samples are limited thus we have analysed the small intestinal and faecal microbiota of adult celiac patients and first-degree relatives.

Community level comparisons showed significant differences in the alpha and beta diversity between the small intestinal and faecal microbiota irrespective of the diagnosis groups. Differences in the bacterial community structure based on diagnosis groups were more visible in biopsy than in the faecal samples using constrained ordinations. Known opportunistic pathogens such as, *Comamonas*, *Acinetobacter* and *Brevundimonas* are enriched in the biopsies of celiac disease group. In biopsy samples, the inferred taxon-taxon interaction network suggested negative interactions between *Akkermansia* and *Acinetobacter* in the celiac disease samples.

The differences in the bacterial community between the biopsy and faecal samples highlight the importance of the study for investigating microbiota changes at the site of disease manifestation. The results of the *alpha* and *beta* diversity analysis indicate subtle changes at community level between the disease subjects and first degree relatives of celiac disease patients. At genus level, we see negative correlations between the beneficial mucus colonizer, *Akkermansia* and the opportunistic pathogen *Acinetobacter* in the small intestine. Changes in abundances of several important bacteria which may have the potential to regulate the immune response highlights the need for further research to unravel their potential role in the pathogenesis of celiac disease.

The origins and process of microbiota development in different geographic areas: creating new nutritional tools for microbiota modulation

The human gut microbiome is a complex bacterial community whose composition is responsive to a number of host characteristics such as host genetics and conditions at birth. These play a crucial role in the compositional development of gut microbiota. Though well documented in western pediatric population, little is known about how these host conditions affect populations in different geographic locations such as the Indian subcontinent. In view of the impact of distinct environmental conditions, we designed a study to assess the gut bacterial diversity of Indian and Finnish children and investigated the influence of FUT2 secretor status and birth mode on the gut microbiome within these populations.

Using multiple profiling techniques, we show that the gut bacterial community structure of 13-14 year old Indian (n=47) and Finnish (n=52) children differs significantly. Specifically, Finnish children possessed higher *Blautia* and *Bifidobacteria*, while genus *Prevotella* and *Megasphaera* were predominant in Indian children. These differences were also reflected in the functional attributes of the bacterial communities. Our study demonstrates a strong influence of FUT2 and birth mode variants on specific gut bacterial genera. Further the dynamics of gut microbial composition in relation with these elements (FUT2 secretor state and birth mode) was noticed to differ between the two populations under study. Collectively, our results implicate that FUT2 secretor state and birth mode influence the gut bacterial composition in children, the pattern of which differs between the Indian population as against the well characterized Finnish cohort. This study highlights the need for exploring such other confounding factors in detail in the underexplored and heterogeneous population like India.

Future Research Plans

- Proposal has been submitted to DBT for the pan India mapping of human microbiome of persons with different ethnicities, residing in different geographical regions and having varying dietary and cultural practices.

Shailza Singh

singhs@nccs.res.in

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

Participants

Vineetha Mandlik, *SRF (DBT)*
Milsee Mol, *SRF (CSIR)*
PruthviRaj Bejugam, *SRF (UGC)*
Ritika Kabra, *JRF (DBT)*
Bhavnita Soni, *JRF (DST-Inspire)*
Pramila Pal, *JRF (CSIR-JRF)*
Anurag Kumar, *JRF (UGC-JRF)*
Dipali Kosey, *Project JRF*
Baishali Sarmah *(Project trainee)*
Dr. Nutan Chauhan, *Post-Doctoral Fellow (DST)*

Collaborators

Dr. Basu, *IISER Pune*
Dr. Kanaujia, *IIT Guwahati*

Background

With the advent of synthetic biology in medicine many synthetic or engineered proteins have made their way to therapeutics and diagnostics. Synthetic biology started with bacterial platforms, but since the application of prokaryotic systems is limited in treating human diseases, newer and better strategies are being developed to use mammalian systems for transgene expression which forms the basis for synthetic mammalian network. Some examples are cited as follows: Metabolic syndrome is characterized by the three interlinked and inter-dependent metabolic disorders of hypertension, hyperglycemia, and obesity which are treated independent of each other. A multifunctional synthetic gene circuit and a combination of drugs can improve the clinical manifestations of this syndrome. Guanabenz is an antihypertensive drug which controls the expression of a fusion hormone GLP-1-Fc-Leptin in a dose dependent manner which simultaneously attenuates hypertension by guanabenz, hyperglycaemia by GLP-1 and obesity by Leptin. A transgene circuit with a dual-promoter integrator was developed that discriminate and kills cancer cells. Tumor specific two different promoters drive the expression of DocS-VP16 and Coh2-GAL4 which are chimeric proteins. When both are expressed (AND logic gate) in the presence of a cancer specific signal, they dimerize to form an active PGAL4 promoter tarnsactivator that triggers the expression of thymidine kinase. It converts the prodrug ganciclovir to the active drug that kills the cancer cells. T cell reprogramming for immunotherapy in chronic lymphocytic leukaemia was done by designing a chimeric antigen receptor (CAR) made up of four functional domains: an anti-CD19 single chain fragment, a human CD137-derived co-stimulatory domain, a CD3f signaling domain and a CD8a-derived transmembrane domain. The chronic lymphocyte leukaemia cells expressing the CD19 marker were recognized by the transgenic T cells expressing the CAR and were activated to proliferate, and kill the cancer cells. Antibodies are fused

to the therapeutic proteins which show targeted activity. For example therapeutic proteins like cytokines when fused to antibodies or receptor ligands, are targeted to cells that express the corresponding antigen or receptor, respectively.

Similarly, a therapeutic protein can be engineered and embedded into a synthetic circuit for resolving leishmaniasis. Different forms of PKCs have a broad range of substrates and many overlapping substrates that can be activated, for the various physiological functions they are involved in. It is proposed here that the chimeric PKC would activate NFkB Rel A by phosphorylation of the IKKb, which in turn will phosphorylate IkB and thus unlocking the Rel A for nuclear translocation and gene expression. Synthetic circuits have been used in different configurations of control mechanism to achieve tunable response when uploaded into the target cellular system e.g. with a negative or positive feedback loop. Downstream of these regulators are placed the gene of interest whose expression can then be tuned with the introduction of inducible molecules like IPTG, tetracycline, doxycycline etc. The regulators are often combined to give rise to logical gate circuits for more complex integration of signals.

Aims and Objectives

- Design a chimeric PKC for immune modulation through NFκB.
- Embed the designed chimeric PKC into a synthetic negative autoregulatory circuit.

Work Achieved

Chimeric PKC, its Homology Modeling and Molecular Dynamics (MD) simulation

The sequence of amino acid of the chimeric PKC_ζα was analyzed by the ProtParam tool for the physiochemical properties, which are listed below.

Molecular weight: 52626.4 Da

Theoretical pI: 5.26

Amino acid composition:

Aa	%	Aa	%	Aa	%	Aa	%
Ala	4.5%	Leu	8.9%	Gln	4.3%	Ser	4.3%
Arg	4.3%	Lys	7.4%	Glu	8.0%	Thr	3.7%
Asn	2.6%	Met	3.5%	Gly	7.4%	Trp	0.9%
Asp	7.6%	Phe	5.8%	His	2.8%	Tyr	3.0%
Cys	2.4%	Pro	5.6%	Ile	5.6%	Val	7.4%

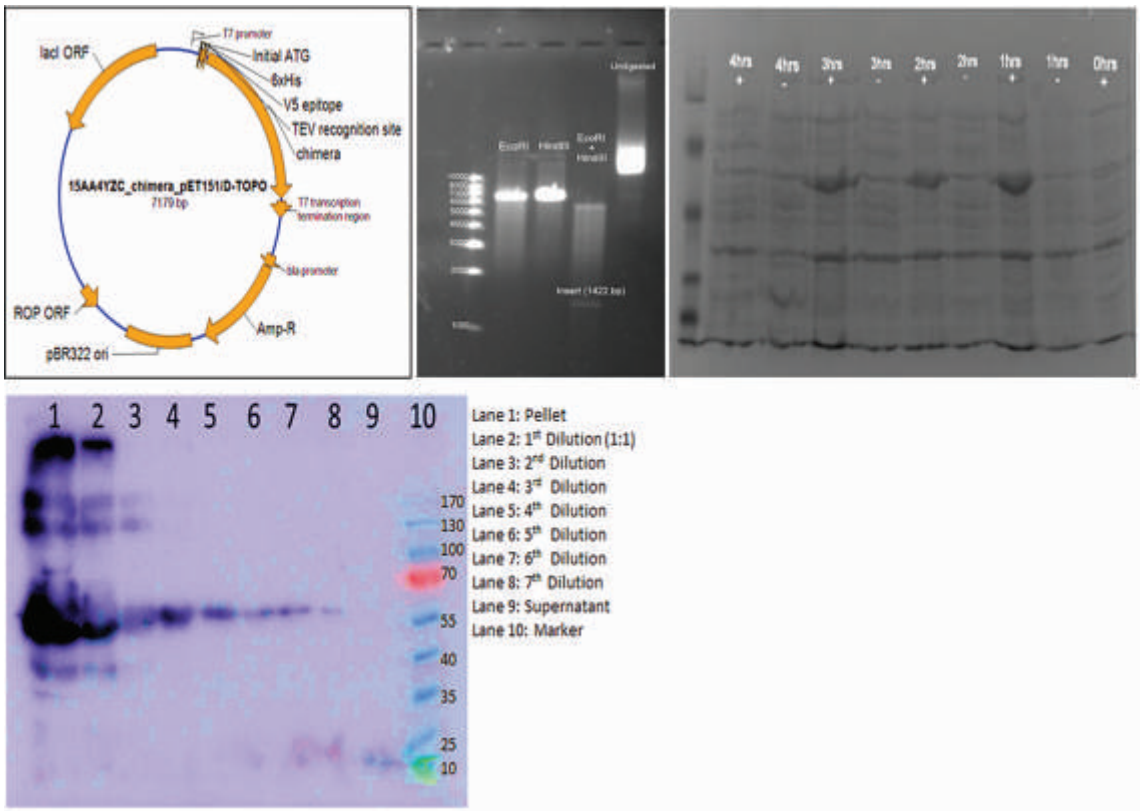


Fig. 1: Plasmid map, restriction digestion, induction with 1mM IPTG from zero to 4 hours (SDS PAGE), and His probing of the His-tagged chimeric PKC

Instability index: The instability index (II) is computed to be 36.47, classifying the protein as stable.

Aliphatic index: 82.45

Grand average of hydropathicity (GRAVY): -0.316

Homology modeling of the chimeric PKC_ζα was performed using 1WMH and 4RA4 as templates. The modeled chimeric PKC_ζα, has about 96.5% of amino acid residues in the favored region and 2.6% in the allowed region. Eleven amino acids were in the outlier region and so loop refinement of the structure was done after which the number of outlier amino acids reduced to 4 i.e. 0.9%. This model was used as the starting model for MD and the protein shows stable dynamics over the 15ns simulation. All the physical parameters viz temperature, volume and pressure were maintained during the 15ns MD run. RMSD plot of 15ns MD simulation for showing the mean fluctuation was at 8 Angstrom and stabilized after 10ns while RMSF plot of the 462 amino acids of the Chimeric PKC_ζα shows that for apart from the hinge/loop of the chimeric PKC all the other amino acids show a mean fluctuation within the 1 Angstrom range.

To check for any modelling errors, the 3D model obtained from modeller and the 3D model after MD simulation was checked on ProSa-Web which returns the z-score and energy plots for the chimeric PKC. The z-score indicates overall model quality and any deviation in the energy structure from random conformations. Z-scores (-9.13) of the chimeric PKC is well within the z-scores of all the experimentally (X-ray, NMR) determined protein structures in PDB. Similarly, the energy plot shows the local model quality by plotting energies as a function of amino acid sequence at a particular position. If most of the amino acids in the structure show a negative energy value then it can be said that the structure is error free, which is the case with the chimeric PKC.

Synthesis, expression and identification of the chimeric PKC

The construct was analyzed by restriction digestion and confirmed the presence of the insert. The chimeric PKC was successfully transformed into BL21DE3 and induced for the expression of chimeric PKC which was confirmed by MS analysis. The His-tagged chimeric protein was also confirmed by western blotting using the anti-His antibody (Figure 1).

Negative autoregulatory circuit design and its quasipotential landscape

The negative autoregulatory circuit *in silico* simulation was done in Berkeley Madonna, using the equation from the toggle switch

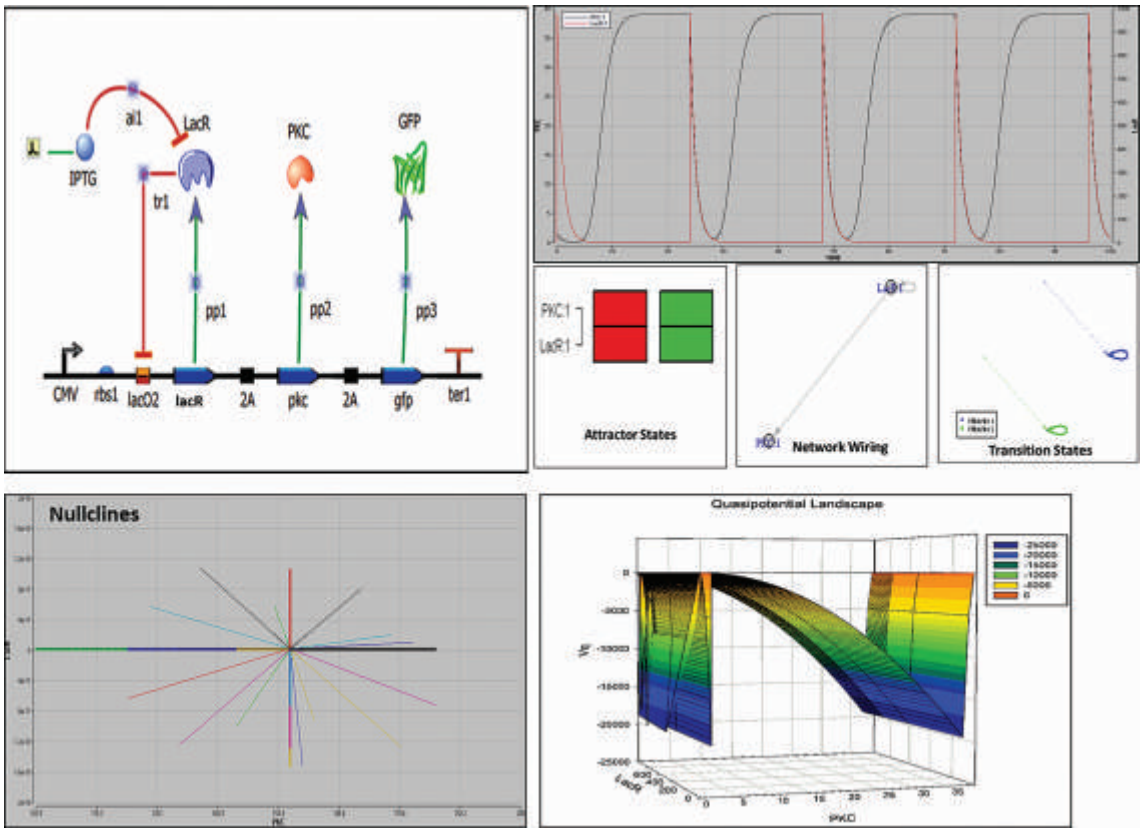


Fig. 2: Synthetic circuit design, BoolNet Analysis, Nullclines and Quasi-potential landscape.

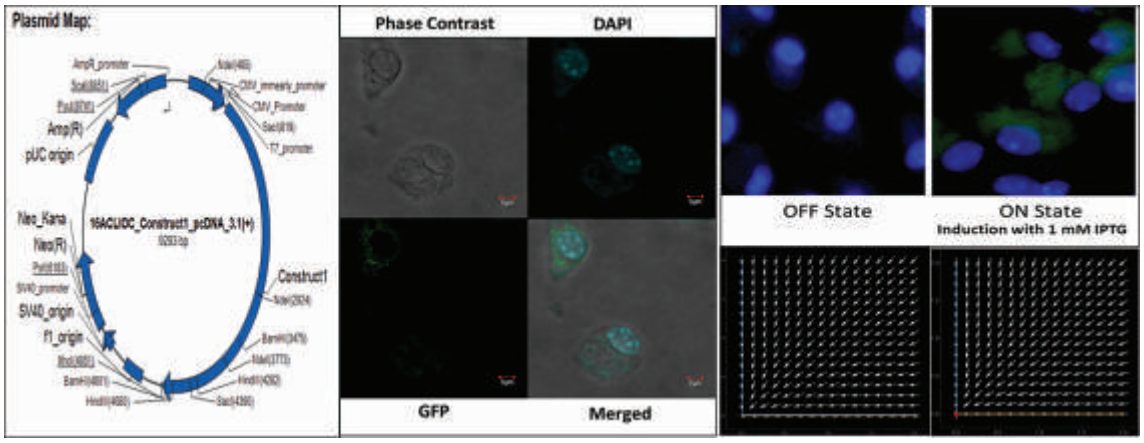


Fig. 3: Synthetic construct map, transfection, expression of GFP and working of the circuit

of Gardner et al. The circuit has only a single negative feedback loop by the LacI repressor protein, whose activity dictates the level of Chimeric PKC in the system. The synthetic circuit is represented in Figure 2. The simulation shows the oscillations generated due to impulse of IPTG at 24hrs time point at a concentration of 1mM.

The time series data generated in Berkeley Madonna was further used for R analysis, which shows that the system has two attractor states, i.e. either ON or OFF state. An attractor state suggests the stable gene expression pattern of the Boolean network. This system of a single negative autoregulator can oscillate between two stable states ON and OFF in the presence or absence of the inducer IPTG. This helps in tuning the circuit response to the IPTG concentration. The network wiring shows how the components are wired with respect to each other i.e. LacI R regulates itself and the expression of PKC. A state transition network depicts the binary representation of the attractors and the transitions between the attractor states. The total number of states in the circuit was 2.

Nullcline for the two ODEs of the synthetic circuit show an asymptotically stable state. The trajectory of the systems converge at a single point indicating that the system can be in either ON or OFF state and never in between (intermediate state doesn't exist). The point of intersection of nullclines is the saddle point or the equilibrium point.

The global dynamics of the system can be inferred by assigning potential to the attractor states. Hence the potential landscape states how forces on the system drive the system to the stable states. For the single negative auto regulatory circuit the force that drives the ON and OFF mechanism is the input of IPTG (inducer). The quasipotential landscape shows how the system crosses the thermodynamic barriers to move from one state to the other. Moreover, it can be visualized in Figure 2 that the attractors remain in a state of low potential and upon addition of inducer, the system moves towards a higher concentration of attractors. Potential valleys correspond to the attractors and peaks to the transition states.

Transfection and *in vitro* verification of the working of the synthetic circuit

The chimeric PKC was introduced into the negative autoregulatory circuit and transfected into peritoneal macrophages. The transfection was successful with an efficiency of over 60%. The working of the circuit was established with 1mM IPTG induction for 24 hours and fluorescence of GFP protein, when the IPTG was withdrawn, the circuit was in the off state i.e. there was no GFP production (Figure 3).

Future Research Plans

Interfacing the computational and synthetic biology tools accelerate our understanding of complex biological systems and also our ability to quantitatively engineer cells that may aid as therapeutic device. Cells are basically engineered either to aid in various delivery platforms or to sense a specific molecule(s) that may distinguish the diseased vs healthy state of the infection model system chosen for the study. The crux, here, lies into having a hierarchical architecture of the functional modules (parts assembled in a way to form an integrated biological system). The biochemical parameters related to transcription factor (TF) promoter binding is quantitatively determined and specifies which promoter drives the expression

of which gene so that the network can be easily rewired. The system is robust to changes in parameter values as this is a context dependent system we have focused on, while laying the basic principle design of the synthetic signaling circuit.

The study began with a motivation when we identified that a negative auto regulatory module is embedded within a large transcriptional network. Like, electronic circuits are designed so that the connection of new inputs or outputs does not in any way impact the properties of a given module, so are the synthetic biological circuits. The promoter binds and sequesters protein that has pleiotropic effects over the entire network. If the total promoter concentration is small compared to the TF-promoter equilibrium constant, sequestration effects are negligible and we do expect the total promoter concentration to be reliable. The promoter concentration ensures the synthetic device component reliability very much like the electronic circuits.

In addition, by designing synthetic systems which are translated and transcribed by completely orthogonal gene expression machinery offers another way of taking advantage of a host cell without interfering with its components. The effects a host plays on the synthetic circuit behavior should be either minimized or where applicable should be quantified in order to characterize and validate parts/modules so as to minimize crosstalk between an engineered circuit and a host's machinery. Thus, in the cellular context the designed synthetic circuit has better orthogonality (decoupling) with higher predictability and robustness.

In nutshell, we present paradigms for the design, construction and validation of robust synthetic circuits which may act as an implantable therapeutic device with more scale-ups or reuse of the modules in future.

Sandhya Sitasawad

ssitaswad@nccs.res.in

Nrf2 activation prevents cardiac fibrosis by modulating oxidative stress and inflammatory response in diabetic hearts

Background

Diabetic cardiomyopathy (DCM), characterized by abnormal cardiac structure and function, poses a high risk of cardiac failure in diabetic patients. Various molecular mechanisms have been proposed to understand the pathogenesis of DCM. However cardiac fibrosis is a major pathological event in the progression and is a crucial etiological factor involved in the pathogenesis of diabetic cardiomyopathy. Cardiac fibrosis is characterized by a high turnover of extracellular matrix (ECM). The increased production of ECM leads to myocardial stiffness and remodeling. These cardiac derangements precede cardiac dysfunction, ultimately causing heart failure.

Transforming growth factor-beta 1 (TGF-β1) is a key mediator of organ fibrosis and it stimulates fibroblast to myofibroblast transformation, thereby promoting production of extracellular matrix (ECM). Persistent myofibroblast activity leads to excessive scarring and loss of tissue compliance. Excess reactive oxygen species (ROS) generation and activation of NF-κB regulates TGF-β1 production in various disorders. TGF-β1 is also known to work synergistically with connective tissue growth factor (CTGF) during the development of organ fibrosis. Experimental models have evidenced the elevation of CTGF levels in the heart of diabetic rats.

Oxidative stress and inflammation have been extensively implicated in diabetes-associated complications. NF-E2-related factor (Nrf2) is a master regulator of endogenous antioxidant defense machinery and imparts protection against oxidative, electrophilic and xenobiotic stress. Besides, Nrf2 is also found to attenuate the NF-κB mediated inflammatory response in various disorders such as cancer, pulmonary diseases, neurodegenerative disorders,

Participants

Prachi Umbarkar, *SRF*
Sarojini Singh, *SRF*
A. Silpa, *SRF*
Dinisha Kamble, *SRF*
Rohini Dhat, *JRF*
Deepali Badhane, *JRF*
Megharani Mahajan, *JRF*
Suneeta, *JRF*
Vishi Sharma, *JRF*

and aging. Thus we speculated that Nrf2 may be a connecting factor in the interplay between oxidative stress and inflammation particularly during the development of cardiac fibrosis under diabetic condition.

Aims and Objectives

- To study the fibrotic remodeling in the heart under diabetic conditions as well as to understand the interplay between the Nrf2, NF-κB, and TGF-β signaling pathways involved in fibrosis.

Work Achieved

Our previous work provided strong evidence that mitochondrial MAO-A is an important source of oxidative stress in the heart and that MAO-A-derived ROS contribute to DCM. Further, we have also shown that diabetes induced cardiomyopathy can be prevented by elevating Prx-3 levels thereby providing extensive protection to the diabetic heart.

In this part of the study, we sought to understand the role of Nrf2 in cardiac remodeling. We explored the potential role of Nrf2 in

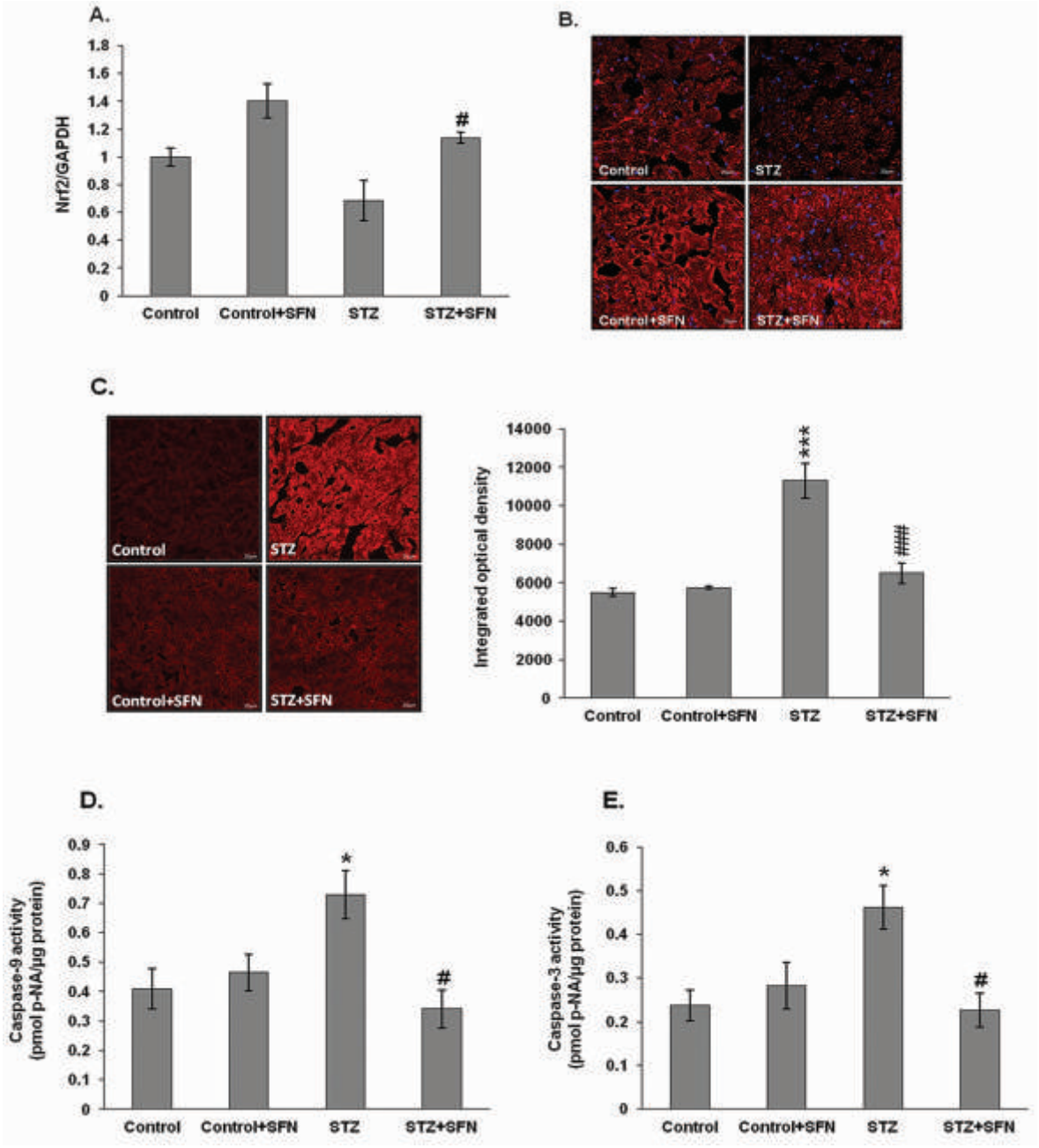


Fig. 1: Diabetes-induced oxidative stress and cardiac apoptosis was prevented by SFN mediated Nrf2 activation. (A) Nrf2 gene expression determined by real time PCR. (B) Nrf2 (red) protein expression. (C) Immunofluorescence staining for 4-HNE (left) and quantification of integrated optical density, (D) Caspase-9 activity, (E) Caspase-3 activity All values are given as the mean ± SEM, (n=3-5/group). *p<0.05, ***p<0.001 vs control group; #p<0.05, ###p<0.001 *vs* STZ group.

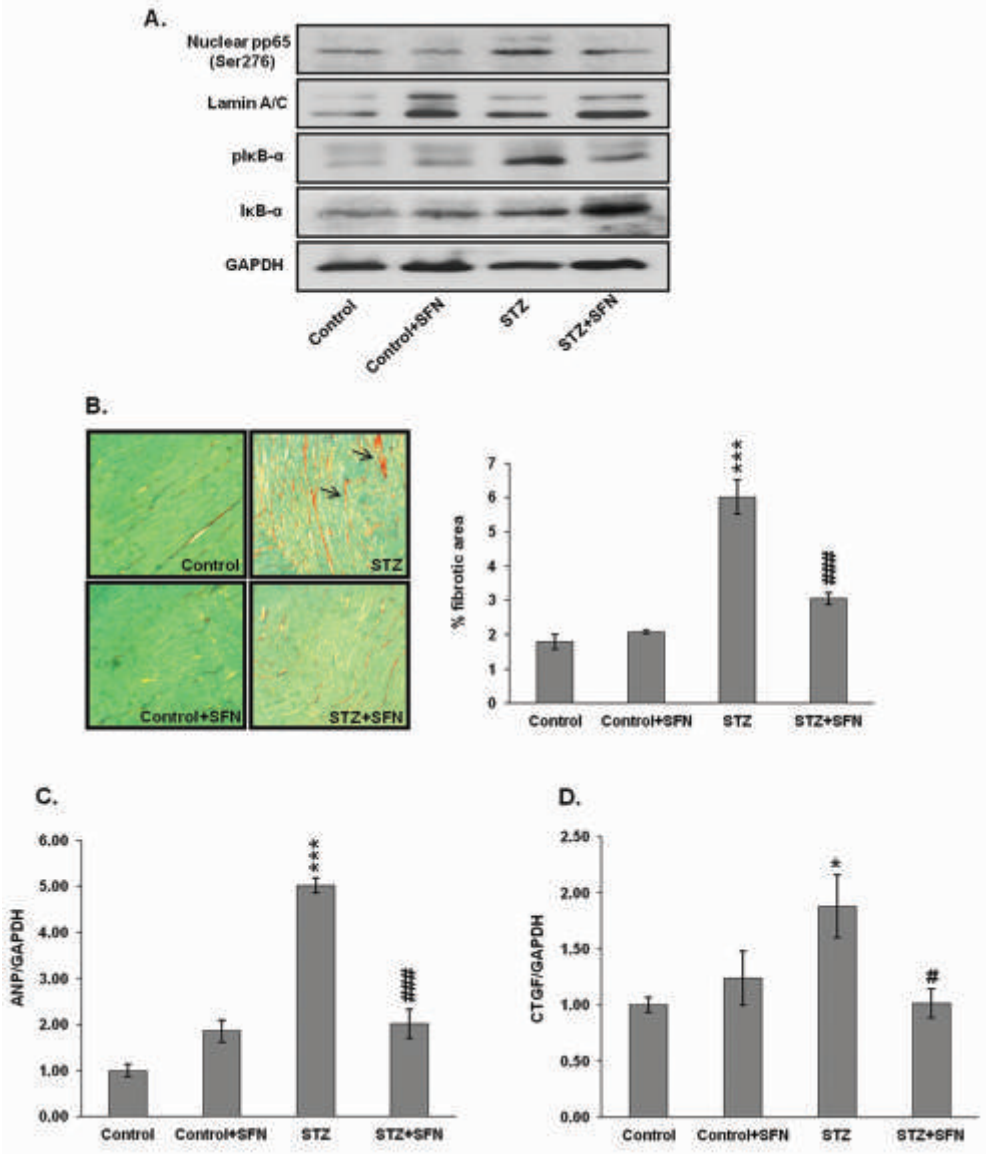


Fig. 2: Nrf2 induction prevents inflammatory response, cardiac hypertrophy and fibrosis in diabetic hearts. (A) Protein expression of nuclear pp65, pIκB-α, and IκB-α. (B) Cardiac fibrosis. Representative image of Sirius Red stained heart tissue sections showing collagen accumulation (left) and quantification of the same (right). Gene expression levels of (C) ANP and (D) CTGF. All values are given as mean ± SEM (n=4-5/group); *p < 0.05, ***p< 0.001 vs control group; #p< 0.05, and ###p< 0.001 vs STZ group.

the development of cardiac fibrosis using an experimental diabetic rat model. A decrease in myocardial Nrf2 activity with concomitant increase in oxidative stress was seen in streptozotocin (STZ)-induced diabetic rats (Fig. 1A-1C). Moreover, chronic oxidative stress induces caspase-dependent apoptosis and DNA fragmentation in various diseases. Therefore, we analyzed the effect of Nrf2 activation on caspases-dependent cardiac cell death. A marked increase was observed in activities of caspase-9 and -3 ($p<0.05$) in diabetic hearts (Fig. 1D-1E). Interestingly, pharmacological induction of Nrf2 by sulforaphane (SFN) could ameliorate these changes significantly. Systemic chronic inflammation is another imperative factor involved in the pathophysiology of diabetes-related complications. Cardiac levels of activated NF-κB (pp65), a pro-inflammatory signaling protein, were found to be elevated after diabetes induction. We also observed an increase in phosphorylation of IκB-α, suggesting its inactivation under hyperglycemic condition. It was observed that Nrf2 activation restored these changes to normal levels thus reducing the inflammation in diabetic condition (Fig 2A).

Since cardiac fibrosis and pathological hypertrophy are key triggers for the development and progression of DCM, thus myocardial hypertrophy was assessed by examining the levels

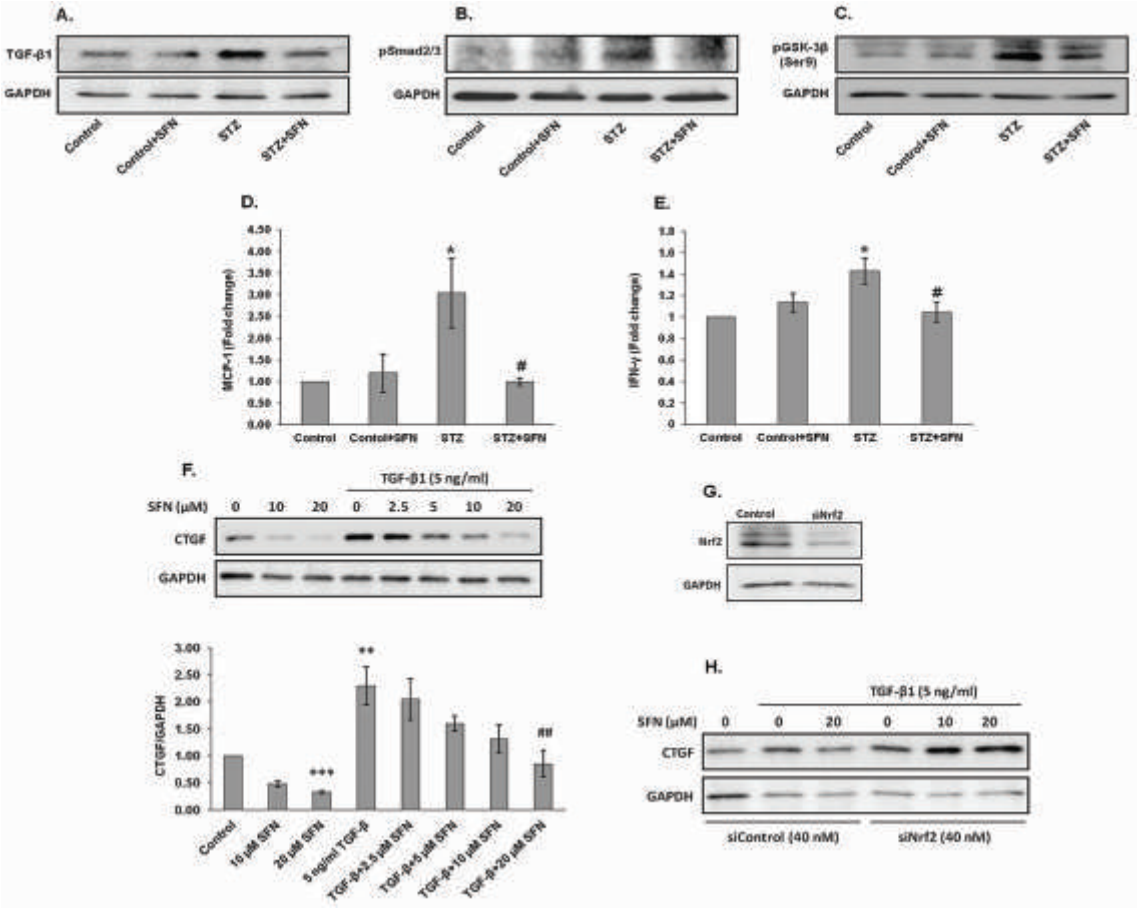


Fig. 3: Nrf2 prevents cardiac remodeling by inhibiting TGF-β/Smad3 signaling pathway. Representative western blot image for (A) TGF-β1, (B) pSmad2/3 and (C) pGSK-3β protein expression. GAPDH was used as internal control. Serum levels of (D) MCP-1 and (E) IFN-γ, measured by ELISA. All values are given as the mean ± SEM, (n= 4-5/group). *p<0.05 vs control group; #p<0.05 vs STZ group. (F) Protein expression of CTGF after TGF-β1 treatment to rat cardiomyoblasts. Representative western blot image (Upper panel) and densitometric analysis (lower panel) (G) western blot analysis for Nrf2 silencing (H) evaluation of CTGF protein expression after Nrf2 silencing. Images are representative of three independent experiments. All values are given as the mean ± SEM, (n= 3). **p<0.01, ***p< 0.001 vs control; ##p<0.01 vs TGF- β1.

of atrial natriuretic peptide (ANP). Significant increase (p<0.001) in ANP was observed in diabetic rat hearts (Fig 2C). Further, myocardial interstitial and perivascular collagen deposition and expression of connective tissue growth factor (CTGF) (Fig 2B and Fig 2D) was also increased in diabetic rats confirming cardiac fibrosis. These changes were significantly alleviated in SFN-treated diabetic rats.

TGF-β1 signaling plays a crucial role in cardiac fibrosis following injuries. Significant increase in the levels of TGF-β1 and its modulators, such as Smad3, pGSK-3β (Ser9), IFN-γ and MCP-1was observed in the heart tissues of diabetic rats (Fig 3A-3E). Treatment with SFN normalized the levels of TGF-β1 signaling modulators and prevented cardiac fibrosis in diabetic animals. Moreover, in TGF-β1-treated H9c2 cells, Nrf2 knockdown could reverse SFN-mediated down-regulation of CTGF, thus confirming the crucial role of Nrf2 in the development of cardiac fibrosis (Fig 3F-3H). Additionally, reduction in cardiac fibrosis by Nrf2 induction was found to counteract the progression of diabetes-induced left ventricular dysfunction (data not shown). In conclusion, our study implies that Nrf2 mitigates cardiac fibrosis by regulating redox homeostasis and inflammation, thereby restoring cardiac structure and function in diabetes.

Future research plans

- Further, efforts are under way to investigate the role of epigenetic mechanisms in the oxidative stress related enzymes playing an important role in the pathophysiology of diabetic cardiomyopathy.

Deepa Subramanyam

deepa@nccs.res.in

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, the hows, whys, whens and whats of this process remain to be worked out. The research carried out in our laboratory aims to address these issues.

Aims and Objectives

- To uncover components of the vesicular transport machinery that play a role in reprogramming and maintenance of pluripotency.
- Establishing tools and techniques to generate genetically modified mice using CRISPR-Cas9 technology.

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 were included in this screen. These included mock transfected cells that

Participants

Shalmali Bivalkar, *PDF*
YV Narayana, *SRF*
Ramaraju Ambatti, *JRF*
Sarita Kumari, *JRF*
Apurv Solanki, *JRF*
Suryabansi Singh, *JRF*
Ridim Mote, *Project JRF*
Jayashree Jagtap, *Technician*

Collaborators

Raghav Rajan, *IISER Pune, India*

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

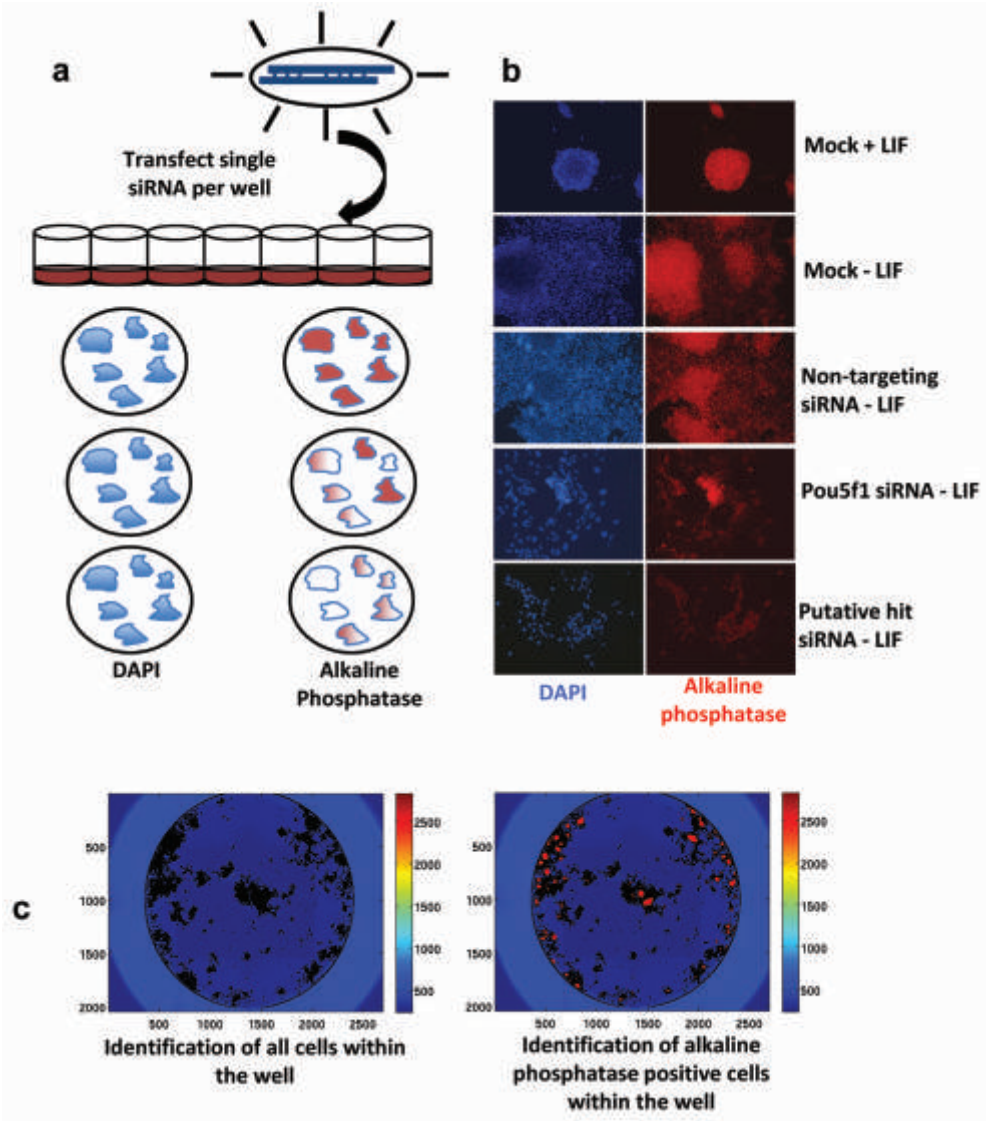


Fig. 1: Schematic of siRNA screen to knockdown genes involved in endocytosis in mouse embryonic stem cells:

(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.

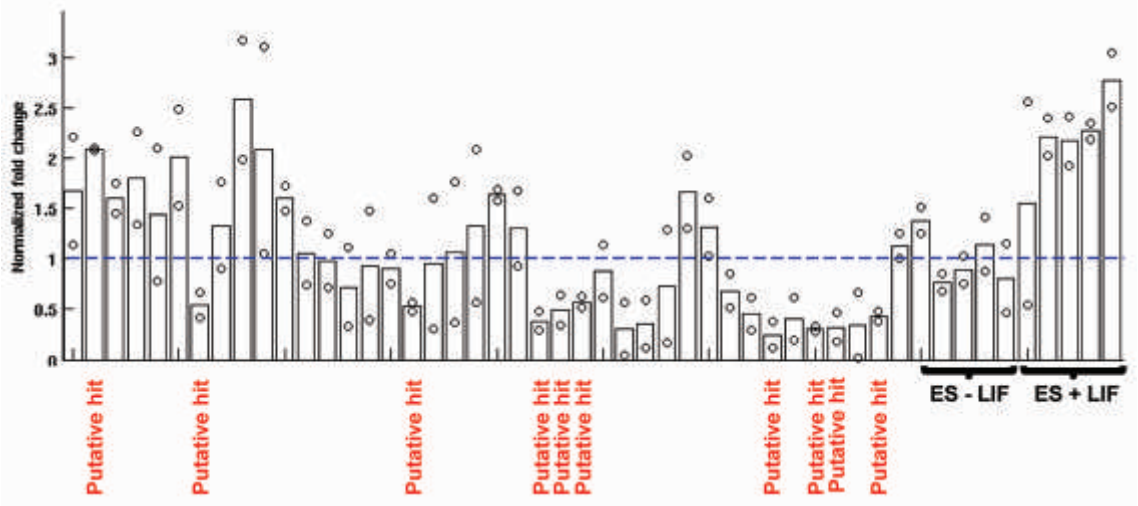


Fig. 2: Knockdown of genes involved in endocytosis and vesicular trafficking impact pluripotency of mouse embryonic stem cells: Graphs showing normalized fold change in Alkaline phosphatase staining compared to staining observed in mESCs grown under ES-LIF conditions. Each screen was repeated twice and 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.

knockdown of that particular gene did not affect differentiation or pluripotency compared to the control. If the value was 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.

Future Research Plans

- Development of knockout embryonic stem cell lines and mice using CRISPR-Cas9 technology to understand the role of endocytic genes in pluripotency and during early mouse embryonic development.

Vidisha Tripathi

tvidisha@nccs.res.in

Gene regulatory functions of mammalian long noncoding RNAs [lncRNAs] during quiescence-proliferation axis

Background

In eukaryotes, cells integrate cues from their surrounding environment and interpret these signals in order to retain their capacity to transition between a cycling state and a resting state, termed quiescence. The ability of cells to retain viability during quiescence and their entry or exit into cell cycle when needed, is necessary for complex multicellular processes. This transition is associated with changes in gene expression accompanied by alterations in chromatin modifications. Dysregulation of this balance can lead to a wide range of hypo and hyper-proliferative pathological conditions such as fibrosis, autoimmune diseases, cancer and ageing. Instead of just being passive, quiescent cells are transcriptionally active and express a set of genes that are distinct from those in proliferating cells, or in cell cycle arrested cells. Several important transcriptional regulators of quiescence have been described, eg. Myc, E2F, HES1, SALL2, MXI1. Additionally, various miRNAs have also been implicated to regulate the expression of their target genes during quiescence. Despite these proposed factors, the drivers and the exact mechanisms by which this balance of gene expression is maintained, is not completely understood.

Another class of regulatory molecules, long noncoding RNAs [lncRNAs], has been implicated in regulating various cellular processes including terminal differentiation and senescence. In contrast to the extensive evidence indicating that lncRNAs participate in a wide-repertoire of cellular contexts, their potential role in controlling mature tissue homeostasis and differentiation remains largely uncharacterized. Moreover, their involvement in the regulation of cellular quiescence and cell-state transition has not been firmly established.

Participants

Sonali Jathar, *JRF*
Juhi Srivastava, *JRF*
Vikas Dongardive, *JRF*
Vikram Kumar, *JRF*
Abhishek Keer, *JRF*

Aims and Objectives

- Characterization of complete lncRNA signature associated with cellular quiescence and proliferation.
- Delineation of the regulatory mechanisms through which lncRNAs orchestrate these processes.

Work Achieved

Characterization of the complete lncRNA signature associated with cellular quiescence and proliferation.

Whole transcriptome sequencing of human fibroblasts during quiescence: A detailed systematic time course analysis to determine lncRNA signatures in cells undergoing quiescence would help to identify factors mediating the entry and exit of cells into this state and also delineate the pathways activated. A detailed expression analysis was performed at three stages: early, late and intermediate response to growth factor [serum] deprivation [SD] and stimulation [SS]. The data obtained were further validated by qPCR for candidate showing most significant changes. To delineate the unique pathways employed during the transition to quiescent and proliferation state, we focused on the lncRNAs induced early in response to SD or SS.

Characterization of unique lncRNA expression program in response to SD/SS: The data obtained in Aim 1.1 is being further validated by qRT-PCR for candidates showing most significant changes. To delineate the unique pathways employed during transition to quiescent or proliferation state, we focus on the lncRNAs induced early in response to SD or SS. Further, comparison of the candidates induced in early response with the sets induced by prolonged entry would be performed. This would help understand the transcriptional regulation of initiation or maintenance of quiescence or proliferation.

Delineation of the regulatory mechanisms through which lncRNAs orchestrate these processes.

We have identified a novel lncRNA, LNC11q that is induced at early response to growth factor stimulation. LNC11q is approximately 3kb long nuclear retained noncoding RNA. This RNA is highly conserved among mammals and is expressed in wide variety of cells/tissues, indicating its potentially important function. Quiescent fibroblasts express extremely low levels of LNC11q, when induced with serum the LNC11q levels increase and start to reduce approx 12hrs of serum stimulation.

Determine the effect of depletion or overexpression of candidate lncRNAs on cell state transition: In order to understand the physiological function of LNC11q and its involvement in driving the cells to proliferation, we examined the effect of its depletion on cell cycle progression in HDFs that have a finite life span. Flow cytometry data revealed that the cells depleted of LNC11q showed slower S-phase progression with concomitant G2 arrest. These results suggest that LNC11q is required for proper entry of quiescent cells into cell cycle upon serum stimulation. To further investigate the mechanism of action of LNC11q and the pathways regulated by this RNA, a detailed gene expression analysis is in progress.

Identificatin of protein targets of candidate lncRNAs: LncRNAs normally act together with specific proteins. To identify protein targets of relevance to quiescence and proliferation, that would shed light on the possible functions of LNC11q, we performed an in vitro RNA pull down assay. Using this approach, we identified several RNA binding proteins, HuR, AUF1, PTB, PUF60, hnRNPU as interacting partners to LNC11q. The results were further confirmed by a second RNA-pull down followed by western blotting. Using HuR antibody, we performed a reverse IP and confirmed the specific interaction of HuR with LNC11q. In order to understand the direct effect of LNC11q on HuR, we analyzed HuR levels in LNC11q depleted extracts and found no significant change in its levels. Interestingly, depletion of HuR also leads to no significant change in the levels of LNC11q. Further experiments to understand the functional significance of interaction of HuR with LNC11q and its activity during cellular proliferation are under progress.

Future Research Plans

- Understanding the mechanism of action of LNC11q mediated through HuR.
- Identification of protein interactors of the candidate lncRNAs in early stages of quiescence.
- Studies on the effect of depletion or overexpression of the candidates in regulation of quiescence and cellular proliferation.
- Functional characterization of lncRNAs induced at early stages of cellular quiescence.
- Functional validation of the candidates in other quiescence models.
- Mechanism of action of selected candidates in driving cellular proliferation.
- Understanding the temporal regulation of lncRNAs during the quiescence-proliferation axis.

Mohan Wani

mohanwani@nccs.res.in

Studies on role of IL-3 in regulation of RANKL and OPG expression in osteoblasts

Participants

Kanupriya Singh, SRF
Vikrant Piprode, *SRF*
Suhas Mhaske, *SRF*
Anil Kumar, *SRF*
Shubhanath Behera, *JRF*
Adrita Guha, *JRF*
Juilee Karhade, *JRF*
Garima Pandey, *JRF*
Amruta Barhanpurkar, *BioCARe Scientist*
Lekha Goyal, *Research Associate*
Satish Pote, *Tech. Officer A*

Collaborators

Gyan C. Mishra, *NCCS, Pune*
Rajesh Gokhale, *IGIB, New Delhi*

Background

Bone maintains its structural and functional integrity by physiological process of bone remodeling that comprises integrated activities of bone resorbing osteoclasts and bone forming osteoblasts. Bone remodeling is regulated by various factors including hormones, growth factors and cytokines. Osteoblasts regulate bone remodeling by producing both stimulatory and inhibitory factors that tightly regulate osteoclasts formation and activity. The stimulatory factors include receptor activator of NF-κB (RANK) ligand (RANKL). RANKL protein is found in two functional forms, a transmembrane glycoprotein on the surface of cells, and a secreted soluble protein. Binding of RANKL to its receptor RANK on the surface of osteoclast precursors initiate signals, which lead to fusion, maturation, activation, and survival of osteoclasts. The inhibitory factor secreted by osteoblasts is known as osteoprotegerin (OPG), which is a decoy receptor for RANKL that prevents the binding of RANKL to RANK, thereby inhibiting osteoclast differentiation and activation. Thus, any alterations in the RANKL/OPG ratio modulate bone remodeling and homeostasis in skeletal diseases such as osteoporosis, Paget's disease and osteoarthritis.

Cytokines secreted by immune and other cell types play an important role in regulation of bone remodeling. Previously, we have reported that interleukin-3 (IL-3), a cytokine secreted by activated T cells, is a potent inhibitor of osteoclastogenesis and inhibits both RANKL and TNF-α-induced osteoclast differentiation and bone resorption. We have also demonstrated that IL-3 enhances in vitro osteoblast differentiation and matrix mineralization from human mesenchymal stem cells (MSCs); and also enhances the ectopic bone formation in immunocompromised mice. However, the role of IL-3 in regulation of osteoblast-osteoclast interactions and underlying mechanism(s) is

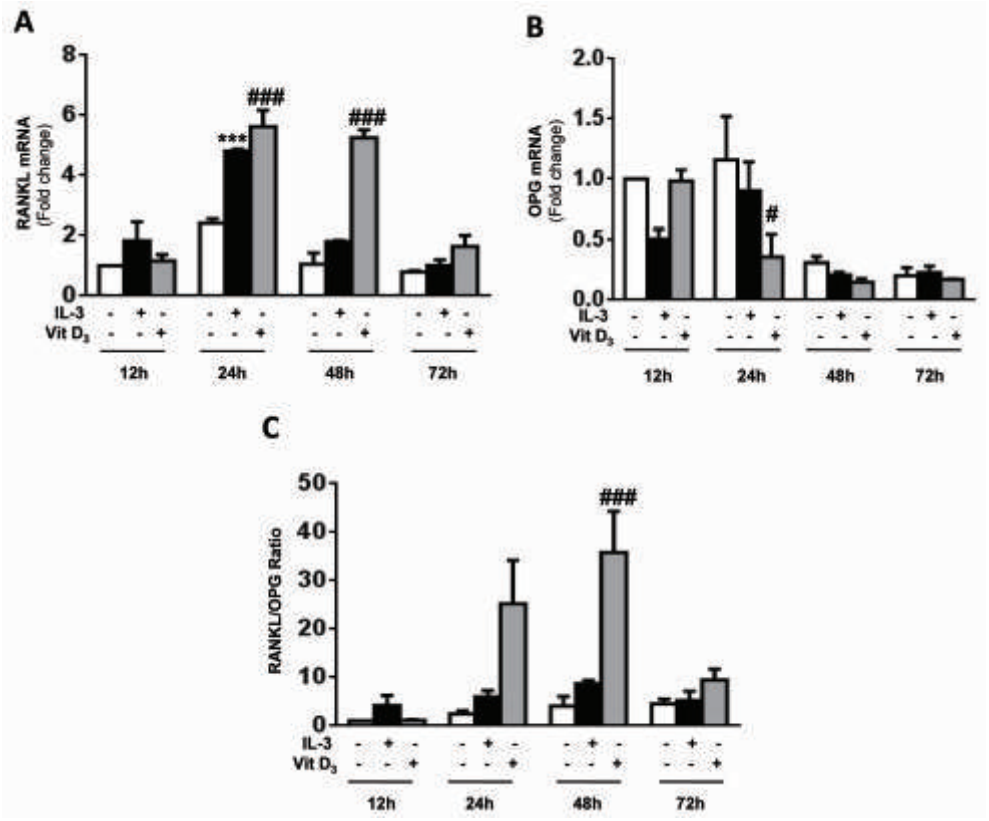


Fig. 1: Effect of IL-3 on RANKL and OPG expression at gene level. Mouse calvarial osteoblasts were cultured for 12, 24, 48 and 72 hours in osteogenic media in presence of recombinant mouse IL-3 (100 ng/ml) or vitamin D3 (10^{-8} M) and gene expression of RANKL (A) and OPG (B) was determined by qPCR. (C) Represents the ratio of quantitative value of RANKL and OPG genes expression. Bar graphs are expressed as mean ± SEM of three independent experiments. Significance was calculated by a one-way ANOVA followed by post hoc bonferroni's multiple comparison test. ***$p < 0.001$ IL-3 versus untreated controls and #$p < 0.05$, ###$p < 0.001$ vitamin D3 versus untreated controls.

not yet delineated. In this study, we investigated the role of IL-3 in modulation of RANKL and OPG expression in osteoblasts.

Aims and Objectives

- To study the role of IL-3 in regulation of RANKL and OPG expression by calvarial osteoblasts.
- To investigate the molecular mechanisms(s) for regulation of RANKL and OPG expression by IL-3.

Work Achieved

IL-3 enhances RANKL expression without affecting OPG

To evaluate the role of IL-3 on RANKL and OPG expression, we cultured mouse calvarial osteoblasts for 12, 24, 48 and 72 hours in osteogenic media in presence of IL-3 or vitamin D_3, and the expression of RANKL-OPG was examined by real time PCR. Vitamin D_3 was used as a positive stimulator of RANKL expression. It was observed that vitamin D_3 significantly increased RANKL expression at 24 and 48 hours. IL-3 increased RANKL expression at all the time points and significant effect of IL-3 was seen at 24 hours (Fig. 1A). Vitamin D_3 significantly decreased OPG expression at 24 hours. However, IL-3 did not show any effect on OPG expression at any time points (Fig. 1B). Vitamin D_3 significantly increased RANKL/OPG ratio, however, the effect of IL-3 on RANKL/OPG ratio was not significant (Fig. 1C).

Next, we observed that vitamin D_3 and IL-3 significantly enhanced RANKL expression at protein level (Fig. 2A and 2B). It was observed that both IL-3 and vitamin D_3 significantly increased expression of total and membrane bound RANKL (data not shown). We observed that IL-3 showed no effect on both intracellular and functional OPG expression. All these results suggest that IL-3 increases RANKL at both gene and protein levels and has no effect on OPG expression.

IL-3 differentially regulate RANKL expression through metalloproteases and JAK2/STAT5 pathway

We further observed that IL-3 increased the number of mononuclear osteoclast precursors, however, it was unable to induce the formation of multinuclear osteoclasts. These results

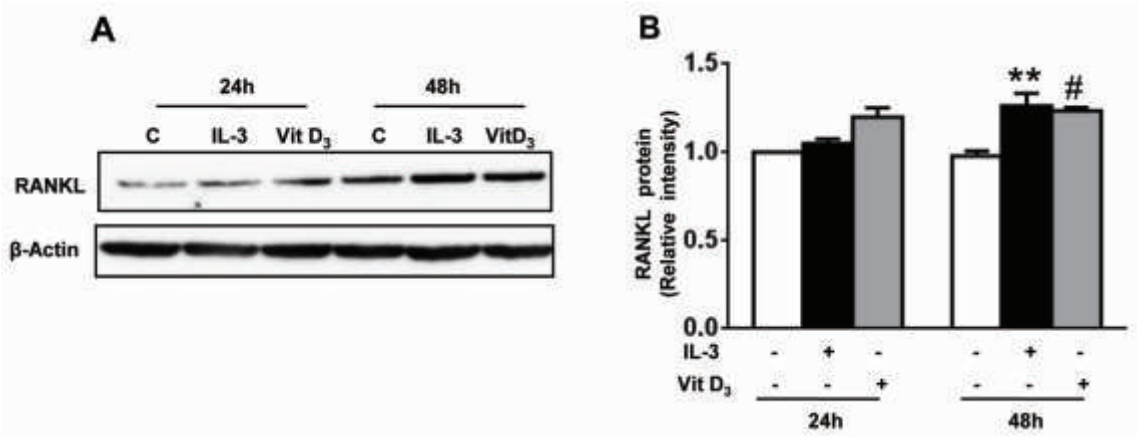


Fig. 2: Effect of IL-3 on RANKL expression at protein level. Mouse calvarial osteoblasts were cultured for 24 and 48 hours in presence of IL-3 or vitamin D_3. RANKL expression was analyzed by western blotting (A) and relative intensities (B) were calculated by densitometry using ImageJ software. Bar graphs are expressed as mean ± SEM of three independent experiments. **p < 0.01 IL-3 versus untreated controls and #p < 0.05 vitamin D_3 versus untreated controls.

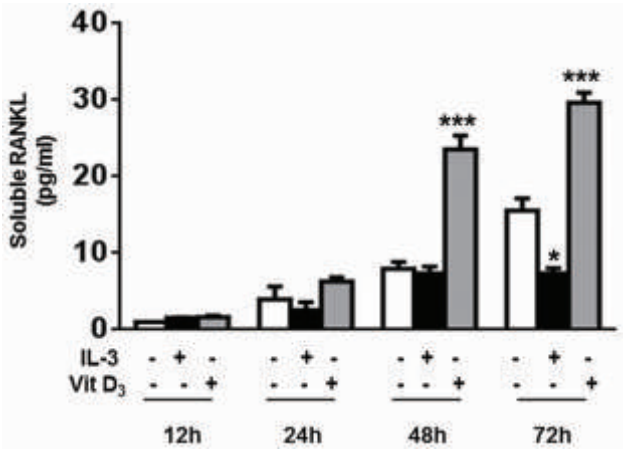


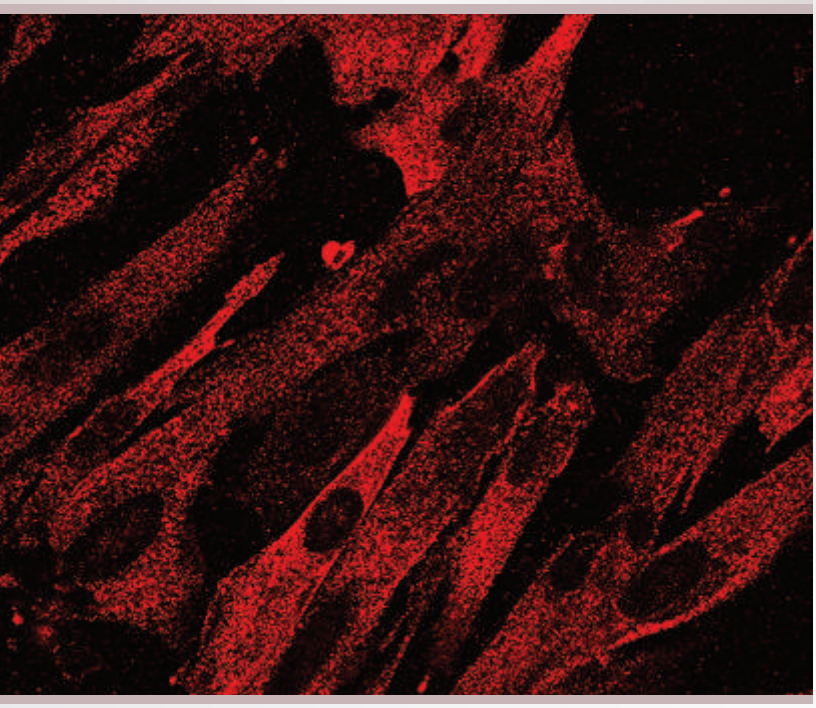
Fig. 3: Calvarial osteoblasts were cultured for 12, 24, 48 and 72 hrs with IL-3 or vitamin D_3 and secretion of RANKL protein in culture supernatants was analyzed by ELISA. *p < 0.05 IL-3 versus osteogenic media control. ***p < 0.001 vitamin D_3 versus osteogenic media control.

suggest that in spite of increasing RANKL expression, IL-3 was unable to induce formation of mature osteoclasts. To investigate the possible reasons for this action of IL-3, we evaluated the effect of IL-3 on soluble form of RANKL in culture supernatants. Interestingly, we found that IL-3 decreases the secretion of soluble RANKL at 24 and 72 hours whereas vitamin D_3 increases the soluble RANKL (Fig. 3). These results suggest that IL-3 differentially regulates membrane and soluble RANKL; and hence, it was unable to induce mature osteoclasts formation.

The soluble RANKL is formed by the proteolytic cleavage of membrane bound RANKL. This process is called as ectodomain shedding which is regulated by various ADAMs and MMPs. Interestingly, we found that IL-3 significantly down-regulated the expression of ADAM10, ADAM17, ADAM19 and MMP3. These results suggest that although IL-3 is capable of increasing RANKL expression at transcript and membrane bound protein levels, it decreases soluble RANKL by down-regulation of metalloproteases that eventually hinder the cleavage of soluble RANKL from its membrane form. Furthermore, IL-3 increases membrane RANKL by activating JAK2/STAT5 pathway. Thus, our results indicate that IL-3 differentially regulates two forms of RANKL through metalloproteases and JAK2/STAT5 pathway.

Future Research Plans

- Our in vitro studies using mouse osteoblasts strongly suggests that IL-3 has an important role in regulation of RANKL. In future studies, we will investigate the in vivo role of IL-3 on RANKL and OPG expression.



Support Units & Other Facilities

Experimental Animal Facility

Dr. Ramanamurthy Boppana
(Facility In-Charge)

The Experimental Animal facility (EAF) at the National Centre for Cell Science is a scientific support service department providing a variety of services in the area of laboratory animal experimentation for the research and development programs of the Institute. 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 and supply of small laboratory animals, viz. inbred and mutant mice, rats, rabbits etc., for the ongoing research projects of the institute. The following is the list of various laboratory animals maintained at the facility:

MICE:

- BALB/cJ
- C57BL/6J
- DBA/2J
- DBA/1/J
- 129/SvJ
- FVB/NJ
- SWISS#
- BALB/c*
- NZB
- AKR#
- CF1
- CD1

Genetically engineered mutant mice (knock-out, transgenic and mutant mice - 41 lines)

RATS:

- WISTAR

The Team

Dr. Rahul M. Bankar
Mr. Md. Shaikh
Mr. A. Inamdar
Mr. Prakash 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

RABBITS:

NEWZEALAND WHITE

* BALB/c with cataract mutation # Outbred

Defined operating procedures are followed in the maintenance of the laboratory animals.

The breeding program for the propagation of the inbred mice is planned and executed to meet the needs of the 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. Complete scientific support and advice is extended as per demand to the scientists and their group members, for the conduct of experiments under IAEC-approved projects.

The total number of mice strains, inbred, outbred, and mutant and hybrids, being maintained at the Experimental Animal Facility, stands at 54. The foundation/nuclear colonies of mice are housed in individually ventilated caging systems. Genetic monitoring using standard protocols for mutant mice and select microsatellite markers for the major inbred strains is carried out regularly by PCR.

As a part of human resource development, 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

Dr. Varsha Shepal *(Technical officer)*

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 283 samples including 100 external samples from April-2016 to March-2017.

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 161 samples including 10 external samples from April-2016 to March-2017.

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 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 MICRO-LC

EKSIGENT NANO-LC and SPOTTER

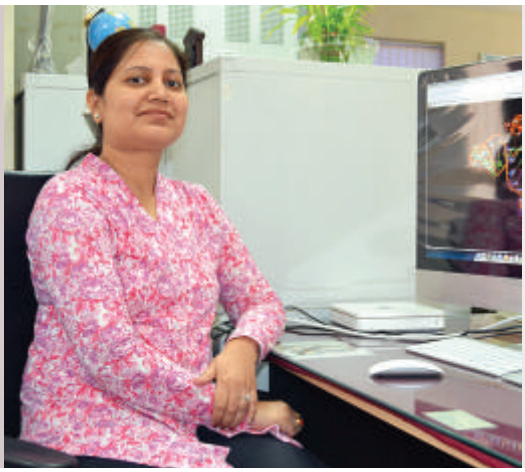
AGILENT GC-MS

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

Integrated 4 port SATA 6GBs controller

Integrated 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 (4 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

HP Elite Desktop of Intel i7 processor, 3.4GHz with 16GB RAM, 2TB SATA storage and 21.1" LCD wide display with Windows 8.1 Professional 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

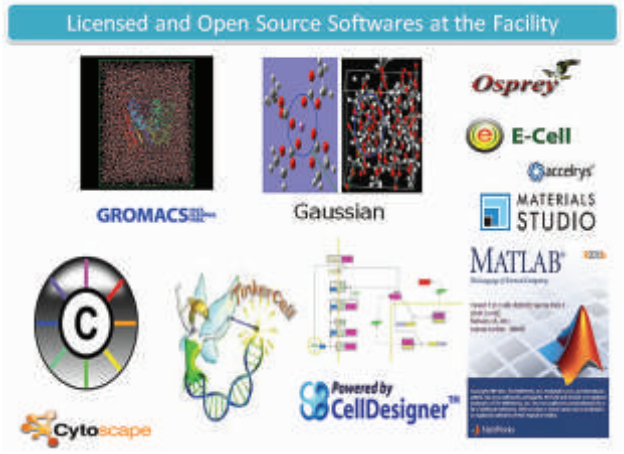
The Bioinformatics Facility at NCCS has procured several software for scientific research having commercial and/or academic license. These are:

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

Molecular Modeling: Modeler

Molecular Docking: AUTODOCK, HADDOCK, ClusPro

Pharmacophore Modeling: Auto Pharmacophore generation,



Receptor-ligand pharmacophore generation, 3D QSAR pharmacophore generation, Steric Refinements with excluded volumes.

Network Modeling: CellDesigner

Toxicity Prediction: Molinspiration, DSSTox, PreADMET Toxicity Prediction

QSAR: Create Bayesian Model, Recursive Partioning Model, Multiple Linear Regression Model, partial least squares model, genetic function approximation model, 3D QSAR model. 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

Workshops conducted by the Bioinformatics and High Performance Computing Facility:

Three workshops on diverse topics related to applications of computational biology were conducted by the Bioinformatics and High Performance Computing Facility of NCCS, during 2016-17. Two of these were in-house workshops organized for the graduate students and project staff of NCCS, while the third one was conducted for students enrolled in the Ph.D. coursework of the S. P. Pune University (Dept. of Biotechnology), which is conducted annually by NCCS. The workshops were designed to train the participants to develop a computational framework for gene survey of biological sequences, including structure prediction, phylogenetic analyses, motif prediction, network modeling, molecular docking, protein-protein interaction, etc., to help them develop inferences about biological mechanisms and hypotheses for further experimental testing.

Library

The NCCS library is listed in the Union Catalogue of Biomedical Serials in India created by the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi and continues to be a member of the Medical Library Association of India.

The NCCS library has a collection of publications in frontier areas of biotechnology. The library's priority is to support the research activities of NCCS. Therefore, the collection is expanded in consultation with the NCCS faculty. The library's print collections are growing by approximately 400 volumes per year. The library holds approximately fourteen thousand five hundred bound journals, three thousand five hundred books, and two hundred and thirty five NCCS Ph.D. theses. It subscribes to twenty scientific journals and thirty other periodicals in print form. The staff and students are provided access to 734 online publications, including journals and the online book series, Methods in Enzymolgy, which are published by various publishers, including Springer, John Wiley, Nature Publishing group, Mary & Libert, Oxford, Elsevier Science Direct, through DeLCON, the online journal consortium of DBT. The library also subscribes to eight additional online journals related to research areas of interest to the NCCS faculty. Furthermore, the library regularly purchases books and magazines in Hindi for general reading.

The library has the Linux-based SLIM21 library software for its housekeeping operations, with an RFID-interfaced book security system, and Web-OPAC for online searching of the library documents. Additional facilities in the library include CD-ROMs for a number of books and a local area network providing access to the internet for PubMed search and other associated activities.

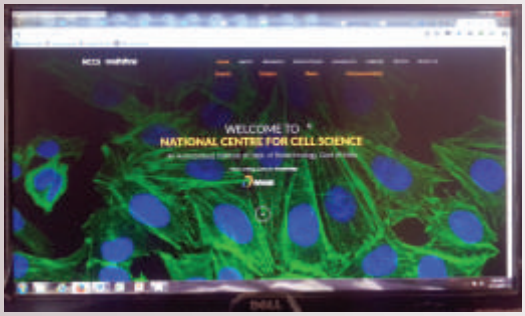
The library personnel are involved in providing library-related information for the NCCS website, including library holdings, services, useful links and other relevant information. During the period under review, they have created a digital archive of the Ph.D. theses submitted by the NCCS research scholars to the University, which are accessible through the NCCS intranet.

In addition to the above, the library also provides in-house services for scanning documents using the iThenticate Anti-Plagiarism Software. It also uses the Turnitin Anti-Plagiarism Software for scanning Ph.D. theses prior to their submission to the Savitribai Phule Pune University. The library has also set up an open access repository for the research publications of the NCCS faculty, which is available through the link: http://nccs.sciencecentral.in

The Team

Dr. K. S. Behera *(Technical Officer)*

Rameshwar Nema *(Technical Officer)*



Computer Section

Dr. M.V. Krishnasastry

(Facility In-Charge)

The Computer Department provides various computing and network infrastructure services to the scientists, technical and administrative users of NCCS. The department helps in keeping the infrastructure up-to-date in both hardware and technical support, as well as network services.

The department provides secured network services, including the design of campus-wide LAN/WAN solutions and internet /intranet solutions, besides providing computing services to ongoing R&D projects. The department has installed three internet links, viz. high speed STM-1 (155Mbps bandwidth) from the PowerGrid Corporation India Limited, 100Mbps bandwidth from NKN, and 10Mbps from Tata communications Ltd. The internet facilities are extended to all institute users and visitors, including visitors in the guest house. The present Network security system has been upgraded with the latest Sophos UTM firewall CR-1500XP and Sofos Antivirus for desktops to provide a cohesive secured environment.

Technical Support Services:

- Wired and wireless networking solutions & services to desktops, laptops and mobile phones.
- Internet connectivity for all scientists, staff and students.
- Computer hardware infrastructure procurement, installation and maintenance.
- Web services include design and maintenance of the intranet and NCCS website and its management.
- User support services including software and hardware installations, for printers , scanners and all other computer-related devices.
- E-mail service for all staff members including scientists, technical and administrative staff and research scholars.
- Technical support for video conferencing / SKYPE / DROPBOX / VPN.
- Virtualised high performance servers managing services like WWW, DHCP, DNS and proxy.
- Network management of high speed routers, switches and WL access points.

The Team

Mr. Rajesh Solanki *(Technical Officer)*

Mr. Shivaji Jadhav *(Technical Officer)*

Mrs. Rajashri Patwardhan *(Technical Officer)*

Mrs. Kirti Jadhav *(Technical Officer)*

New Initiatives:

1. New NCCS website

The new NCCS website was designed, developed and hosted with standards conforming to the Digital India initiative. It was developed in the php Codeignitor framework. A new server having Linux CentOS7.0 was also configured for hosting the TransFlux application.

2. New Cyberom Firewall

A new UTM firewall named Cyberoam CR1500ING-XP was installed and configured according to the NCCS security policy. This firewall has advanced features like wireless network protection, Web application firewall, Gigabit fibre channel module etc. This firewall has been working efficiently.

3. Wifi Network connectivity in the new hostel

As per the requirement of research fellows, a new wifi network has been commissioned with configuration of 3 CISCO switches and 12 APs on the three floors of the new hostel. This wifi network is centrally controlled and managed from a secured Cyberoam firewall. The wifi service to access the internet is configured for use by about 60-100 students.

4. Storage capacity augmented

The capacity of the HP storage server (HP MSA2040) was increased by installing 18 SAS hard disk drives (HDDs) of 1.2TB. These HDDs are configured in RAID 6 to avoid any data loss in case of single drive failure and is presented to the HP Blade Server.

5. Sophos Antivirus

A new virtual Win 2008 server is configured for hosting the Sophos Antivirus for local upgrades and updates of virus definition. The cloud-based client software has been installed on all desktop PCs. This software provides real time protection against latest malware, robots, ransomware, threats etc.

6. VMware upgradation

The VMWARE std. edition and Vsphere was upgraded from ver.5.0 to 6.0. for managing virtual servers. The new version has support for large storage capacity, 128vCPU, 4TB vMemory etc.

7. Store & Purchase Management Software

The software specification document for computerization of the stores & purchase (S&P) department was prepared. This software will computerise the S&P department and all associated procedures, starting from budget allocation to final payment. This system will have the option of exporting / importing data into it in the CSV, XL text formats, and will provide linkages to other department modules in the future, thereby enabling the computerization of all the departments of the institute.

8. Renewal of Tata Internet Connectivity

The 10 Mbps(1:1) ILL from TATA Communications was renewed for 1 year, i.e. for the time period, 2016-2017.

Other Facilities

1) FACS Core Facility

The Team

- **Dr. L. S. Limaye** (Facility In-charge)
- Hemangini Shikhare
- Pratibha Khot
- Amit Salunkhe
- Ashwini Kore
- Dnyaneshwar Waghmare
- Atul Khirwale *(Operator provided by BD and posted in NCCS under the BD-NCCS Stem Cell COE from Dec.2011)*

There are seven instruments in the FACS core facility of the institute, under the supervision of Dr. L. S. Limaye. These are operated on rotation basis by six dedicated operators. The usage of the instruments in this facility for the period under consideration is summarized below:

Table 1: IMMUNOPHENOTYPING & CELL CYCLE analysis

Equipment	Surface / Intracellular staining	DNA Cell cycle	CBA flex	CBA	Total Samples Acquired
FACS Calibur	1638	5419	---	---	7057
FACS Canto II (Old)	8123	231	---	30	8384
FACS Canto II (New)	8005	14	---	182	8201

Table 2: STERILE SORTING

EQUIPMENT	SORTING	ACQUISITION **
FACS Aria II SORP	203	644
FACS Aria III SORP	330	1342
FACS Aria III Standard	145	609

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

BD Pathway 855:

With the BD Pathway 855, imaging of samples can be done for live cell staining (96 well plates) as well as for fixed cells in the form of sections on slides. Images

are captured in montage form (e.g. 8X8, 10X10) with a mercury arc lamp and transmitted light. Macros are done as per the user's request.

Analysis of samples from extramural users:

In addition to the faculty and students of NCCS, researchers from other institutions also utilize the services of the NCCS FACS facility. Due to an increase in the number of samples received from such researchers, the charges for samples analysed at this facility for these end-users were revised in June 2012. The charges are lower for academic and research institutions than those for private institutes/ companies. Researchers from ARI, IRSHA, S. P. Pune University (departments of Biochemistry and Zoology) and Sinhagad college of engineering have availed themselves of this facility during the year 2016-17. A total of 242 samples were received from them for applications like Surface/ Intracellular staining and DNA cell cycle analysis.

Activities under the BD-NCCS COE programme:

i. 'BD HorizonTM Global Tour' Symposium

Under the BD-NCCS COE programme, BD organized a 'BD HorizonTM Global Tour symposium' at NCCS on 20th April 2016. Over 200 participants attended this symposium, including researchers from Pune and Mumbai.

This symposium included several talks by international application experts from BD, including their staff scientists and other senior personnel, which were aimed at creating awareness and updating the audience about several aspects of flow cytometry. The topics covered included strategies to design the best panels to answer experimental questions, analysis of multiple data sets for maximizing the resolution of the cell population of interest, sorting of highly pure cell populations through optimized panel design, and the future of flow cytometry.

The talks were widely appreciated and proved especially valuable to end-users of flow cytometry, such as the graduate students. The enthusiasm of the participants was evident from their highly interactive engagement in Q &A.

ii. Canto-II training and examination

Training was organized in batches on Canto-II during the period under consideration. A total of 19 students from NCCS received training in batches in July 2016, September 2016 and January 2017. An examination is scheduled in April 2017, for the trained students. The BD application specialists will conduct this examination with help from the NCCS FACS operators.

Science Day Activities:

On the occasion of the National Science Day, the facility operators, Hemangini Shikhare and Pratibha Khot, explained the 'NCCS Flow Cytometry Core Facility' to visitors with the help of a poster, as part of the open day at NCCS on 28 February 2017.

2) Imaging facility

The Team

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

The facility has three confocal microscopes. The details of these microscopes are given below:

i) Zeiss LSM510 META

Advanced Spectral Confocal Microscope, Zeiss LSM510 META: This system comprising of fully motorized and computer controlled Inverted Fluorescence microscope is used for confocal imaging regularly. The Lasers available are Blue Diode laser (405nm), multi-line Argon laser (458/477/488/514 nm), 543nm He-Ne and 633nm He-Ne. The spectral detector permits separation of upto eight emission signals, even if the fluorescence spectra are strongly overlapping.

ii) Leica SP5 II

This is a high-end Broadband Confocal Laser Scanning Microscope with 3 PMTs, 2 Hybrid detectors and AOBS technology equipped with CO2 incubator, fully motorized, automated and computer controlled microscope Leica DMI 6000. The Lasers are Blue Diode Laser 405nm, Ar Laser with 458nm, 488nm, 476nm, 496nm, 514 nm lines, DPSS 561nm, HeNe 594nm and HeNe 633nm with incubation chamber for live cell experiments as well as FRET and FRAP experiments. The software for Confocal imaging 3D imaging and reconstruction, Time lapse, colocalization, FRET (SE & AB), FRAP are also available.

iii) Olympus Fluoview 10i

This is an easy to use and self-contained confocal microscope equipped with four lasers [405, 473, 559 and 635nm].

The numbers of samples imaged during this year were approximately 7000 in-house, plus 50 samples received from various other institutes.

On the occasion of the National Science Day on 28 February 2017, facility was open to all for demonstration. Students from various colleges and faculties visited the facility.

3) DNA sequencing facility

The Team

- Dr. Yogesh Shouche *(Facility In-Charge)*
- Dr. Kamlesh Jangid *(Scientist In-Charge, NCMR)*
- Dr. Sarang Satoor *(Technical Officer)*
- Mr. Mandar Rasane *(Technician)*
- Mr. Vikas Patil *(Technician)*
- Mr. Sunil Dhar *(Technician)*

The central sequencing facility is located at the National Centre for Microbial Resource (NCMR) and houses two instruments (3730 and 3730xl) from Applied Biosystems along with all sequence and data analysis software. The facility offers services related to the identification of microorganisms; sequencing of plasmids, PCR products and cloned inserts; primer walking; and genotyping and fragment analysis to researchers from NCCS and other organizations. In addition, the facility serves as the back-bone of culture authentication and identification for NCMR's preservation activities. During the period 2016-17, a total of 22570 sequencing reactions were run and 3924 cultures of bacteria and fungi were identified by sequencing the 16S rRNA gene and ITS regions, respectively, at the facility.

4) IVIS Imaging System

The Team

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

The IVIS imaging system is a common central facility. This instrument provides bioluminescent and fluorescent imaging of cells or whole small animals under in-vitro and in-vivo conditions, respectively. The IVIS imaging instrument was used by over 30 research scholars and scientists of NCCS, as well as collaborators from other institutes. Bioluminescence as well as fluorescence imaging was used by them with different strains of mice (NOD/SCID/ NUDE/ C57/Balb/C etc) as well as with different types of tissue culture plates (i.e. 96 well, 24 well and 12 well etc).

The Xenogen IVIS-Spectrum System is capable of imaging bioluminescence and fluorescence in living animals. The system uses a novel in-vivo biophotonic imaging 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.

The IVIS imaging system includes a custom lens with a 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 allows 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 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 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

Benefits and Features if the IVIS Imaging System:

- 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 standard set of four filter pairs for fluorescent imaging are included 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

IVIS Imaging System

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*

This facility is an infrastructure service department of the institute. It provides essential support services to the research laboratories, cell repository, media section and other service departments. These services include washing, packing and sterilization of glassware and other research materials, thus making available a supply of sterile glassware and other material required for research. It also supplies high grade distilled water to all sections of the institute. In addition to this, some of the technical staff members of this facility are involved in ensuring the safe disposal of radioactive and biohazardous waste materials.

Microbial Culture Collection

Yogesh Shouche
yogesh@nccs.res.in

Participants
Tapan Chakrabarti, *Consultant*
Dilip Ranade, *Consultant*
Kamlesh Jangid, *Scientist*
Omprakash Sharma, *Scientist*
Dhiraj Dhotre, *Scientist*
Amaraja Joshi, *Scientist*
Neeta Joseph, *Scientist*
Rohit Sharma, *Scientist*
Amit Yadav, *Scientist*
Mahesh Chavdar, *Scientist*
Avinash Sharma, *Scientist*
Praveen Rahi, *Scientist*
Prashant Singh, *Scientist*
Shrikant Pawar, *Technical Officer*
Vishal Thite, *Technician*
Sonia Thite, *Technician*
Mahesh Sonawane, *Technician*
Madhuri Vankudre, *Technician*
Shalilesh Mantri, *Technician*
Vikram Kamble, *Technician*
Umera Patawekar, *Technician*
Vikas Patil, *Technician*
Sunil Dhar, *Technician*
Yogesh Nimonkar, *Technician*
Shraddha Vajjhala, *Technician*
Vipool Thorat, *Technician*
Archana Suradkar, *Technician*
Prachi Karodi, *Technician*
Mrinal Mishra, *Technician*
Mandar Rasane, *Technician*
Abhijeet Pansare, *Technician*
Swapnil Kajale, *Technician*
Mitesh Khairnar, *Technicain*
Tushar Ghole, Technician

Background

The Department of Biotechnology established a Culture Collection in June 2008 with broader charter to preserve, characterize and authenticate microbial resources. These are valuable raw materials 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 economic 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 (now known as National Centre for Microbial Resource, NCMR) is currently functioning in the interim laboratory facility at Sai Trinity, Pashan, since 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

Since its establishment, MCC received ~200000 cultures collected from various ecological niches as part of the microbial prospecting project undertaken by Department of Biotechnology (DBT) in collaboration with nine participating institutes/universities. 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.

Culture Preservation Status

All cultures received from the nine participants preserved in -80 °C freezers. In addition, all three star and re-fermented cultures and a significant proportion of the normal cultures also 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. Since then, MCC has finished the 1st passage of all three star and re-fermented cultures.

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 6710 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. During the year 2016, MCC has supplied 363 bacterial and 75 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. During the year 2016, MCC has authenticated 1023 cultures (833 bacterial and 190 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. During the year 2016, MCC has accessioned 21 bacterial cultures under IDA and 20 safe deposits. 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, phenotypic characterization, 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, during the year 2016 a total of 2901 cultures for bacteria and fungi together (including 34 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 4 requests for bacterial identification and phylogenetic analysis during the year 2016. 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.

MALDI-TOF MS based Biotyping:

Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometer (MALDI-TOF MS) is being used for the fast and accurate identification of microorganisms. The biggest challenge for MALDI-TOF MS based microbial identification is the availability of adequate database, especially for microorganisms of non-clinical origin. Since the installation of MALDI-TOF MS in April 2013 at NCMR, the protocols for sample preparation and analysis have been standardized. During the year 2016, a total of 15055 samples have been processed successfully for various organizations on payment basis. In addition to this, NCMR is also involved in the development of MALDI-TOF MS spectral database.

FAME Analysis

MCC has start providing FAME analysis as a service in February 2013. Along the year 2016, 76 (paid services) bacterial cultures have been analyzed on the MIDI system.

DNA-DNA hybridization and GC (mol %)

DNA-DNA hybridization (DDH) and G+C content (mol %) is 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. Over the year 2016, MCC has analyzed 3 paid bacterial cultures for DNA-DNA hybridization and 3 for % GC content.

Cyanobacteria in MCC Collection

MCC has started accepting cyanobacteria and is working actively in the identification and preservation of cyanobacteria belonging to diverse groups. Till now, MCC has received a total of 106 cultures for preservation under general deposits.

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.

Future Research 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.

Anaerobic microbes

The facility for the cultivation of anaerobic microbes is ready and we propose to start receiving anaerobes for deposit in all the three categories.

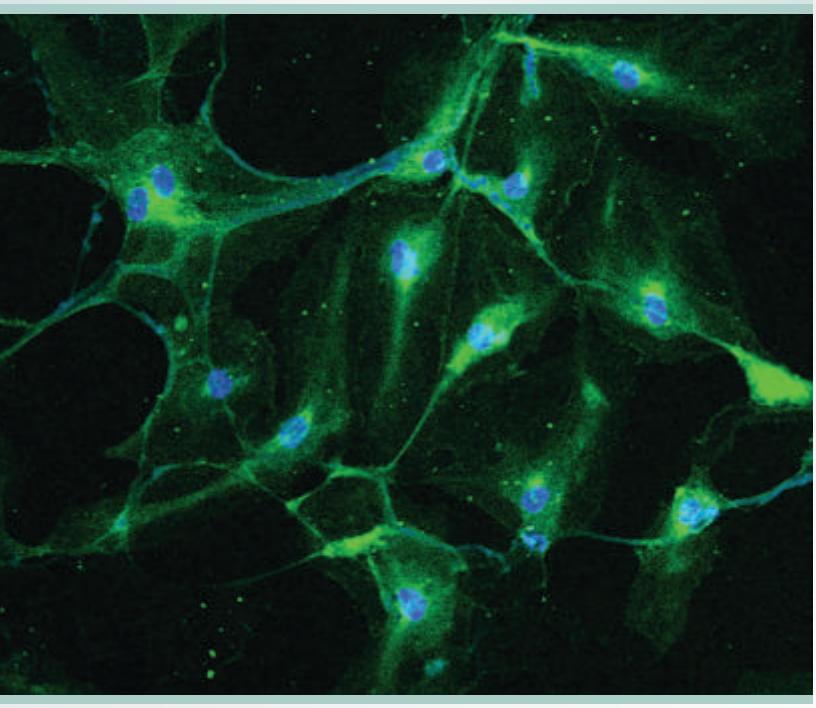
Supply of cultures to Industry

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.

Conferences / Events Organized by MCC

- Workshop on Nomenclature of Archaea and Bacteria by Prof. Aharon Oren Dates: September 9-10, 2016.

- Bergey's International Society for Microbial Systematics third meeting on 'Microbial Systematics and Metagenomics' at MCC-NCCS, Pune on 12-15 September 2016.
- MCC in association with AMI arranged 'International Symposium on Microbial Ecology and Systematics' at NCL, Pune on September 16-17, 2016.
- Lecture and hands on session on 'Gene annotation' arranged by MCC-NCCS for MSc second year students on 24th November 2016.
- Workshop on 'Microbial Identification and Preservation; 5-9 January 2017.
- A hands-on training workshop was organized from 1-10 March, 2017 on 'Microbial Identification, Preservation and Genome Analysis'.
- More than 500 hundred students from various colleges visited MCC during 2016. For every batch of students MCC staff explains the various facilities and services available at MCC.
- On the occasion of the National Science Day, students from schools were invited to visit MCC and were introduced to basic microbiology. Dr. Ranade delivered a talk on 'Bioenergy and biofuels' in English and Marathi.)



Other Information

Publications / Book Chapters / Patents

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Book Chapters / Invited Reviews / Editorials (NCCS Faculty)

1. Bussas V, Sharma A, Shouche Y (2017) IP and the Budapest Treaty - Deposit Biological material for patent purposes. In 'Microbial Resources: From Functional Existence in Nature to Applications'. Ipek Kurtboke (Ed.); Elsevier Inc. DOI: 10.1016/B978-0-12-804765-1.00014-X.

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5. Panda Suchismita, Shiras A, Bapat SA. Long Noncoding RNAs: Insights into their roles in normal and Cancer Stem Cells. In: Cancer and Non-coding RNAs, 2016, Chakrabarti J. and Mitra S. (Eds.), Elsevier Publishers.

6. Suresh A., Naik R.R., Bapat S.A. (2017) Role of Cancer Stem Cells in Oral Cancer. In: Kuriakose M. (ed) Contemporary Oral Oncology. Springer, Cham.

Publications of MCC Scientists

1. Ghosh, D. K., Bhose, S., Sharma, P., Warghane, A., Motghare, M., Ladaniya, M. S, Reddy M.K. Thorat Y, Yadav, A. First Report of a 16SrXIV Group Phytoplasma Associated With Witches'-Broom Disease of Acid Lime *(Citrus aurantifolia)* in India. Plant Disease. May 2017, 101(5): 831.
2. Jangid, K., Kao, M.H., Lahamge, A., Rathbun, S.L., Williams, M.A., and Whitman, W.B. K-shuff: A novel algorithm for characterizing structural and compositional diversity in gene libraries. PLOS One. 2016 Dec 2;11(12):e0167634. doi: 10.1371/journal.pone.0167634. eCollection 2016.
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4. Shanmugam, S.G., Magbanua, Z.V., Williams, M.A., Jangid, K., Whitman, W.B., Peterson, D.G., and Kingery, W.L. Bacterial diversity patterns differ in soils developing in sub-tropical and cool-temperate ecosystems. Microbial Ecology. 2017; 17: 556.
5. Sharma R, Girish Kulkarni, Sonawane MS. *Alanomyces*, a new genus of *Aplosporellaceae* based on four loci phylogeny. Phytotaxa. 2017; 297 (2): 168–178.
6. Sharma R, Prakash O, Sonawane MS, Nimonkar Y, Golellu PB, Sharma R. Diversity and distribution of phenol oxidase producing fungi from soda lake and description of *Curvularia lonarensis* sp. nov. Frontiers in Microbiology. 2016; 7:1847.
7. Suradkar, A., Villanueva, C., Gaysina, L. A., Casamatta, D. A., Saraf, A., Dighe, G, Mergn G, Singh, P. *Nostoc thermotolerans* sp. nov., a soil dwelling species of *Nostoc* (Cyanobacteria) isolated from Madhya Pradesh, India. International Journal of Systematic and Evolutionary Microbiology. 2017 May; 67(5):1296-1305. doi: 10.1099/ijsem.0.001800.
8. Yadav, V., Thorat, V., Mahadevakumar, S., Janardhana, G. R., & Yadav, A. First Report of the Association of the 16SrII-D Phytoplasma Subgroup with Little Leaf Disease of *Crotalaria* in Karnataka, India. Plant Disease. December 2016, Volume 100, Number 12 Page 2523.

Book Chapters / Invited Reviews / Editorials (MCC Scientists)

1. Ghosh, M., Saha, A., Jangid K., Joshi, A.A., and Chaudhuri, S.R. (2016) A polyphasic approach of species identification for genus *Bacillus*. In Chaudhuri, S.R. (ed), Life Science: Recent Innovations & Research. International Research Publication House, India.
2. Sharma R (2017) Ectomycorrhiza: Symbiotic fungal association with plant their diversity, ecology and practical applications. In: Mycorrhiza - Function, Diversity, State of the Art (eds: Varma A, Prasad A, Tuteja N). Springer International Publishing DOI: 10.1007/978-3-319-53064-2.

Patents resulting from research carried out by NCCS faculty

NCCS faculty inventor: Manas Santra

- *SRPX for treatment of cancer*'

  United States Patent Application: US 2016/0206693 A1; Publication date: 21 July, 2016

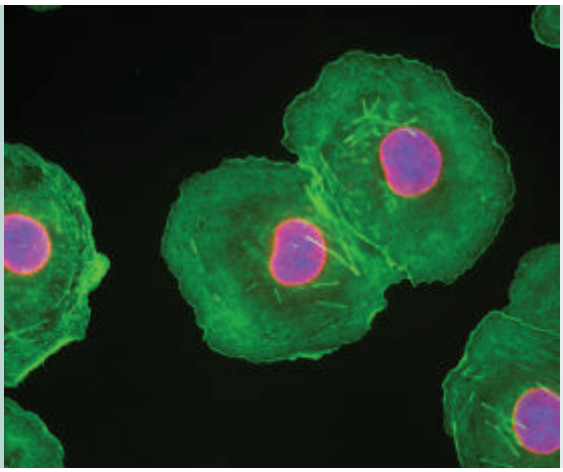
NCCS faculty inventor: Samit Chattopadhyay

- *An ER-specific reagent for monitoring HNO in biological objects*'

  India. Provisional Application No: 201611041925; Provisional filing date: 12/8/2016.

- *Ruthenium (II) complexs, preparation and uses thereof*'

  US Patent Granted: No. US 9505794 B2 , Publication date 29.11.2016



Awards / Honours / Memberships

Awards / Honours / Memberships - NCCS Faculty

Janesh Kumar

- EMBO short-term fellowship (February 2017)

Gopal Kundu

- Adjunct Professor, Curtin University, Perth, Australia (2015-till date)
- Elected as Associate Editor, Molecular Cancer (2015-till date)

Girdhari Lal

- International Congress of Immunology 2016 (ICI2016) travel grant by American Association of Immunologists (AAI), USA.
- Member, Society of Leukocyte Biology (SLB), USA.
- Member, American Association of Immunologists (AAI), USA.
- Member, European Academy of Tumor Immunology (EATI), France.
- Member, Molecular Immunology Forum, India.

Nibedita Lenka

- Nibedita Lenka, Chairperson, Institutional Ethical Committee, OCT Therapies & Research Pvt. Ltd. Mumbai.
- Nibedita Lenka, Member, Institutional Committee for Stem Cell Research (IC-SCR), National Institute for Research in Reproductive Health (NIRRH), Mumbai.
- Nibedita Lenka. Life Member, Indian Academy of Neuroscience.
- Nibedita Lenka. Active Member, International Society for Stem Cell Research (2005 - present).
- Nibedita Lenka. Member, Indian Society of Developmental Biology (2016-present).

Lalita Limaye

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

Amitabha Majumdar

- SERB early career research award, 2017-2019 : 'Studying the translational homeostasis landscape in Drosophila Fragile-X syndrome model'.

Srikanth Rapole

- Executive council member, Proteomics Society, India (PSI)
- Life member, Indian Society for Mass Spectrometry (ISMAS)
- Member, American Society for Mass Spectrometry (ASMS)
- Editorial member, Journal of Proteins and Proteomics (JPP)

Anjali Shiras

- Elected to Executive Committee: Indian Association of Cancer Research (IACR) (2016-18)
- Elected to Executive Committee: Indian Society of Neuro-oncology (ISNO) (2016-18)
- Elected as Secretary and Vice-President Moving Academy of Medicine and Biomedicine (2016-19)

Shailza Singh

- 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

Sandhya Sitaswad

- Member of the Governing Council of International Academy of Cardiovascular Sciences - India section (IACS-India)

Deepa Subramanyam

- Trust-DBT India Alliance Intermediate Fellow (2013-2018).

Mohan Wani

- Member, Stem Cell Task Force, DBT (2017).
- Academic Editor, PLOS ONE (2016-17).
- Chief Guest in STPI Conference on "Toxicologic Pathology of Nervous and Musculoskeletal System" held at Pune, October 21, 2016

Awards & Honours - NCCS Students

- **Nakka Kiran Kumar** (former student of Samit Chattophadhyay): INSA Medal for Young Scientist, 2016.
- **Ananth P. Burada:** Newton-Bhabha PhD Placement fellowship" to work in Prof. Elena Orlova's lab at Department of Biological Sciences, University of London, London, UK for 4-months.
- **Jyoti Kumari:** Fellowship to work with Prof. Peter J. Peters at Maastricht University, Maastricht, Netherlands for 3-months (2017)
- **Rajesh Vinnakota:** SERB National Post-Doctoral Fellowship (March2017).
- **Dr. Dhiraj Kumar:** Best Poster award at the '2nd International Conference on Translational Research: Applications in Human Health & Agriculture' organized at KIIT University, Bhubaneshwar, India (14-16 Oct, 2016).

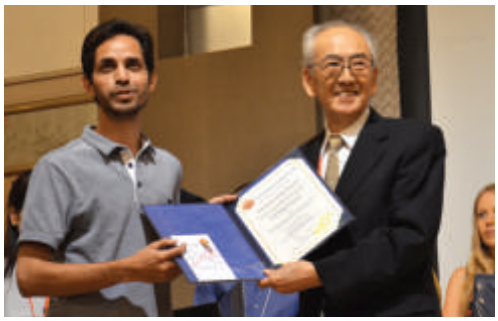
- **Dr. Dhiraj Kumar** (centre) with Prof. Marco Falasca (left) & Dr. Dev Mukhopadhyay (right)
- **Shilpi:** 'Best Oral Presentation' award at the 43rd Indian Immunology Society Conference, Visakhapatnam (16-18 Feb, 2017).
- **Neeraja Kulkarni:** Travel Award from DBT-CTAP to attend the Cytokines 2016 conference held at San Francisco, USA (16-19 October 2016).
- **Sandip Sonar:** Travel award by American Association of Immunologists (AAI), USA, to present his work at the International Congress of Immunology 2016 (ICI2016) in Melbourne, Australia (21-26 August 2016).
- **Sourav Paul:** Travel Awards from DBT-CTAP and DST-SERB, to attend the International Congress of Immunology 2016 (ICI2016) in Melbourne, Australia (21-26 August 2016).
- **VarunHaran M:** Best poster Award for 'Wnt-BMP at the cross road to specification of various Mesodermal

Derivatives. Students'; 2nd Mini-symposium on Cell Biology, NCCS, Pune. 2016.

- **Ankita C Dhenge :** Nature Travel Grant Award (to present a poster at the Gordon Research Conference on Cell Biology of Megakaryocyte and Platelets; Italy; 26 Feb-3 March, 2017.

- **Mr. Venkatesh Chanukuppa:** Travel award in "8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health" organised by NIPGR, New Delhi from 14-17 December 2016 at New Delhi.

- **Mr. Tushar More:** Poster selected for oral presentation in "8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health" organised by NIPGR, New Delhi from 14-17 December 2016 at New Delhi.



- **Hemendra Singh Panwar:** Best poster award at the XXVI International Complement Workshop, Kanazawa, Japan (September 4-8, 2016).

- **Hina Ojha:** Best poster award at the XXVI International Complement Workshop, Kanazawa, Japan (September 4-8, 2016).

- **Mangesh Suryawanshi:** Young Investigator Award 2016 in Yakult India Microbiota and Mangesh Suryawanshi. Probiotic Foundation- Probiotic Symposium 2016- "Probiotics in Health - Emerging Opportunities", Chennai, India (3rd and 4th December 2016)

- **Mangesh Suryawanshi:** 2nd prize for Poster in Yakult India Microbiota and Probiotic Foundation- Probiotic Symposium 2016- "Probiotics in Health - Emerging Opportunities", Chennai, India (3rd and 4th December 2016).

- **Mangesh Suryawanshi:** 2nd Prize for Poster in International Diabetes Summit 2017, JW Marriot Hotel, Pune, India (10-12th March 2017).

- **Dhiraj Paul:** Young Scientist Award 2016 by Association of Microbiologist of India (AMI)

- **Dhiraj Paul:** DST-International Travel Grant for attending the 16th International symposium on Microbial Ecology ISME 2016 held in Montreal, Canada (21-26th August 2016).

- **Suhas Mhaske:** "International Travel Grant" from Department of Science and Technology (DST), New Delhi and Centre for International Co-operation in Science (CICS), Chennai to attend the "4th Annual Meeting of the International Cytokine and Interferon Society (ICIS) Cytokines 2016" at San Francisco, CA, USA, October 16-19, 2016.

- **Anil Kumar:** "International Travel Grant" from Department of Biotechnology (DBT), New Delhi and Centre for International Co-operation in Science (CICS), Chennai to attend the "4th Annual Meeting of the International Cytokine and Interferon Society (ICIS) Cytokines 2016" at San Francisco, CA, USA, October 16-19, 2016.

Awards / Honours / Memberships - MCC Scientists

All members of the MCC faculty are members of the Association of Microbiologist of India (AMI) and the Bergey's International Society for Microbial Systematics (BISMiS).

Dr. Avinash Sharma

- Indian National Science Academy (INSA) Fellowship under International Scientific Collaboration and Exchange of Scientists in 2016.
- The Young Scientist Award in Environmental Microbiology by the Association of Microbiologists of India (AMI) for the year 2016.

Dr. Kamlesh Jangid

- Appointed as Associate Chief Editor in 2016 for Soil Biology & Biochemistry, Elsevier Science.
- Convener for 3rd Meeting of the Bergey's International Society for Microbial Systematics. 12-15 September 2016. Microbial Culture Collection, Pune, India.

Dr. Om Prakash Sharma

- Selected to coordinate the "Global fecal coliform sampling campaign" project from the Indian side (from December 2016). Forty three different laboratories from 21-countries are working in this programme.
- Selected as Fellow of Indian National Science Academy (INSA) in Environmental Microbiology to develop the Indo-Israel bilateral program in wastewater treatment for the year 2016.
- Selected for ICMR, Human Resource Development (HRD) Long term Fellowship (2016), to conduct research in the area of human gut microbiology in collaboration with Collage of Medicine, School of Biomedical Sciences, Florida State University, USA.

Dr. Shrikant Pawar

- Convener of International Symposium on Microbial Ecology and Systematics. 16-17 September 2016. National Chemical Laboratories, Pune, India.
- Convener of Workshop on Nomenclature of Archaea and Bacteria. 9-10 September 2016. Microbial Culture Collection, Pune, India.

Other Awards

NCCS received six awards at the 'Akhil Bhartiya Rajbhasha Sammelan Evam Chintan Shivir' held at Munnar, Kerala (1st to 3rd June, 2016):

1 Dr. Shekhar C. Mande, Director, NCCS, received the "Rajbhasha Shree" Samman for overall Hindi Implementation in the Institute.

2. Best Hindi patrika award for 'Meemansa' (2015 issue).

3. Best Hindi website award.

4. Three awards for scientific articles written in Hindi, which were published in 'Meemansa':

a) 1st prize for the articles written by Dr. Kale & Dr. Limaye (joint award).

b) Dr. Kale's and Dr. Limaye's articles also received special prizes

(photographs are on the right side)

Dr. Samar Roy Chowdhury:

- 'Jury of congress award' for outstanding achievement & excellent contribution in the noble cause of biotech management (awarded by the Honb'le Minister Smt. Krishnaraj at the World Science Congress during the 6th International Science Conference held at PGIMER, New Delhi, 23-25 Dec, 2016).
- 'Acharya P.C. Roy Memorial Award 2016' (awarded by the Indian Chemical Society at the 53rd Annual Convention of Chemists, GITAM University, VSKP, 27-29 Dec, 2016).

Extramural Funding (NCCS Faculty)

Manoj Kumar Bhat

- Assays for the hypoglycemic and hypolipidemic effects of ITC Herbal substances using specific *in vitro* cellular models'. Technical services on metabolic health (NCCS/Admin/ 9(6)2014). Project completed in March 2017

Samit Chattopadhyay

- Role of Nuclear Matrix Protein SMAR1 as Regulator of Suppressor T Cell in Inflammatory Bowel Disease (IBD)' 2015 – 2017 (DBT, India).
- J C Bose Fellowship' 2013 – 2018 (DST, India).
- Tumor suppressor SMAR1 regulates transcription of β-catenin and protects from metastatic colon cancer' 2016 - 2019 (DST, 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 Nup93Nup205 complex of the nuclear pore complex. 2013 - 2016. (DST-SERB EMR funded).
- Reconstitution and structural studies on Nup93•Nup62•Nup54•Nup58 quaternary complex 2015-2018. (DBT-basic sciences EMR funded).
- Structural and functional role of NPC in HIV infections 2016-2021. (DBT-Centre for excellence)

Jomon Joseph

- Role of Nup358 in the regulation of cytoplasmic mRNP granules. 2016-2019 (DST, India)

Vaijayanti Kale

- Identification of aging-induced epigenetic changes causing hematopoietic stem cell dysfunction: Rescue using in vitro niche (IVN)-technology. Feb 2017 – Jan 2020. (DBT).

Janesh Kumar

- Molecular Mechanisms for Regulation of Ionotropic Glutamate Receptors by their Auxiliary Subunits. 2014 - 2019. (Wellcome Trust/DBT India Alliance, India).
- Centre of Excellence in Biomolecular Structure and Function on Host-Pathogen Interactions. 2017 – 2022. (DBT, India) (Co-investigator).

Gopal Kundu

- Chitosan nanoparticle mediated Andrographolide and/or Raloxifene delivery in breast cancer and its implication in multi-targeted therapy. 2016-2019 (Department of Biotechnology, Government of India).
- Translational development of protein nanomedicine and multifunctional hydroxyapatite nano-contrast agent. 2016-2019 (Department of Biotechnology, Government of India).
- Multilayer Nano-capsules and Targeted DNA Vaccines for Immunotherapy of Cancer. 2016-2019 (EU/Inno-Indigo, Department of Science and Technology, Government of India).
- Multi-Omics Analysis to Decipher Mechanisms of Hormone Resistance in Breast Cancer 2017-2022 (VNCI, Department of Biotechnology, Government of India).

Girdhari Lal

- Role of gamma-delta T cells in the generation and maintenance of transplantation tolerance. from Department of Biotechnology, Government of India. (2016-2019).

Nibedita Lenka

- Nibedita Lenka. NER Twinning Project, DBT (2016-2019).

Lalita Limaye

- "Studies on generation of induced pluripotent stem cells from umbilical cord tissue derived adult stem cells -[BT/PR12696/MED/31/287/2014]"-Project sanctioned by DBT in Feb. 2017 for 3 years.

Amitabha Majumdar

- Understanding the mechanism of persistence of memory, WT-DBT India Alliance intermediate fellowship 2014- 2019.

Shekhar Mande

- An omics approach for diagnosisng tuberculosis. 2013-2016 (DBT).
- International Associated Laboratory in the area of Systems Immunlogy and genetics of infectious diseases(LIA-SIGID) 2015-2019 (DBT).
- SysTB: A network program for resolving the intracellular dynamics of host pathogen interactions in TB infection. 2012-2017 (DBT).
- Center for Excellence in Biomolecular Structure and function on host-pathogen interactions in TB infections. 2016-2021 (DBT).

- Elucidation of gene regulatory networks in *Mycobacterium tuberculosis* from the available high throughput data and prediction of transcription regulation. 2014-2016 (DST)

Debashis Mitra

- Identification of novel cellular targets and new lead molecules to inhibit HIV-1 infection. (2012-2017) Tata Innovation Fellowship grant, Department of Biotechnology, India.
- Cellular Stress Proteins in HIV infection: Biochemical and Functional Characterization.
- Under the Center of Excellence in Biomolecular Structure and Function on Host-Pathogens Interactions led by Dr. Shekhar C. Mande (2016-2021).

Srikanth Rapole

- An attractive and promising strategy for early cancer diagnosis through the assembly of the human cancer volatome. 2013-2017 (DBT New INDIGO).
- Metabolomic profiling for identification of novel potential biomarkers in breast cancer using mass spectrometry and bioinformatics. 2013-2017 (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. 2013-2017 (DBT Basic Science).
- Acquisition of modern Orbitrap mass spectrometer for establishing state-of-the-art proteomics facility at National Centre for Cell Science. 2016-2021 (DBT Basic Science).
- Exploring the volatome of noncommunicable diseases as a promising, innovative and integrating approach for its rapid diagnostics. The case study of cáncer and neurodegenerative diseases. 2016-2019 (DST Inno Indigo).

Arvind Sahu

- Soluble mediators of the immune system against Aspergillus fumigatus. 2014-2017 (Department of Science and Technology, India 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).

Manas Santra

- Understanding the role of post translation modification(s) on apoptotic activity of PUMA' (2015-2018, CSIR, India).
- Development of Novel Inhibitors of AKT: An Unorthodox Approach Targeting the Pleckstrin Homology Domain' (2016 -2019 Twinning project- DBT, India).
- Identification of Ring - Finger E3 ubiquitin ligases involved in NF-B pathway activation and deciphers the molecular mechanism' (2017 - 2020 DST-SERB, India).

Vasudevan Seshadri

- Post transcriptional gene regulation in Plasmodium falciparum. 2015-18. (Department of Science and Technology, India)

Anjali Shiras

- Identification of Biomarkers for diagnosis and prognostication by Next Gen sequencing of Oligo-dendroglial tumor exome. 2012-16. (Department of Biotechnology, India).
- A novel strategy for reprogramming of somatic cells to induced pluripotent stem cells 2014-16. (Department of Science and Technology-UKIERI, Indo-UK).
- Cis-acting pair of novel non-coding RNAs - Ginir and Giniras in Cell growth Regulation and Transformation of mouse and human cells. 2015-18. (Department of Biotechnology, India)
- Studies on Exosome Mediated Regulation of Angiogenesis in Glioblastoma. 2015-18. (Department of Biotechnology, India).
- Altered microRNA and its targets in glioblastoma cell lines 2016-19 (Department of Biotechnology, India).
- Derivation of functional hepatocytes from human induced Pluripotent Stem Cells. 2017-20. (Indian Council of Medical Research, India).
- Examine cross-talk between epidermal and dermal skin cells through in vitro models in order to regulate hyper-pigmentation 2017-19 (Unilever Industries Ltd, India).

Yogesh Shouche

- Establishment of Microbial Culture Collection and Biological Research Centre. 2009-2015 (Department of Biotechnology, India).
- Maharashtra Gene Bank, 2014-2019 (Rajiv Gandhi Science and Technology Commission, India).
- Tracking the shift in gut microbiome from healthy to diabetic state: an omic approach. 2015-2016 (Unilever, India).

Shailza Singh

- Molecular motors as nanocircuits in Leishmaniasis: System cues guiding synthetic biology device construction (2016-19), funded by Department of Biotechnology, Ministry of Science and Technology, Government of India.

- Understanding the mechanism of ABC-type metal sequestering proteins: structure-based novel drug development against human pathogens.(2017-2020), funded by Department of Biotechnology, Ministry of Science and Technology, Government of India.

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).

Vidisha Tripathi

- Investigating the role of long noncoding RNAs in mammalian gene expression regulation. 2015-2020 (DBT, India).
- Understanding the role of mammalian long noncoding RNAs (lncRNAs) in regulating cellular quiescence. 2016-2019 (DST, India).

Mohan Wani

- "Development of Ni free nitrogen stabilized austenitic stainless steel for biomedical applications" (a collaborative initiative between IIT-BHU, Varanasi and NCCS, Pune) funded by The Ministry of Steel, Govt. of India, New Delhi.
- "Preclinical study on development of therapeutic adjuvant based on Ayurveda" (a collaborative initiative between S. P. Pune University and NCCS, Pune) funded by The Ministry of AYUSH, Govt. of India, New Delhi.

Extramural Funding (MCC Scientists)

Dr. Amit Yadav

- Understanding genomic factors associated with pathogenicity and transmission of 16SrII group phytoplasmas, their taxonomy and role of insect gut microbiome in their vectoring ability'. 2017- 2020. (DST-SERB, India).

Dr. Om Prakash

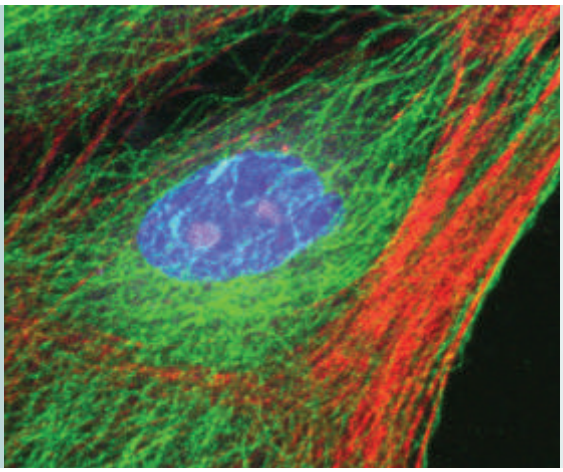
- Study of biomethanation potential of landfill methanogens and their prospective role in global climate change, carbon sequestration and generation of bioenergy'. 2016-2019. (DBT, India).

Dr. Praveen Rahi

- Population structure of root-nodulating bacteria associated with pea cultivated in different agroclimatic regions of India. 2015-2018. (DST- SERB, India).

Dr. Prashant Singh

- Decoding Indian Cyanobacterial Diversity using the polyphasic approach. 2016-2019. (DST-SERB, India).
- Young Investor Grant. 2016-2019. (DST, India).



Conferences / Meetings / Workshops

Talks Delivered by / Events Participated in by the Faculty

Manoj K. Bhat

- Invited Talk: 'Weight Control Interventions in Melanoma'. Progression and therapeutic outcome in Obesity: Global Summit on Melanoma & Carcinoma, Brisbane, Australia, July 14th -15th , 2016.
- Invited: Talk: 'Diet Induced Obesity and its ramification on cancer cells'. National Symposim on Current Research in Cancer Biology and Therapy, UIAR, Koba, Gandhinagar, Gujarat, October 7th-8th, 2016.
- Invited: Talk: 'Metabolic Disorders: An Unexpected Role In Cancers'. 35th Indian Association for Cancer Research (IACR) Conference, New Delhi April 8th - 10th, 2016.
- Invited Talk: 'Obesity and Cancer: Implications in Cell Growth and Chemotherapy'; 2nd International Conference on Translational Research (ICTR), KIIT University, Bhubaneswar, Odisha, October 14th- 16th, 2016.
- Invited Talk: 'Cancer Chemotherapy: Involvement of Membrane Cholesterol'; MHRD- Global Initiative of Academic Network (GIAN) work shop on Lipid Signaling in Health and Diseases, School of Life Sciences, University of Hyderabad, December 8th-9th, 2016.
- Cancer Chemotherapy: Involvement of Membrane Cholesterol'; 2nd National Conference of Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, January 24th -25th, 2017.
- Invited Talk: 'Cellular and Metabolic Factors Influencing Chemotherapy'; International Symposium on Breast Cancer Research. NCCS, Pune, February 27th, 2017.
- Invited Talk: 'Obesity and Cancer: Implications in Cell Growth and Chemotherapy'; National on Recent Advances Modern Biology and Bioinformatics (RAMBB), DPU, Pune, 16th -17th March 2017.
- Invited Talk: 'Exploiting Metabolic Plasticity of Cancer Cells: An Emerging Strategy to Induce Synthetic Lethality'; Advances in Enzymology: Implications in health, disease and therapeutics - INDO-US conference cum workshop, ACTREC, Navi Mumbai, 17th -19th January, 2017.

Sharmila Bapat

- Cellular and Network Approaches to molecular stratification and therapeutic prediction in high-grade serous ovarian adenocarcinoma (HGSC)' - Invited Talk at the National Cancer Institute, Rockville, MD, USA on April 4, 2016.
- Attended the Accelerating Anti-Cancer Drug Development and Validation workshop organized by the National Institute of Health, Bethesda as a participant, from May 3-5, 2016.
- Varying Levels of Tumor Heterogeneity in Ovarian Cancer' - Invited talk at the Oregon Health & Science University, Portland USA on 9th May, 2016.
- Molecular classification of cancer'' - Invited talk at the 3rd Indo-British Advanced Master-Class in Oncoplastic Breast Surgery and Breast Cancer organized by International School of Oncoplastic Surgery and Prashanti Cancer Care Mission, Pune, India on 5-7th August, 2016.
- Tumor Heterogeneity - not just noise!' - Invited talk at "Ovarian Cancer Workshop: Challenges and Provocative Questions" at the Penn State Hershey Cancer Institute, PA, USA on May 16, 2016.
- Tumor initiating Cells in Ovarian Cancer' - Invited talk at The 32nd International Kumamoto Medical and Bioscience Symposium on "The Ovary" at Yamasaki Memorial Hall, Kumamoto University, Japan on November 3rd, 2016.
- Cancer Stem Cells and stress induced evolution - understanding the drug recalcitrance phenomenon" Invited

Talk at the 8th East Zonal Oncology Symposium, 21st January, 2017, Kolkatta.

- "Cancer Stem Cells and their Clinical Implications' - Invited talk at "Department of Biotechnology (DBT)-North East Region (NER) Hands-on-Training Workshop on Stem Cell Biology"at ACTREC-TMC, Kharghar on 16th February, 2017.
- Identification and relevance of fusion transcripts using *in vitro* models of high-grade serous ovarian cancer' - Invited talk at the 2nd Annual Conference on "Next Generation Sequencing" (NGS) conducted at IICT Auditorium, Hyderabad by CCMB and Bioserve, 23 February, 2017.
- Expression based networks and functional pathways in molecular classification of ovarian cancer' - Invited Talk at CDFD, Hyderabad on 24 February, 2017.

Radha Chauhan

- Structural studies on Nup93 subcomplex of the human nulcear pore complex (Invited talk); 2nd Membrane structural biology workshop, IISc Bangalure, 15-16th December 2016.

Jomon Joseph

- Animal viruses and nucleoporins (Jomon Joseph), New Horizons in Biology-Indian Institute of Science, 16-17, June 2016, Bengaluru, India.
- Advanced microscopic techniques in cell biology research (Jomon Joseph). EMSI-meeting-Aug 11-13, 2016, CSIR-NCL, Pune, India.

Musti Krishnasastry

- Survival Strategies of Mycobacterium tuberculosis: 'The Game' and 'The Strategies' on Table': Invited talk; National Seminar on Recent Trends in Biology; Department of Zoology, S.P. Pune University, Pune, India, February 2017.
- Strategies for TB Vaccine': Invited talk; Yashraj Biotechnology Pvt Ltd, Navi Mumbai; Navi Mumbai, India, March 2017.

Janesh Kumar

- Marvel of Nature's Design: Glutamate Receptor Ion channels as Sophisticated Nano Machines': Invited talk; Saturday lecture series of Microbiology Department, Pune University; Pune, India, September 2016.
- Strategies for Expression and Purification of Membrane proteins from Eukaryotic Expression system': Invited Talk; M4I, Maastricht University; Maastricht, Netherlands, December 2016.
- Ionotropic Glutamate Receptors and their Regulation by Cognate Auxiliary Subunits': Invited Talk; M4I, Maastricht University; Maastricht, Netherlands, December 2016.
- Structural Biology of Ionotropic Glutamate Receptors: Current Trends and Future Directions': Invited Talk; Bhabha Atomic Research Center; Mumbai, India, February 2016.

Gopal Kundu

- Indian Association for Cancer Research (IACR) Conference, The Ashok Hotel, New Delhi, 8-10th April, 2016 (Invited Talk).
- Indian Society of Translational Research (ISTR) Conference, Bose Institute, Kolkata, 16-18th April, 2016 (Invited Talk).
- Cancer and Cancer Stem Cell Workshop, Curtin University, Perth, Australia, 6th May, 2016 (Invited Talk).
- Department of Pharmaceutical Science, National University of Singapore, Singapore, 9th May, 2016 (Invited Talk).
- CSIR-IHBT, Palampur, Himachal Pradesh, 23rd May, 2016 (Invited Talk).
- Poornaprajna Institute of Science & Research, Bangalore, 26th August, 2016 (Invited Talk).
- National Conference, Bharathiar University, 5th October, 2016 (Invited Talk).
- 2nd International Conference on Translational Research, KIIT University, Bhubaneswar, 14th-16th October, 2016 (Inaugural Address & Invited Talk).
- International Conference on Stem Cell and Cancer Conference, Goa, 21-23rd October, 2016 (Invited Talk).
- Transcription Assembly Conference, Bose Institute, Kolkata, 8th November, 2016 (Invited Talk).
- Mayo Clinic Angiogenesis Conference, Jacksonville, Florida, 18th-20th November, 2016 (Invited Talk & Session Chair).
- AFRRI, Bethesda, Maryland, USA, 23rd November, 2016 (Invited Talk).
- IASST, Guwahati, 5th December, 2016 (Invited Talk).
- International Conference on Molecular Medicine, Bose Institute, Kolkata, 9th January, 2017 (Invited Talk).
- Indian Association of Cancer Research (IACR) Conference, Amala Cancer Centre, Kochi, 9-11th February, 2017 (Invited Talk).
- National Conference, Gurudas College, Kolkata, 17th February, 2017 (Invited Talk).
- Department of Zoology, University of Calcutta, Kolkata, 17th February, 2017 (Invited Talk).
- National Conference, University of Burdwan, Burdwan, 18th February, 2017 (Invited Talk).
- IISER, Trivandrum, 22nd February, 2017 (Invited Talk).

- International Conference on Breast Cancer Research, NCCS, Pune, 27th February, 2017 (Invited Talk).
- Yashraj Scientific Advisory Committee Meeting, Mumbai, 4th March, 2017 (Invited Talk).
- System Oncology Conference, Kochi, 11th March, 2017 (Invited Talk).
- National Conference, D Y Patil University, Pune, 16th March, 2017 (Invited Talk).
- NIBMG, Kalyani, 30th March, 2017 (Invited Talk).
- National Conference, University of Kalyani, Kalyani, 30th March, 2017 (Invited Talk).
- IISER, Kolkata, 31st March, 2017 (Invited Talk).
- Board of Study, SIT, Tumkur, Karnataka, 28th April, 2016 (Meeting).
- Science on the Swan Conference, Perth, Australia, 3-5th May, 2016 (Participant).
- International Convention, Ujjain, Madhya Pradesh, 12-14th May, 2016 (Participant).
- DBT Nanotechnology Project Meeting, Amrita Nano Science Centre, Kochi, 18-19th May, 2016 (Meeting).
- DBT Nanotechnology Meeting, AIIMS, New Delhi, 22nd and 23rd June, 2016 (Meeting).
- Inno-Indigo, EU Meeting and Talk, NCCS, Pune, 12th January, 2017 (Meeting).
- Amrita Nano Science Centre, Kochi, DBT Meeting, 9th March, 2017 (Meeting).

Girdhari Lal

- Lal G (2017) Effect of inflammatory signals on blood-brain barrier in transmigration of inflammatory CD4 T cells. *Molecular Immunology Forum* (MIF) on 10-11 March organized at Chandigarh.
- Sonar S, and Lal G (2017) Transendothelial migration of Th17 cells across the blood-brain barrier during inflammation and autoimmunity. 43rd Annual Conference of Indian Immunology Society, Immunocon 2016 held on 16-18th February 2017 in Visakhapatnam. *(Invited lecture).*
- Sonar S, Kulkarni N. and Lal G (2016) Role of chemokine receptor signaling in the inflammation and autoimmunity. Maastricht University, Netherlands and NCCS Workshop held at NCCS Pune on 10th March.
- Kulkarni N. and Lal G (2016) CCR6 intrinsic signaling promotes Th17 cell differentiation during autoimmunity. International Congress of Immunology 2016 held from 21-26 August 2016 at Melbourne, Australia. *(Mini-Oral Presentation; AAI travel Award 2016). Eur. J. Immunol. 2016, 46(Suppl.1):359.*
- Lal G (2016) Transendothelial migration of Th17 cells across the blood-brain barrier during inflammation and autoimmunity. Symposia on Pathogens and Host Response organized by National institute of Immunology (NII) New Delhi on 10-12th August. *(Invited Talk).*
- Lal G (2016) Role of intratumoral natural killer cells in shaping the effector CD4 T cell response. Presented in International mini-conference on 'Novel Therapeutic Approaches Targeting Cancer and Cancer Stem Cell' held at NCCS, Pune on 18th March 2016.
- Lal G. (2016) Non-chemotactic function of chemokine receptor CCR6 in the inflammation and autoimmunity. System Immunology and Genetics of Infectious Diseases (SIGID) meeting on 29-31 May 2016 at Nimes, France. *(Invited Talk).*

Nibedita Lenka

- Neurogenesis: Default or instructive?'; 9th World Congress on Preventive and Regenerative Medicine. KIIT University, Bhubaneswar, Odisha, 2016 (Invited Speaker).
- Generation and Characterization of NSCs and DA neurons from Embryonic Stem Cells and exploration of their therapeutic efficacy'; IJO-CARVES (Indian Journal of Ophthalmology-Clinical and Research in Vision and Epidemiological Sciences) - 2016 organized by Indian journal of Ophthalmology, Aditya Jyot Research in Vision & Ophthalmology & Eye Advance, Mumbai (Invited Speaker).

Lalita Limaye

- ω-3 (n-3) and ω-6 (n-6) polyunsaturated fatty acids stimulate the bone marrow stem cell population in mice', Limaye L.S., Limbkar K.R. and Kale V.P. Poster presented at the ISSCR , 22- 25th June 2016, San Francisco,USA.
- Cell therapeutic applications of Haematopoietic (HSCs),Mesenchymal (MSCs) and induced pluripotent stem cells (i PSCs) derived from cord tissues'; National Symposium on "Recent Advances in Modern Biology & Biotechnology 2017 (RAMBB 2017)" March 16-17, 2017; Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune.

Shekhar Mande

- A Systems Biology Approach in Mycobacterium tuberculosis infection'. 2nd SIGID Steering Committee Meeting held at Nimes, France. Nimes, France. 30/05/2016.
- Biotechnology in India'. YIM Meeting, University of Cambridge, UK. 16/09/2016.

- Mapping Protein Flexibility. Combination of Crystallographic and Computational Approaches'. CSIR Foundation Day talk at CSIR- Indian Institute of Chemical Biology, Kolkata. 28/09/2016.
- Indian Structural Biology'. Inauguration of XRD-2 beamline at Elettra synchrotron radiation, Trieste, Italy, 20/10/2016.
- Indian Human Microbiome Initiative'. Annual meeting of the Indian Academy of Sciences, Bangalore held at the Indian Institute of Science Education and Research, Bhopal, 05/11/2016.
- Role of Interdisciplinary Sciences in Modern Biology'. IISF outreach programme held at the CSIR- National Chemical Laboratory, Pune, 02/12/2016.
- Mycobacterial GroEL's: Moonlighting in non-specific Binding'. New Indigo meeting held at ITQB, Oeiras, Portugal, 20/12/2016.
- Computational Approaches in Genome Analyses'. Ramalingaswamy Conclave held at the Indian Institute of Science Education and Research, Pune, 05/01/2017.
- Development of Analysis Tools for Large Scale Genomic Data'. Bioinformatics Workshop held at the Centre for Bioinformatics, Savitibai Phule Pune University, Pune, 10/01/2017.
- Context-dependent Regulatory Networks: Motifs and Combinatorial Searches'. Institute of Advanced Study in Science and Technology, Guwahati, 06/03/2017.
- Computational Approaches in Biology'. Talk delivered at Recent Advances in Modern Biology and Biotechnology, DY Patil University, Pune, 17/03/2016.

Debashis Mitra

- Stress proteins in HIV infection, Invited Talk, System Immunology and Genetics of Infectious Diseases (SIGID) Meeting, Nîmes, France, 29-31st May 2016.
- Hsp70 binding protein-1 (HspBP1) inhibits HIV-1 replication by suppressing NF-κB mediated activation of viral transcription, Invited Talk, Virology Conference, IISc, Bangalore, 20-21st June 2016.
- HspBP1 inhibits HIV-1 replication by suppressing NF-κB mediated activation of viral transcription, Invited Talk, Transcription Assembly, Bose Institute, Kolkata, 8-9th November 2016.
- Stress proteins in HIV-1 infection, Invited Talk, Guha Research Conference-2016, Diu, 8-12th December 2016.
- Targeting cellular stress proteins and signalling pathways: a novel strategy in the fight against HIV/AIDS, Invited Talk, 3rd International Conference of Perspectives of Cell Signaling and Molecular Medicine, Bose Institute, Kolkata, 8-10th January, 2017.
- Cyclin F regulates HIV-1 infectivity through ubiquitination and proteasomal degradation of Vif, Invited Talk, 5th Molecular Virology Meeting, THSTI, Faridabad, 11-12th February 2017.
- Cellular factors and signalling pathways: novel targets in the fight against HIV/AIDS, Invited Talk, National Symposium "Recent Advances in Modern Biology & Biotechnology 2017 (RAMBB 2017)" Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Pune, 16-17th March 2017.

Srikanth Rapole

- Investigation of Proteomic, Metabolomic, and Volatomic Alterations towards Potential Biomarkers in Breast Cancer. Invited talk at Mini symposium on metabolomics and volatomics towards disease markers organized NCCS, May 16, 2016 at Pune.
- HCV Project in India – Results and Future Prospects. Invited talk at Final European Indian Human Cancer Volatome (HCV) meeting organised by University of Rostock, June 6-8, 2016 at Rostock, Germany.
- Investigation of Proteomic, Metabolomic, and Volatomic Alterations towards Potential Biomarkers in Breast Cancer. Invited talk at Human Volatome (Indigo) meeting organised by CDFD, 31st October - 4th November, 2016 at CDFD, Hyderabad.
- Targeted proteomics for clinical applications. Invited talk at 8th annual meeting of proteomics society of India and education day organised by NIPGR, December 13, 2016 at New Delhi.
- Quantification and validation of potential biosignatures for intrinsic subtypes of breast cancer. Invited talk at 8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health organised by NIPGR, December 14-17, 2016 at New Delhi.
- Identification of Novel Potential Biomarkers for Breast Cancer using Mass Spectrometry based Proteomic and Metabolomic Approaches. Invited talk at DBT workshop on Recent advancement in cellular and nano-biotechnology: Applications in cancer research organized by Sanjivani college of pharmaceutical education and research, Shingnapur March 10-11, 2017.
- Quantitative Proteomics and Metabolomics towards Novel Potential Biomarkers for Breast Cancer. Invited talk at

Proteomics day mini symposium organized by NCL, March 18, 2017 at Pune.

- Mass spectrometry based proteomics and metabolomics. Invited talk at LC-MS workshop cum training organized by Vikram Sarabhai Institute of cell and Molecular Biology, March 23-24, 2017 at MSU Baroda.
- Final European Indian Human Cancer Volatome (HCV) meeting organised by University of Rostock, June 6-8, 2016 at Rostock, Germany.
- Workshop on cancer proteogenomics jointly organized by RGCB and Broad institute, September 26-30, 2016 at RGCB, Thiruvanthapuram.
- Human Volatome (Indigo) meeting organised by CDFD, October 31 - November 4, 2016 at CDFD, Hyderabad.
- 8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health organised by NIPGR, December 14-17, 2016 at New Delhi.
- DBT workshop on Recent advancement in cellular and nano-biotechnology: Applications in cancer research organized by Sanjivani college of pharmaceutical education and research, March 10-11, at Shingnapur.
- Proteomics day mini symposium organized by National Chemical Laboratory, March 18, 2017 at Pune.
- LC-MS workshop cum training organized by Vikram Sarabhai Institute of cell and Molecular Biology, March 23-24, 2017 at MSU Baroda.

Arvind Sahu

- Complement - Role in innate immunity and beyond; Invited talk on the occasion of the World Immunology Day, Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, April 29, 2016.
- Structural basis of complement regulation by Kaposica, the complement regulator of Kaposi's sarcoma-associated herpesvirus; Invited talk; Symposium on Biology and Molecular Pathogenesis of Viruses, Indian Institute of Science, Bangalore, June 20, 2016.
- Structural basis of complement regulation by Kaposica, the complement regulator of Kaposi's sarcoma-associated herpesvirus; Invited talk; 12th Indo-Australian Biotechnology Conference at Bhubaneswar, August 9, 2016.
- Complement: a viral target for immune evasion; Invited talk; Institute of Bioinformatics and Biotechnology (IBB), S. P. Pune University, Pune, October 22, 2016.
- Synergy between the classical and alternative pathways of complement is essential for conferring effective protection against the pandemic influenza A(H1N1) 2009 virus infection; Invited talk; National Symposium on "Recent Advances in Modern Biology & Biotechnology 2017 (RAMBB 2017)", Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, March 16, 2017.

Manas Santra

- F-box protein FBXO31 and cell cycle check points/ Symposium'; One day symposium; The Society of Biological Chemists of India Mumbai Chapter, October 1, 2016.
- F-box protein FBXO31 protects from oncogenic transformation through activation of cell cycle check points', Indian Institute of Chemical Biology, November 24, 2016.
- F-box protein FBXO31 is a dedicated checkpoint protein to facilitate cell cycle arrest through activation of regulators in radiation-induced DNA damage/Conference'; SFRR-INDIA-17 Conference on 'Basic and Applied Aspects of Health Management Using Radiation, Antioxidants and Nutraceuticals' Mumbai, India, 9-12 January, 2017.
- Research in Cancer Biology - Breast Cancer biomarkers', 17 Feburary 2017, SDM College of Medical Science & Hospital, Dharwad, Karnataka.
- Protein phosphatase 1 regulatory subunit p90/MAPK pathway feedback loop regulates breast cancer malignancy', International Breast Cancer Research Symposium, NCCS, Pune, 27 February 2017.
- Future Trends and Innovation in Biochemistry', 4 March 2017, Department of Biochemistry, Saurashtra University, Gujrat.
- Recent Advancement in Cellular & Nano-Biotechnology: Applications in Cancer Research', 10 March 2017, Sanjivani College of Pharmaceutical Education and Research, India.

Anjali Shiras

- Evolving Dynamics of Brain tumor stem cells; 9th Annual Conference Indian Society of Neuro-Oncology, 1st-3rd April, 2016, Hyderabad, India.
- Tumor Suppressive microRNAs in Glioblastoma: Role in Glioma Progression; 2nd International Conference on Translational Research, 14th-16th October, 2016, KIIT University, Bhubaneswar, India.
- Novel Strategy for Generation of human induced pluripotent stem cells and there in vitro differentiation, 9th World Congress on Preventive and Regenerative Medicine,

13th-15th November 2016. KIIT University, Bhubaneswar, India.

- Cancer Stem Cells –Facts and Fallacies; DBT-NER Hands-on-Training workshop on Stem Cells 23rd-27th January, 2017, ACTREC, Mumbai, India.
- Research Trends in Glioma Biology; Neuro-oncology Research Meeting; Wallace Cancer Centre, Bangalore, India, March 2017.
- Induction of human pluripotent stem cells and there in vitro differentiation: looking forward to regenerative medicine; African Academy of Sciences (AAS) and the Stellenbosch Institute for Advanced Study (STIAS) Workshop On Cell Biology and Regenerative medicine (CBRM) 27th June to 1st July 2016. Stellenbosch, South Africa.

Dr. Yogesh Shouche

- Megaculturomics of Microbial Biodiversity from Diverse Ecological Niches in India, Invited talk delivered in Bergey's International Society for Microbial Systematics third meeting on Microbial Systematics and Metagenomics. 12-15, September, 2016, Pune India.
- Exploring the outer space for microbial life, Invited talk delivered in International symposium on Microbial Ecology and Systematics, 16-17 September 2017, Pune, India.
- A tale of two Gangas Next-Gen Genomics & Bioinformatics Technologies, Invited talk delivered in (NGBT) Conference 3-5 October 2016 Kochi, India.
- Microbial Culture Collection (MCC) at National Center for Cell Science (NCCS) Pune, invited talk delivered in Conference on Microbial Resource Centers and Conservation of Microbial Diversity" and 13th meeting of the "Asian Consortium For the Conservation and Sustainable Use of Microbial Resources (ACM)" from 8-10 November 2016, Chandigarh India.
- Thank you, mother: Maternal transmission of microbiome, invited talk delivered in international Conference and outreach program on Environment and Ecology: Sustainability and challenges, 4-6 January, Delhi India.
- Human Microbiome: Indian perspective, invited talk delivered in 104th Indian Science Congress, 3-7 January 2017 Tirupati India.
- Human Microbiome: the other genome, invited talk delivered in International Conference on interface of Physical, Chemical & Biological Sciences, 11-13 January 2017, Sagar, India.

Shailza Singh

- Invited talk on "Synthetic Bioengineering for Neglected Tropical Disease: A Computational Pipeline" at Interdisciplinary Biotechnology Unit, AMU, Aligarh, 16th-17th March 2017.
- Invited Talk on "Structure based design of a new series of coumarins as anti-leishmanial inhibitors" at Department of Biochemistry, MGIMS, Wardha, 9th-10th March 2017.
- Invited Talk on "Deciphering lipid metabolism in Leishmaniasis using systems perspective" Lipid Meet 2016, Amity University, Gurgaon, 14th-15th Dec 2016.
- Invited Talk on "Structureomics of the sphingolipid metabolism of the parasite Leishmania", National Symposium on Omics to Structural Basis of Diseases, 30th September-1st October 2016, MSU Vadodara, Gujarat.
- Invited Lecture in DST Inspire Camp, Dayanand Science College, Latur, "Cells governing the Life in Science" (Four Lectures), 26th-27th September 2016.
- Invited talk on "Dissecting and Optimizing Druggability through Network Modeling and Liposomal formulations" at National Seminar on Drug Development and Diagnostic Approaches for Tuberculosis Infection, July 29-30, 2016, Bhanuben Nanawati College of Pharmacy, Vile Parle, Mumbai.
- Invited talk on "Bioinformatics - Tool for Drug development of Natural products" at Garware College, 23rd March 2016, Pune.

Sandhya Sitasawad

- Mitochondrial Peroxiredoxin-3 protects against hyperglycemia induced myocardial damage in Diabetic cardiomyopathy'; International Conference on Recent Advances in Cardiovascular Research: Impact on Health and Disease; 9-11, February 2017, Vallabhbhai Patel Chest Institute, University of Delhi.

Deepa Subramanyam

- Traffic control in embryonic stem cells; / Invited talk/ IIT Madras, December 27th, 2016.
- Traffic control in embryonic stem cells; / Invited talk/ IISER Thiruvananthapuram, March 20th, 2017.

Mohan Wani

- Immunomodulatory properties of mesenchymal stem cells and its therapeutic effects in collagen-induced arthritis animal model of rheumatoid arthritis' during 85th Annual

Meeting of Society of Biological Chemist (SBC) at CFTRI, Mysure, November 21-24, 2016.

- New targets for the treatment of degenerative diseases of musculoskeletal system' during Seminar on "Drug Discovery through Enhanced Drug Targeting" at Sinhgad Institute of Pharmacy, Pune, January 3, 2017.
- Opportunities for international collaborations' during Brainstorming Session on "Strategy for developing appropriate animal models to establish safety and toxicity of stem cells for human diseases" at NIAB, Hyderabad, March 28, 2017.
- 1st Annual Symposium on "Cell and Gene Therapy" at Christian Medical College (CMC), Vellore, August 5-6, 2016.
- Workshop on "Equine Colic, Fracture Repair and Radiology" at KNP College of Veterinary Medicine, Shirval, October 24-25, 2016.
- Guha Research Conference (GRC) 2016 meeting at Diu, Island, December 08-12, 2016.

Participation by Students / Other Scientists / Project Staff in Conferences / Workshops

- Gaurav Soman; Development and characterization of a cytotoxic monoclonal antibody that targets Cancer Stem Cells - Poster Presentation at Conference on Systems Networks Approaches for Therapeutics, Kochi, India, March 2017.
- Gaurav Soman, Sagar Varankar, Ancy Abraham, Madhuri More and Snehal Gulhane participated in 2nd Mini-Symposium on Cell Biology organized by NCCS, Pune on 11th May 2016.
- Gaurav Soman, Ancy Abraham, Madhuri More and Snehal Gulhane participated in 'Science Communication and Career Workshop' organized by Nature India and Nature Jobs in partnership with Wellcome Trust/DBT India Alliance on 14th July 2016.
- Gaurav Soman, Ancy Abraham, Madhuri More, Snehal Gulhane and Neha Thakre participated in 'International Symposium on Breast Cancer Research' organized by NCCS, Pune in partnership with Prashanti Cancer Care Mission on 27th February 2017.
- Sonal Patel: 'Role of nuclear matrix binding protein SMAR1 in vertebrate embryogenesis' (Sonal Patel, Aftab Alam, Samit Chattopadhyay), 18th Australia and New Zealand Zebrafish Meeting, 30th January,2017- 2nd February, 2017, Waiheke Island, New Zealand.
- Parshuram Sonawane: Simpifying the complex encompassing the gateway of information. (Bhawna Burdak, Kriti Chopra, Pankaj Madheshiya, Parshuram Sonawane, Pravin Dewangan, Sangeeta Niranjan, Shrankhla Bawaria, Virashree Raorane & Radha Chauhan). 3rd NCCS Retreat, October 4-5, 2016 Bhor, India.
- Pravin Dewangan: Structural Studies on the central channel of the nulcear pore complex. (Pravin Dewangan, Sangeeta Niranjan & Radha Chauhan). 44th National Seminar on Crystallography, 10-13th July 2016. Indian Institute of Science education and research (IISER) Pune, India.
- Kriti Chopra: In-silico prediction of coevolved residues of the interacting nucleoporins. (Kriti Chopra and Radha Chauhan). 44th National Seminar on Crystallography, 10-13th July 2016. Indian Institute of Science education and research (IISER) Pune, India.
- Sangita Niranjan: Exploring Structural and Biochemical basis of Nup155 in NPC assembly (Sangeeta Niranjan & Radha Chauhan). 44th National Seminar on Crystallography, 10-13th July 2016. Indian Institute of Science education and research (IISER) Pune, India.
- Bhawna Burdak: Structural studies on Nup93-Nup188 complex of vertebrate Nuclear pore complex (Bhawna Burdak & Radha Chauhan). 44th National Seminar on Crystallography, 10-13th July 2016. Indian Institute of Science education and research (IISER) Pune, India.
- Parshuram Sonawane: Analysis of Interaction Network of Channel Nucleoporins with Adapter Ring of Vertebrate Nuclear Pore Complex. (Parshuram Sonawane, Pankaj Kumar Madheshiya and Radha Chauhan). 44th National Seminar on Crystallography, 10-13th July 2016. Indian Institute of Science education and research (IISER) Pune, India.
- Swati Gaikwad, Maitreyi Ashok, Manas Sahoo: 'Identification of a new interaction motif for Ago family of proteins' (Swati Gaikwad, Maitreyi Ashok, Manas Sahoo and Jomon Joseph); NCCS Mini-Symposium on Cell Biology, 11 May 2016.
- Rohan Kulkarni ; Rejuvenation of old HSCs by young in-vitro niche (IVN) via transfer of microvesicles carrying youth signals. (Rohan Kulkarni, Manmohan Bajaj Suprita Ghode, Lalita Limaye and Vaijayanti P. Kale), 44th "EMBL Conference Hematopoietic Stem Cells: From the Embryo to the Aging Organism 2016 (3rd June 2016 to 6th June 2016)." Heidelberg, Germany.

- Sheetal Kadam; Curcumin rescues high glucose- and diabetes-induced EPC dysfunction in vitro and in vivo. (Sheetal Kadam, Meghana Kanitkar, Kadambari Dixit, Rucha Despande and Vaijayanti Kale), International Diabetes Summit 2017 from 10th to 12th March 2017, Pune, India.
- Anshul Assaiya: Structural and Functional studies on Drosophila Ionotropic Receptors (Anshul Assaiya and Janesh Kumar), 44th National Seminar on Crystallography, 10-13, July, 2016, Pune, India.
- Surbhi Dhingra: Expression, Purification and Crystallization of Prokaryotic GluTamate Receptors (Surbhi Dhingra and Janesh Kumar), 44th National Seminar on Crystallography, 10 - 13, July, 2016, Pune, India.
- Pratibha Bharti: Structural and Functional Characterization of cystine-knot AMPAR modulating protein (CKAMP44) (Pratibha Bharti and Janesh Kumar), 44th National Seminar on Crystallography, 10 - 13, July, 2016, Pune, India.
- Shivam Shukla: Structural and Functional Studies on YbeY (Shivam Shukla, Ravi Singh, Janesh Kumar and Shree Prakash Pandey), 44th National Seminar on Crystallography, 10 - 13, July, 2016, Pune, India.
- Jyoti Kumari: CEM3DIP 2016: The 1st CryoEM and 3 Dimensional Image Processing Course at IISER Thiruvananthapura, 2 - 13, July, 2016, Thiruvananthapura, India.
- Ananth P. Burada: CEM3DIP 2016: The 1st CryoEM and 3 Dimensional Image Processing Course at IISER Thiruvananthapura, 2 - 13, July, 2016, Thiruvananthapura, India.
- Anshul Assaiya: Latest Methods in X-ray Crystallography: Lecture Series and Practical Course at JNU, 14 - 25, November, 2016, New Delhi, India.
- Surbhi Dhingra: Latest Methods in X-ray Crystallography: Lecture Series and Practical Course at JNU, 14 - 25, November, 2016, New Delhi, India.
- Pratibha Bharti: Latest Methods in X-ray Crystallography: Lecture Series and Practical Course at JNU, 14 - 25, November, 2016, New Delhi, India.
- Pratibha Bharti: Practical aspects of membrane protein crystallization-A workshop, 15 - 16, Dec 2016, IISC, Banglore, India.
- Ramesh Butti: Oral talk entitles "Osteopontin Induces Fibroblast to Myofibroblast Transition to Promote Breast Tumor Progression" (Ramesh Butti and Gopal Kundu). 2nd International conference on translational research. 14th -16th October, KIIT, Bhubaneswar, India.
- Ramesh Butti: Attended "International Symposium on Breast Cancer Research". 27th February 2017, NCCS, Pune, India.
- Ramesh Butti: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune, India.
- Ramesh Butti: "BD HorizonTM Global Tour" 20th April, 2016, NCCS, Pune, India.
- Deepti Tomar: Attended "International Symposium on Breast Cancer Research". 27th February 2017, NCCS, Pune, India.
- Deepti Tomar: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune, India.
- Deepti Tomar: "BD HorizonTM Global Tour" 20th April, 2016, NCCS, Pune, India.
- Deepti Tomar: "Workshop for Women in Science Journalism". Level 1: March 5th -7th, 2017, CoESME at IISER Pune in association with the Newton Bhabha Fund at IISER, Pune, India.
- Amit Singh Yadav: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune, India.
- Totakura V S Kumar: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune, India.
- NNV Radha Rani: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune, India.
- Ramakrishna Nimma: "2nd mini symposium on cell biology 2016". 11th May 2016, NCCS, Pune.
- Sumit Das: Attended Hands-On Training Course in Flow Cytometry organized by C-CAMP at NCBS, Bengaluru 14-17 June, 2016.
- Sumit Das: Attended "International Symposium on Breast Cancer Research", 27th February 2017, NCCS, Pune, India.
- Shilpi and Lal G (2017) Gamma-delta (γδ) T cells help in the generation of costimulatory blockade-induced transplantation tolerance. 43rd Annual Conference of Indian Immunology Society, Immunocon 2016 held on 16-18th February 2017 in Visakhapatnam. *(Best Oral Presentation Award).*
- Kulkarni N, Sharma, PK, Mujeeb VR, Srivastava S, Puri P and Lal G (2016) CCR6 intrinsic signaling in the CD4 T cells promotes the differentiation of inflammatory Th17 cells during autoimmune colitis. 57th Annual Conference of Indian Society of Gastroenterology held on 15-18 December at New Delhi. India. *(Poster Presentation).*
- Kulkarni N, Sethi A, Shaikh S and Lal G (2016) Non-chemotactic function of chemokine receptor CCR6 in the differentiation of Th17 and Treg cells. Cytokine2016 held on 16-19th October 2016 in San Francisco, USA. *(Poster Presentation).*

- Sonar S and Lal G (2016) IFN- signaling at the blood-brain barrier (BBB) endothelial cells has inflammatory function but in parenchyma plays a regulatory role during autoimmunity. International Congress of Immunology 2016 held from 21-26 August 2016 at Melbourne, Australia. *(Oral Presentation, Travel Award by AAI). Eur. J. Immunol. 2016, 46(Suppl.1):563.*
- Paul S and Lal G (2016) Intratumoral Natural Killer (NK) cells possess inhibitory phenotype and suppress Th1 effector function. International Congress of Immunology 2016 held from 21-26 August 2016 at Melbourne, Australia. *(Mini-Oral Presentation) Eur. J. Immunol. 2016, 46(Suppl.1):89.*
- Patta I, Lal G and Galande S (2016) Role of chromatin organizer SATB1 during development of regulatory T cells. 43rd Scandinavian Society for Immunology Meeting, Turku, Finland organized on 10-13 May. *(Poster presentation).*
- Ankita Dhenge. Presented poster on Regulation of platelet biogenesis by Notch3 induction through actin polymerization by valproic acid. Dhenge A.C , Kale V.P. and Limaye L.S.* in the following conference: Gordon research conference on "Cell biology of Megakaryocytes and Platelets on" held in Italy from. 26th Feb to 3rd March 2017.It was supported by DBT travel grant and partial support from NCCS, Pune, India.
- Swastik Phulera; 61st Annual Meeting of the Biophysical Society. February 11-15, 2017, New Orleans, Louisiana, USA.
- Lumbini Yadav; Latest Methods in Structural Biology Conducted by GIAN-JNU November 15-25, 2016, New Delhi, India.
- Ashwani Kumar; Structural and Functional study on hypoxia protein Rv0081 in *Mycobacterium tuberculosis* during latency (Ashwani Kumar, C.M. Santosh Kumar, Swastik Phulera, Parshuram Sonawane, and Shekhar C. Mande), 44th National Seminar on Crystallography (NSC44), July 10-13, Pune, India.
- Sapna Sugandhi; Structural and Functional characterization of redox proteins from *Mycobacterium tuberculosis* (Sapna Sugandhi, Shekhar C. Mande), 44th National Seminar on Crystallography (NSC44), July 10-13, Pune, India.
- Shekhar Made's entire research group attended the 44th National Seminar on Crystallography (NSC44), July 10-13, 2016, held at IISER Pune.
- Jay Trivedi and Debashis Mitra, Identification of a novel HIV-1 inhibitor targeting NF-κB signalling pathway, SIGID Meeting on "Pathogens and Host Response" 10th-12th August 2016, National Institute of Immunology, New Delhi, India.
- Surya Shrivastava and Debashis Mitra, Nef alleviates YY1 mediated epigenetic silencing of HIV-1 LTR driven gene expression, SIGID Meeting on "Pathogens and Host Response" 10th-12th August 2016, National Institute of Immunology, New Delhi, India.
- Tushar More presented a poster entitled "Comprehensive quantitative metabolomic approach to investigate metabolic alterations in invasive ductal carcinoma of the breast" at 8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health organised by NIPGR, December 14-17, 2016 at New Delhi. (Poster selected for oral presentation).
- Venkatesh Chanukuppa presented a poster entitled "Differential proteomic analysis of multiple myeloma towards new targets and biomarkers" at 8th annual meeting of proteomics society of India and international conference on functional and interaction proteomics: Application in food and health organised by NIPGR, December 14-17, 2016 at New Delhi. (Poster selected for travel award).
- Hemendra Singh Panwar; Domain swapping reveals functional modularity present in the decay-accelerating factor (CD55) (Panwar, H.S., Ojha, H., Ghosh, P., Raut, S. and Sahu, A.), XXVI International Complement Workshop, 4-8, September, 2016, Kanazawa, Japan.
- Hina Ojha; *In silico* identification of CCP sequence motifs allow identification of novel complement regulators (52. Ojha, H., Mahajan, G., Mande, S. and Sahu, A.), XXVI International Complement Workshop, 4-8, September, 2016, Kanazawa, Japan.
- Kunal Jaani : Presented a poster, 'Isolation of bacteriorhodopsin producing haloarchaeon, *Halostagnicola larsenii* ibs (MCC 2956), from Indian black salt' (P. P. Kanekar, S.O. Kulkarni, Y.Shouche, K. Jani, A.Sharma), Bergey's International Society for Microbial Systematics (BISMiS) - Microbial Systematics and Metagenomics' conference (12-15th September 2016), held at MCC-NCCS, Pune, India.
- Kunal Jaani : Presented a poster, 'Metagenomics approach to reveal the microbial community structure of continental shelf, Agatti Island, India' (Kunal Jani, Vinay Rale, Yogesh Shouche, Avinash Sharma); 'Accelerating Biology 2017-Delivering precision' conference (17-19 Jan 2017), organized by C-DAC Pune, India.
- Diptaraj Chaudhari : Poster entitled Comprehensive Human Microbiome Analysis in the Indian Rural and Semi-urban

Subpopulation Reveals Abundance Of Genus Prevotella And Dialister (Diptraj Choudhury, Dheeraj Dhotre, Akshay Gaike, Dheeraj Agarwal, Yogesh Shouche) presented in Yakult India Microbiota and Probiotic Foundation- Probiotic Symposium 2016- "Probiotics in Health – Emerging Opportunities", 3rd and 4th December 2016, Chennai,India .

- Sahabram Dewala: Participated in Transcriptome data analysis workshop organized by Biosakshat on August 16, 2016 at Venture Centre, NCL Innovation Park, Pune, India.
- Sahabram Dewala: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit, September 16-17, 2016, at NCL, Pune,India.
- Deepak V. Khairnar: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit , September 16-17, 2016, at NCL, Pune,India.
- Rahul L. Bodkhe: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit, September 16-17, 2016, in NCL, Pune,India.
- Satish Kumar: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit , September 16-17, 2016, in NCL, Pune,India.
- Abhijit Kulkarni: Participated in Two-day Workshop on Nomenclature of Archaea and Bacteria organized by BISMiS and AMI Pune Unit, September 9-10, 2016, held at MCC-NCCS, Pune, India.
- Abhijit Kulkarni: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit, September 16-17, 2016, in NCL, Pune, India.
- Kusum Dhakar: Participated in 5th National Workshop on - Next Generation Sequencing Data Analysis, 15- 17th March 2017, organized by National Institute of Pathology, ICMR, New Delhi.
- Mithil Shetty: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit, September 16-17, 2016, in NCL, Pune, India.
- Dhiraj Paul: Participated in Two-day International Symposium on Microbial Ecology and Systematics organized by BISMiS and AMI Pune Unit, September 16-17, 2016, in NCL, Pune, India.
- Dhiraj Paul: Presented a poster entitled Microbial diversity and community composition of Lonar Lake: the only hypersaline meteorite crater lake within basalt rock (Dhiraj Paul, Satish Kumar,Yogesh Shouche), at the 16th International symposium on Microbial Ecology ISME 2016, 21-26th August 2016, Montreal, Canada.
- Suhas Mhaske attended "Science Communication and Career Workshop (SciComm101)" at National Centre for Cell Science, Pune, July 14, 2016.
- Suhas Mhaske attended "4th Annual Meeting of the International Cytokine and Interferon Society (ICIS) Cytokines 2016" at San Francisco, CA, USA, October 16-19, 2016. He was awarded "International Travel Grant" from Department of Science and Technology (DST), New Delhi and Centre for International Co-operation in Science (CICS), Chennai.
- Suhas Mhaske attended "International Symposium on Breast Cancer Research" at National Centre for Cell Science, Pune, February 27, 2017.
- Anil Kumar attended "4th Annual Meeting of the International Cytokine and Interferon Society (ICIS) Cytokines 2016" at San Francisco, CA, USA, October 16-19, 2016. He was awarded "International Travel Grant" from Department of Biotechnology (DBT), New Delhi and Centre for International Co-operation in Science (CICS), Chennai, India.
- Amruta Naik attended "Science Communication and Career Workshop (SciComm101)" at National Centre for Cell Science, Pune, July 14, 2016.
- Amruta Naik attended "International Symposium on Breast Cancer Research" at National Centre for Cell Science, Pune, February 27, 2017.
- Adrita Guha attended "Workshop on Science Communication" at National Centre for Cell Science, Pune, March 27-31, 2017.
- Shubhanath Behera attended "Workshop on Science Communication" at National Centre for Cell Science, Pune, March 27-31, 2017.

Talks Delivered by / Workshops Participated in by MCC Scientists

Dr. Amit Yadav

- MLSA of Phytoplasmas Associated with Crops and Insects vectors in India at the 10th Annual Conference of Mycoplasmologists at All India Institutes of Medical Sciences (AIIMS) organized by Indian Association of Mycoplasmologists and American Society of Microbiologist. New Delhi; March 20 & 21, 2017.
- Taxonomy of 'Candidatus' Phytoplasma, an Indian Scenario. Invited talk at 'National Symposium on Phytopathogenic Mollicutes: Indian scenario of diagnosis, epidemiology and disease management' organized by Indian Phytopathological Society (IPS) and Division of Plant Pathology, Indian Agricultural Research Institute, Pusa Campus, New Delhi, India. December 17 and 18, 2016. New Delhi, INDIA.
- Current Scenario and Future Challenges: Journey Behind and the Journey Ahead, Taxonomy, Diagnostics & Characterisation of phytoplasma. Invited talk at Farmers Academia Interface on Phytoplasma Diseases in Horticultural Crops organized by Indian Council of Agricultural Research (ICAR), Directorate of Floriculture Research (DFR), Pune, India. June 29, 2016, Rajahmundry, Andhra Pradesh, INDIA.

Dr. D. R. Ranade

- Delivered 8 lectures on "Biogas Technology" to students undergoing "Post-graduate Certificate Course in Skill Development in Renewable Energy "at MITSKILL, MIT, Pune in December, 2016.

Dr. Kamlesh Jangid

- Delivered talk on K-shuff: A novel algorithm for characterizing structural and compositional diversity in gene libraries. 2016. 3rd Meeting of the Bergey's International Society for Microbial Systematics, Pune, India.
- Delivered talk on Citizen Science: Microbiology in daily life. 2016. Venezia Co-operative Housing Society, Pune, India.
- Delivered talk on Microbial Research at the World's Largest Culture Collection. 2016. DYP Institute of Biotechnology, Pune, India.

Dr. Mahesh Chavadar

- Invited talk on "Need of Microbial Culture collections" organized by Yashwantrao Chavan Institute of Science, Satara on, 6th August. 2016.

Dr. Om Prakash

- Delivered talk on "Polyphasic approach of bacterial classification and determination of Biosafety Classes in Bacteria" at Institute of Soil, Water and Environmental Sciences Volcani Center, Agricultural Research Organization 68 HaMacabim Rd. Rishon Lezion, Israel(July -2016).

Dr. Prashant Singh

- Invited lecture in National conference on Plant Science Technologies: current status and future prospects (NCPST-2017)' during 21-23 March, 2017 in Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India.
- Invited lecture in 2nd National seminar on Current Trends in Life science in Centre for Biological Sciences, Central University of South Bihar (CUSB) on 20th - 21st February, 2017 in Patna, Bihar, India.

Dr. Praveen Rahi

- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) applications in microbial ecology; Invited talk, Frontiers in Biotechnology; Chandigarh, India, March 2017.
- MALDI-TOF MS Applications in Microbial Ecology Studies: the Example of Fenugreek (Trigonella foenum-graecum) Root-Nodulating Bacteria at Microbes and Biosphere: What's New what's Next- AMI2016, Guwahati, Assam, India (24-27 November 2016).

Dr. Rohit Sharma

- Invited Talk on 25th November 2016 titled "ECM Mushrooms: Culturing, preservation and Artificial Synthesis; Characterization of ECM Mushrooms" at 2nd Certificate Course on 'Taxonomy, Biodiversity, Ex situ Conservation and Application of Fungi at National Fungal Culture Collection of India (NFCCI), Pune.
- Invited Talk on 30th September 2016 titled "Species Concept; ECM Mushrooms: Culturing, preservation and Artificial Synthesis; Characterization of ECM Mushrooms" at CAFT Training Programme entitled "Fungal diversity and new

trends in taxonomy through DNA Barcoding and Chemo-profiling" at Division of Plant Pathology, IARI, Pusa, New Delhi.

- Talk on 17th September 2016 titled "Diversity and distribution of phenol oxidase producing fungi from soda lake and description of Curvularia lonarensis sp. nov." at MSI-2016 at National Conference on FUNGAL BIOTECHNOLOGY and 43rd Annual Meeting of the Mycological Society of India, 16-18 November 2016, Jaipur.

Dr. Shrikant Pawar

- Wonders of Microbiology' - talk delivered for the 'Second Saturday talks series' at IUCAA; 12th November 2016.
- Research Opportunities in Biotechnology' - D. Y. Patil College of Arts Commerce and Science, Pimpri.

Dr. Avinash Sharma

- Attended training course of 'Microbial Resources Information Management and Utilization for Developing Countries'; 9-23 September, 2016; funded by Chinese Academy of Science, Beijing, China.

Events Organized

Conferences / Symposia Organized

BD Horizon™ Global Tour symposium *(conducted under the BD-NCCS COE programme)*

20th April 2016






2nd Mini-symposium on Cell Biology *(organized by the Ph.D. students of NCCS)*

11 May 2016

Dr. Kazutoshi Mori, Kyoto University, Japan

Prof. Gaiti Hasan, NCBS, Bangalore, India

Dr. Myriam Gorospe, National Institute on Aging, NIH USA

Dr. Sanjeev Das, NII, New Delhi, India

Dr. Ullas Kolthur-Seetharam, TIFR, Mumbai



Mini-symposium on Metabolomics & Volatomics Towards Disease Markers

16th May, 2016

(funded by the New Indigo programme of the Department of Biotechnology, Govt. of India)







Science Academies' Summer Research Fellows' Symposium

8th July, 2016

44th National Seminar on Crystallography (NSC44)

10-13 July, 2016

(co-organized by NCCS, IISER-Pune, CSIR-NCL & S.P. Pune University)








NCCS Retreat

(Mantra Resort, Bhor; Oct, 2016)





2nd International Conference on Translational Research

14th-16th October, 2016

(co-organized by Dr. G. C. Kundu of NCCS with KIIT University, Bhubaneswar, India)

Biology of the Cell: Challenges and Opportunities *(NCCS Alumni Symposium)*

23rd December 2016















International Breast Cancer (BC) Research Symposium

27th February, 2017

Co-organized by NCCS, Prashanti Cancer Care Mission (PCCM) and Markey Cancer Research Center (MCC), University of Kentucky College of Medicine, USA.

Workshop on Nomenclature of Archaea & Bacteria 9,

10 September, 2016

Organized by MCC; Conducted by Prof. Aharon Oren

BISMiS 2016

12-15 September, 2016

Bergey's International Society for Microbial Systematics - 3rd meeting on Microbial Systematics and Metagenomics (organized by MCC)

International Symposium on Microbial Ecology and Systematics

16-17 September, 2016

(organized by MCC in association with AMI)

The 9_{th} Young Investigator Meeting (YIM) 2017

March 6-10, 2017; Goa

(Dr. Deepa Subramanyam of NCCS was a member of the organizing committee)

Workshops / Hands-on Training Conducted

Workshops on Computational Biology

Three workshops on topics related to applications of computational biology were conducted by the Bioinformatics and High Performance Computing Facility of NCCS during 2016-17.

'Protein-Protein Interaction Network'
in-house workshop, May 2016

Ph.D. coursework workshop

Basic Cell Culture Technology *(Hands-on Training Workshops conducted by the Cell Repository)*

16-19 May, 2016

26-28 September, 2016

Science Communication & Career Workshop

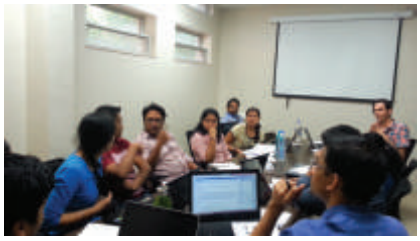
14th July, 2016

(organized in association with the Wellcome Trust/DBT India Alliance and Nature India & NatureJobs)

Science Communication Workshop

Organized in association with the Maastricht University, the Netherlands

27-31 March, 2017



Lecture and hands on session on 'Gene annotation'

24th November 2016.

(organized by MCC for MSc second year students)

Workshop on 'Microbial Identification and Preservation

5-9 January 2017.

(organized by MCC)

Hands-on training workshopon 'Microbial Identification, Preservation and Genome Analysis'

1-10 March, 2017

(organized by MCC)

Workshop on sample preparation for mass spec analysis

October, 2016

(In-house hands-on workshop for NCCS students, conducted by Dr. Srikanths's group)

Research Students of NCCS awarded with Ph. D. Degrees

(01.04.2016 – 31.03.2017)

No.	Research Scholar	Title of the Thesis	Date of award of Ph.D.	Research Guide
1	Ms. G. Manasa Gayatri	Role of mesenchymal stem cells in the amelioration of experimental arthritis.	5th April, 2016	Dr. Mohan Wani
2	Ms. Manasi Talwadekar	Generation of mesenchymal stem cells from human umbilical cord tissues, their characterization and differentiation to neural cells.	12th April, 2016	Dr. L. S. Limaye
3	Mr. Manas Ranjan Sahoo	A novel role for Nup358 in miRNA pathway.	13th April, 2016	Dr. Jomon Joseph
4	Mr. Natesh Kumar	Role of Oncostatin M (OSM)-mediated signaling in tumor progression in gliomas	22nd April, 2016	Dr. Padma Shastry
5	Ms. Shweta Singh	Studies on microenvironment-mediated regulation of hematopoietic stem cells	29th April, 2016	Dr. Vaijayanti Kale
6	Ms. Mithila Sawant	Investigation of anti-cancer activity of AECHL-1 and/or its synthetic analogs on breast cancer cells in vitro and in vivo: Role of mitochondria and redox regulation	2nd May, 2016	Dr. Sandhya Sitasawad
7	Mr. Arya Vindu	Characterization of RNA-binding proteins involved in malarial parasite life cycle	5th May, 2016	Dr. Vasudevan Seshadri
8	Ms. Priyanka Chaudhary	Studies on regulation of heat shock proteins during HIV-1 infection in Tcells	18th May, 2016	Dr. Debashis Mitra
9	Mr. Aman Sharma	Elucidating mechanisms of cancer stem cell (csc)-niche interactions in glioma	20th May, 2016	Dr. Anjali Shiras
10	Ms. Jinumary Mathai	Role of SMAR1 in global gene regulation through interaction with tumor suppressor p53	24th May, 2016	Dr. Samit Chattopadhyay
11	Mr. Naoshad Mohammad	Methyl β cyclodextrin augments the efficacy of chemotherapeutic drugs in solid tumour cells: Molecular mechanism of action	5th July, 2016	Dr. Manoj Kumar Bhat
12	Ms. Suprita Ghode	Identification of signaling modulators in the bone marrow microenvironment regulating HSC fate	12th July, 2016	Dr. Vaijayanti Kale
13	Ms. Darshana Kadekar	Stromal cell mediated ex vivo expansion of hematopoietic stem/ progenitor cells with a special reference to regulation of apoptosis	23rd July, 2016	Dr. Lalita Limaye
14	Ms. Rutika Naik	Study of Tumor Heterogeneity in Ovarian Cancer	3rd August, 2016	Dr. Sharmila Bapat
15	Mr. Dhiraj Kumar	Studies on role of CD133+ cancer stem-like cells in regulation of melanoma growth, angiogenesis and metastasis	31st August, 2016	Dr. Gopal Kundu
16	Mr. Parmanand Malvi	Impact of obesity and adipokines on melanoma progression and the outcome of dacarbazine therapy.	1st September, 2016	Dr. Manoj K. Bhat
17	Mr. S. Satishkumar	CD 40 & vascular endothelial growth factor in anti-tumor immune response	28th September 2016	Dr. B. Saha

No.	Research Scholar	Title of the Thesis	Date of award of Ph.D.	Research Guide
18	Mr. Abdul Khalique	Role of the insulin 5' UTR binding trans acting factors in insulin gene regulation	14th October, 2016	Dr. Vasudevan Seshadri
19	Mr. Ekansh Mittal	Investigation of Topology & Mechanism of Function of Rv1694, TlyA protein *Mycobacterium tuberculosis* H37Rv	24th October, 2016	Dr. M. V. Krishnasastry
20	Mr. Sunil Banskar	Microbial diversity associated with gut of Indian bats (Chiroptera).	3rd November, 2016	Dr. Yogesh Shouche
21	Mr. Brijesh Kumar	Role of slug transcription factor in promoting epithelial to mesenchymal transition in ovarian cancer	10th November 2016	Dr. Sharmila Bapat
22	Mr. Bal Krishna Chaube	Mechanistic insight into the regulation of glucose metabolism in cancer cells: cause and consequence of metabolic reprogramming in tumorigenesis.	15th November 2016	Dr. Manoj Kumar Bhat
23	Ms. Swati Gaikwad	Functional characterization of interaction between the nucleoporin Nup358 and Argonaute	18th November, 2016	Dr. Jomon Joseph
24	Mr. Ajitanuj Rattan	Studies on the role of complement in influenza infection	28th November, 2016	Dr. Arvind Sahu
25	Ms. Poonam Pandey	Translation regulation of insulin by different elements of insulin mRNA and its trans-acting factors	4th January, 2017	Dr. Vasudevan Seshadri
26	Mr. Santosh Kumar	Investigation of transport mechanism of Rv1694 (TlyA) in *Mycobacterium tuberculosis*	10th January, 2017	Dr. Musti Krishnasastry
27	Mr. Anand Kamal Singh	Aberrant epigenetic markers in epithelial ovarian cancer progression	12'h January, 2017	Dr. Sharmila Bapat
28	Mr. Anil Kumar	Investigation of immunoprotective sequences of *P. falciparum* and other species.	25th January, 2017	Dr. Musti V. Krishnasastry
29	Mr. Shivendra Vikram Singh	Role of cellular and physiological heterogeneity on chemotherapeutic outcome in cancer	7th February, 2017	Dr. Manoj Kumar Bhat
30	Ms. A. Silpa	Role of Prx-3 in redox regulation of hyperglycemia-induced contractile dysfunction in diabetic cardiomyopathy.	16th February, 2017	Dr. Sandhya Sitasawad
31	Ms. Prachi Umbarkar	Role of Monoamine Oxidase-A (MAO-A) in hyperglycemia-induced oxidative stress mediated apoptosis and contractile dysfunction in diabetic cardiomyopathy.	16th February, 2017	Dr. Sandhya Sitasawad
32	Ms. Tracy Augustine	Identification and functional characterization of differentially expressed cell cycle and apoptosis associated proteins during HIV-1 infection	20th February, 2017	Dr. Debashis Mitra
33	Ms. Surya Shrivastava	Studies on the role of HIV-1 Nef protein in viral and cellular gene expression	23rd February, 2017	Dr. Debashis Mitra

NCCS Foundation Day

(26th August, 2016)

Foundation Day Oration:

'The Crucial Role of Innovations to Spur Rapid Economic Growth'

Prof. M. M. Sharma

F.R.S., F.N.A., F.T.W.A.S.

Former Director, UDCT/ICT, Mumbai

Felicitation of employees who have completed 20 years of service

Ms. Tanuja Bankar

Mr. D. G. Moundekar

Mr K. Munikrishnan

Other Talks by Invitees

- **Ms. Aparna Thakar**
  'Understanding Stress & Its Management'
  13th April, 2016

- **Dr. Tanweer Hussain**
  MRC Laboratory of Molecular Biology, Cambridge, UK
  'Structural insights into key events during translation initiation'
  9th May, 2016

- **Dr. Ganesh Halade**
  Division of Cardiovascular Disease, The University of Alabama at Birmingham, the USA.
  'Metabolic Transformation of FATS in Cardiac Remodeling and Heart Failure'
  8th June, 2016

- **Dr. Suchi Goel**
  Karolinska Institute, Sweden
  'Molecular switching in Plasmodium: Is it the key to its success?'
  14th June, 2016

- **Dr. Jagan Srinivasan**
  Worcester Polytechnic Institute, the USA
  'The Underground Social Network: Studying Communication in Worms'
  16th June, 2016

- **Dr. Srini V. Kaveri**
  Director, CNRS Office in India, Embassy of France, New-Delhi
  #1 : 'Antibodies: More than mere neutralisers of pathogens'
  #2 : 'Indo-French interactions: More than mere red wines'
  17th June, 2016

- **Dr. Prabha Sampath**
  Senior Principal Investigator, Institute of Medical Biology, A*Star, Singapore
  'Consequences of a Defective Molecular Switch in Cutaneous Wound Healing & Carcinomas'
  20th June, 2016

- **Dr. Sougata Roy**
  EMBL-European Bioinformatics Institute & Wellcome Trust Sanger Institute, Cambridge, UK
  'Timing is the key to survival – Understanding the circadian regulation of gene expression'
  24th June, 2016

- Prof. Santanu Bhattacharya, FNA, FASc, FTWAS
  Director, Indian Association for the Cultivation of Science, Kolkata, India
  'Molecular Design of Cellular Delivery Systems'
  13th July, 2016

- Dr. Geetha Shankar
  Principal Clinical Scientist, PD Oncology, Genentech, Inc., USA
  'Cancer Immunotherapy: Past, present and future'
  4th August, 2016

- Dr. Prayag Murawala
  Center for Regenerative Therapies, Technische Universität, & Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
  'Understanding cellular and molecular cues that drive axolotl limb regeneration'
  6th September, 2016

- Dr. Gaurav Das
  University of Oxford, UK
  'Of Flies and Food: How food choices are driven by nutrient specific memories and motivational states in Drosophila'
  28th September, 2016

- Dr. Kausik Chakraborty
  Institute of Genomics and Integrative Biology, New Delhi. Inda
  'Governing cellular proteostasis'
  3rd October, 2016

- Dr. Sachin Ranade
  Associate Editor, Nature Communications
  'How to get published in Nature titles!'
  14th October, 2016

- Dr. Meghana Tare
  UMass Medical School, USA
  'Exploring the mechanisms of cell death in development and disease'
  7th November, 2016

- Dr. Shambaditya Saha
  Max Planck Institute of Molecular Cell Biology and Genetics, Germany
  'A competition mechanism positions non-membrane-bound organelles in cells'
  10th November, 2016

- Prof. Rohinton Kamakaka
  Dept. of Molecular Cell and Developmental Biology, University of California, Santa Cruz, USA.
  'A Silent Looping Journey has Boundaries'
  29th November, 2016

- Dr. Shruthi Viswanath
  Dept. of Bioengineering and Therapeutic Sciences, Univ. of California San Francisco, USA
  'Integrative structure determination of the yeast centrosome'
  5th January, 2017

- Dr. Chhavi Mathur
  National Institute of Neurological Disorders and Stroke (NINDS), NIH, USA
  'Transcriptome of squid giant axon provides insights into axonal protein synthesis'
  20th February, 2017

- Dr. Gireesha Mohannath
  Department of Biology, Indiana University, USA
  'Chromosomal position determines activity status of rRNA genes on a multi-megabase scale in Arabidopsis'
  14th March 2017

- Dr. Sreeramaiah Gangappa
  Cell and Developmental Biology Department, John Innes Centre, UK.
  'Temperature-mediated regulation of plant growth and immunity'
  15th March 2017

- Dr. Sabari Thirupathy
  Dept. of Bacteriology, University of Wisconsin-Madison, USA.
  'Replication-transcription crosstalk threatens genomic stability'
  29th March, 2017

Technical Seminars

- 'Understanding Complex Lipid Metabolism through Quantitative Lipidomics using LC-MS/MS'
  Dr. Baljit Ubhi [Staff Scientist – Lipidomics & Metabolomics, Sciex, USA]
  25th May, 2016

- 'Orbitrap Fusion Lumos Tribrid Mass Spectrometry designed to advance your Scientific Pursuits'
  Dr. Sangram Pattanaik [DGM (LSMS), Thermo Fisher Scientific]
  21st July, 2016

- 'End to End NGS Data Analysis'
  Ms. Aishwarya Narayanan [Senior Application Scientist - Strand Life Sciences]
  27th July, 2016

- 'Cryo Ultramicrotomy and advanced techniques'
  Dr David Bentley (from USA) [Icon Analytical Pvt Limited].
  27th October,2016

- '3D Imaging at the Nanoscale: Unveiling the wonders of nature at the molecular level'
  Dr. Gaurav Sharma [Thermo Fishcer (FEI) Eindhoven, The Netherlands]
  22nd December, 2016

Outreach and Teaching

Outreach

National Science Day
28 Feb, 2017

a) Popular Science Public Talks

'Snakes, Snake-bite Treatment & Snake Venom Research'
- Antony Gomes
[Former Professor, University of Calcutta]

'Challenges of Communicating Science to the Public: A Journalist's Perspective'
- Pallava Bagla
[Science Editor, New Delhi Television (NDTV)]

b) 'Innovative Ideas in Biological Research' competition - *Presentations by the shortlisted teams*

c) Exhibits & Displays

Public Talk by Lasker laureate, Prof. Kazutoshi Mori *(Kyoto University, Japan):*

'How did a rustic country boy become a Lasker laureate?'

10 May, 2016

'The 2016 Nobel Prizes : A Curtain Raiser to the Award Ceremony'

5th December, 2016

'The discovery of autophagy-specific genes: The contributions of Nobel laureate, Prof. Yoshinori Ohsum'

- Dr. Bhupendra Shravage (ARI, Pune)

'The 2016 Nobel prize in Physics: Topology in Condensed Matter Physics'

'The 2016 Nobel prize in Literature'

- Prof. Sunil Mukhi (IISER-Pune)

'The 2016 Nobel Prize in Chemistry: Conferring Molecular Machines as Engines of Creativity'

- Prof. N. Jayaraman (IISc, Bengaluru)

Edu-Bridge *(ongoing teaching programme at the Jankidevi Bajaj College of Science, Wardha)*

Dr. Janesh Kumar organized a demonstration of 'protein crystallization', and 'protein structure visualization and analysis using PYMOL'

February, 2017

Curtain Raiser for the 2nd India International Science Festival (IISF 2016) - Western Zone

13th November 2016

a) Young Scientists' Meet

b) Science & Technology Expo



Curtain Raiser to the 5th Bharatiya Vigyan Sammelan & Expo

18th November, 2016


Popular Science Talks

Talks in Marathi delivered by Dr. L. S. Limaye for the Marathi Vidnyan Parishad:

"Stem cells che jatan" - at the Science Park, Pimpri-Chinchwad.

Lectures on the topic, 'Stem Cells', were delivered by Dr. Deepa Subramanyam for school and college students, and the general public, at the Muktangan Exploratory Science Centre in Pune.

A talk on 'Marvel of Nature's Design: Glutamate Receptor Ion channels as Sophisticated Nano Machines' was delivered by Dr. Janesh Kumar at the Saturday Popular Lecture Series organized by the Department of Microbiology, S. P. Pune University.

DST INSPIRE Camp: Dr. Shailza Singh and Dr. Nibedita Lenka have delivered talks at the Dayanand Science College, Latur, for students of class 11th and 12th attending this camp.

Visits

A visit to the cell repository and other facilities of NCCS was organized for participants of the CME on 'Basic Techniques in Research Methodology'.

3rd March, 2017

[This CME, which was sanctioned by the Rashytriya Ayurveda Vidyapeeth (RAV), Ministry of AYUSH, Govt. of India, was organized for PG teachers by IRSHA, Bharati Vidyapeeth Deemed University,Pune.]

Teaching

Talks delivered by & hands-on activities / training conducted by NCCS faculty

Scientist	Topic / Symposium	Class / Department	Institution	Date
Sharmila Bapat	'The Problem of Microbial and Cancer Cell Persisters' (Plenary Talk as Chief Guest at the inauguration of Microfest)	B.Sc, M.Sc Microbiology	Abasaheb Garware College	06/01/2017
Sharmila Bapat	'Emerging frontiers in cancer epigenetics' (at the seminar, 'Cancer Biology and Therapy : Recent Developments and Newer Perspectives')	B.Sc, M.Sc Life Sciences	SIES College of Arts, Science and Commerce, Mumbai	27/02/2017
Sharmila Bapat	'Cancer Stem Cells in Tumor Heterogeneity and Drug Resistance' (Invited talk at Recent Advances in Modern Biology & Biotechnology 2017')	B.Sc, M.Sc Life Sciences, Biotechnology and Bioinformatics	Dr. D. Y. Patil Biotechnology & Bioinformatics Institute	16/03/2017
Radha Chauhan	Life under the microscope	Primary-middle school	Govt. Primary school	16/11/2016
Radha Chauhan	Biology: why is it so important?	9-10th grade	Wisdom world school	6/12/2016
Janesh Kumar	Protein Structures: Introduction and General Overview; Examples	B.Sc and MSc	Jankidevi Bajaj College of Science (JBCS), Wardha	24/02/17
Janesh Kumar	Crystals and Crystallization - Theory & Practicals	B.Sc and MSc	JBCS, Wardha	24/02/17
Janesh Kumar	Introduction to Protein data bank	B.Sc and MSc	JBCS, Wardha	25/02/17
Janesh Kumar	Molecular visualization & analysis using Pymol - Theory & Practicals	B.Sc and MSc	JBCS, Wardha	25/02/17
Janesh Kumar	Central dogma : Transcription (Insights from RNA polymerase structures)	B.Sc and MSc	JBCS, Wardha	25/02/17
Janesh Kumar	Central dogma : Translation (Insights from Ribosome structures)	B.Sc and MSc	JBCS, Wardha	25/02/17
Janesh Kumar	Structures of tRNA and tRNA synthatase	Biotechnology	Dept. of Biotechnology, SPPU, Pune	17/01/2017
Janesh Kumar	Functional Insights from Ribosome structures	Biotechnology	Dept. of Biotechnology, SPPU, Pune	21/01/2017
Janesh Kumar	Structural aspects of membrane proteins	Biotechnology	Dept. of Biotechnology, SPPU, Pune	24/01/2017
Janesh Kumar	Structure of RNA polymerase and its role in transcription	Biotechnology	Dept. of Biotechnology, SPPU, Pune	28/01/2017 & 04/02/2017
Gopal C. Kundu	Cancer Awareness in India, INSPIRE Program, DST	XIth Std.	Dhule, Maharashtra	27/09/2016
Gopal C. Kundu	Cancer Awareness in India, INSPIRE Program, DST	XIth Std.	Pondicherry University, Port Blair	26/12/2016
Gopal C. Kundu	Cancer Awareness in India, INSPIRE Program, DST	XIth Std.	Amity University, Jaipur	16/01/2017
Nibedita Lenka	Unfolding Life and Development: A Stem Cell Perspective.	Class 11th & 12th (Mentor, DST INSPIRE Science Camp, Basic Science stream; Biology)	Dayanand Science College, Latur	29/09/ 2016

Scientist	Topic / Symposium	Class / Department	Institution	Date
Shekhar Mande	Nature and Structure	VIIIth - XIIth Std.	Muktangan Exploratory Science Centre	18/04/2016
Shekhar Mande	INSPIRE Camp, title of presentation: "From Molecular Struture to Life Forms"		Manipal University, Manipal	24/05/2016
Shekhar Mande	INSPIRE Camp, title of presentation: "Physics in Biology"		Swami Vivekananda College, Latur	06/06/2016
Shekhar Mande	Structural Biology I & II	Centre for Bioinformatics	SP Pune University	04/04/2016 & 11/04/2016
Shekhar Mande	Computational Bioinformatics I& II	Department of	SP Pune University Biotechnology	09/04/2016 & 16/04/2016
Debashis Mitra	Immunology	Biotechnology Department	SP Pune University	March-April 2017
Debashis Mitra	Virology	Biotechnology Department	SP Pune University	March-April 2017
Manas K. Santra	AKT promotes tumorigenesis by accelerating proteasomal degradation of tumor suppressor FBXO31/Talk	Life Sciences	Saha Institute of Nuclear Physics, Kolkata, India	24/11/2016
Anjali Shiras	Advances in Molecular Biology Shaping Life	B.Sc. Zoology & Biotechnology	Modern College, Ganeshkhind, Pune	31/08/2016
Anjali Shiras	Concepts In Molecular Biology	B.Sc. Zoology	S.P. College, Tilak road, Pune 411030.	27/09/2016
Yogesh Shouche	Microbial Genetics	Biotechnology	SP Pune University	
Yogesh Shouche	Advanced Techniques in Microbial Taxonomy	Microbiology	SP Pune University	
Yogesh Shouche	DNA Fingerprinting RFLP, Next Generation Sequencing	Biotechnology	SP Pune University	
Shailza Singh	Cells governing the Life in Science	Class 11th & 12th (DST INSPIRE Camp)	Dayanand Science College, Latur	Sep, 2016
Deepa Subramanyam	Stem Cells	7th to 12th Std.	Muktangan Exploratory Science Centre	28/01/17 27/03/17
Deepa Subramanyam	New trends in Biology	11th & 12th Std. (Science)	Delhi Public School, Pune	25/08/16
Vidisha Tripathi	Cell Cycle regulation	B.Sc.	JBCS, Wardha	24/02/17

Classes taught by NCCS faculty for the Ph.D coursework (2016)

(for Ph.D. students registered with the S.P. Pune University)

Scientist	Topic
Sharmila Bapat	Tumor Heterogeneity: Implications for effective treatment
Manoj Kumar Bhat	Cancer therapies
Radha Chauhan	Structural Biology (course coordinator)
Jomon Joseph	Microscopic Techniques
	Non-coding RNAs and miRNA
Musti Krishnasastry	Tools and Techniques
Janesh Kumar	Tools and techniques
	Membrane protein crystallization and strategies
	Examples of membrane protein structures
Dr.Gopal C. Kundu	Application of Nanomedicine in Cancer
	Tumor Immunology and Targeted Therapy
Girdhari Lal	Transplantation Immunology
	Tumor Immunology
Amitabha Majumdar	Stem cell course
Shekhar C. Mande	Structural Biology I & II
Debashis Mitra	Immunology
Manas Kumar Santra	Cancer Biology/ Cell cycle and cancer, Tumorsuppressors, Oncogene, Gene silencing, Cellular signaling
	Molecular Biology / transcription
	Proteasomics / Protein - protein interaction
	Project proposals evaluation
Arvind Sahu	Research Methodology
	Proteomics
Vasudevan Seshadri	Biosafety
	Translation and control
	Microarray, Qunatitative PCR and NGS
Anjali Shiras	Hallmarks of Cancer
	Cell Cycle Regulation
Yogesh Shouche	Microbial Ecology
Shailza Singh	Computer Applications and Bioinformatics
Deepa Subramanyam	Stem Cell Biology
Vidisha Tripathi	Tools and Techniques
	Molecular Biology

Teaching by MCC Scientists

Scientist	Subject	Class	Institution
Dr. Shrikant Pawar	Opportunities in Biotechnology	M. Sc. Biotechnology	DY Patil College, Pimri
Dr. Shrikant Pawar	Mechanisms of molecular divergence, Neutral evolution, Molecular distances and phylogenetic distances	M. Sc.	School of Liberal Sciences, Vimannagar, Pune
Dr. Shrikant Pawar	Workshop on 'Basic Techniques in Molecular Biology and Bioinformatics'	Students of the 'Edu-Bridge' programme	JB College of Science, Wardha
Dr. Dhiraj Dhotre	Metagenomics and transcriptomics	M. Sc. Bioinformatics	Dept of Bioinformatics, UoP
Dr. Dhiraj Dhotre	Bioinformatics and data analysis	M. Sc. Botany, Bioinformatics, Biotechnology	Dept of Botany, Bioinformatics, Biotechnology UoP
Dr. Amit Yadav	Methods in Microbial ecology	M. Sc. Microbiology	Dept of Microbiology
Dr. Kamalesh Jangid	Bacterial Sytematics	M. Sc. Microbiology	Dept of Microbiology
Dr. Kamalesh Jangid	Structure and Applications of biofilms	M. Sc. Microbiology	Dept of Microbiology

JRF Coursework taught by MCC Scientists (for Ph.D. students registered with the S.P. Pune University)

Name of Scientist	Subject / Topic
Dr. Amaraja Joshi	What is Microbial Systematics? concept of Identification, Nomenclature and Classification
Dr. Rohit Sharma	The Species concept
Dr. Om Prakash	Importance of morphological, biochemical and physiological differences for species delineation AND Concept of Chemotaxonomy and use of membrane fatty acids, lipid, protein, quinone, peptidoglycan as biomarker
Dr. Dhiraj Dhotre	Tree nomenclature: tree rooting, trees and distances, trees and character evolution, gene trees and species trees, consensus trees
Dr. Dhiraj Dhotre	Molecular chronometers in phylogeny: single gene & multi-gene sequence based microbial typing
Dr. Amit Yadav	Techniques used in microbial ecology
Dr. Dhiraj Dhotre	Model fitting and hypothesis testing AND Sources and types of errors in phylogenetic inference
Dr. Dhiraj Dhotre	Whole genome comparisons. Tree-building algorithms: distance-matrix methods, minimum evolution, LS, maximum parsimony, maximum likelihood and Bayesian inference
Dr Kamlesh Jangid	Concept of diversity (α, β & Y), calculation of diversity indices (richness and evenness) and rarefaction analysis AND Collection of ecological samples for community analysis
Dr Kamlesh Jangid	Modern approaches to study microbial diversity: Omics in diversity analysis (metagenomics + metaproteomics + metatrancriptomics) AND Preserving and Exploiting Microbial Resources

Other Happenings at NCCS

International Day of Yoga

21st June, 2016

'Scientific Benefits of Yoga'
- Talk by Prof. Bhushan Patwardhan
Director, School of Health Science, S.P. Pune University
(organized jointly with the Vijnana Bharti)

Yoga Session
(conducetd by Mr. Sunil Kachare)

Inauguration of the New Seminar Hall

by former Director, Dr. G. C. Mishra, accompanied by the RAP-SAC members

19th August, 2016

Dr. M. S. Patole superannuated on 30 Dec, 2016

NCCS is thankful for his valuable contributions

Screening of the Marathi film, 'Astu'

followed by panel discussions with Dr. Mohan Agashe (lead actor & Producer), Sumitra Bhave (Director & Producer) and Sunil Sukthankar (Director)

21st August, 2016

International Women's Day

(co-organized with IISER-Pune)

8th March 2017

Talk by
Prof. Mangala Narlikar

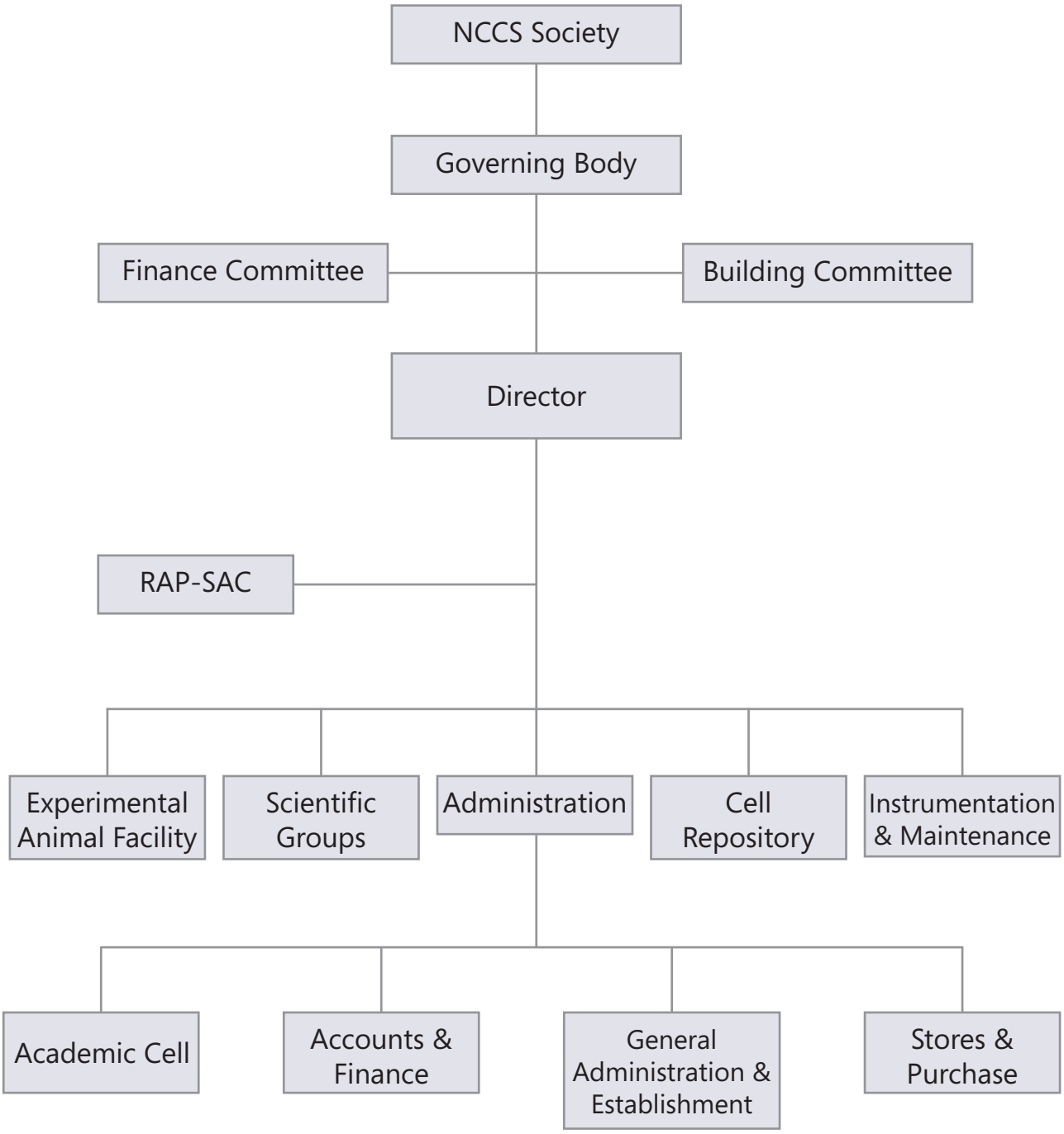
Panel Discussion

Talk by
Ms. Rashmi Shukla
(Commissioner of Police, Pune)

Sitar Recital by **Ms. Sahana Banerjee**

Sports 2017

NCCS Organization



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   Honorable 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,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi -110003.
   Phone - 011-24362950
   Email - vijay.dbt@nic.in

3. **Ms. Sumita Mukherjee** — Member
   Joint Secretary and Financial Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110003.

4. **Dr. Arvind Duggal** — Member
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110 003.
   Phone - 011-24361215
   Email -duggal.dbt@nic.in

5. **Mr. Chandra Prakash Goyal** — Member
   Joint Secretary (Admin)
   Department of Biotechnology
   Block - 2, 7th Floor, CGO Complex
   Lodhi Road, New Delhi - 110003
   Phone - 011-24362982,
   Email - cpgoyal@nic.in

6. **Dr. Soumya Swaminathan** — Member
   Director General- ICMR &
   Secretary Dept. of Health Research,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911,
   New Delhi - 110029
   Email: soumya.s@nic.in,
   doctorsoumya@yahoo.com

7. **Dr. T. Mohapatra** — Member
   Director General,
   Indian Council of Agricultural Research
   And Secretary, Dept. of Agricultural
   Research & Education,
   Krishi Bhavan, New Delhi - 110114.
   Phone: 011-23382629, 23386711,
   E-mail: dg.icar@nic.in

8. **Prof. V. Nagaraja** — Member
   President,
   Jawaharlal Nehru Centre for Advanced
   Scientific Research (JNCASR),
   Jakkur, Bangalore -560064
   Phone : 080 - 22082752
   Email: president@jncasr.ac.in,
   vraj@mcbl.iisc.ernet.in

9. **Prof. Jaya Tyagi** — Member
   Professor,
   Department of Biotechnology,
   All India Institute of Medical Science (AIIMS),
   Ansari Nagar, New Delhi - 110029
   Phone.: 011-26588491
   Email:jstyagi@gmail.com, jstyagi@aiims.ac.in

10. **Prof. W.N. Gade** — Member
    Vice Chancellor,
    Savitribai Phule Pune University,
    Ganeshkhind, Pune - 411 007
    Phone - 020-25693868
    Email - puvc@unipune.ac.in

Governing Body Members

1. **Prof. K. VijayRaghavan** — Chairman
   Secretary,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi -110003.
   Phone - 011-24362950
   Email - vijay.dbt@nic.in

2. **Ms. Sumita Mukherjee** — Member
   Joint Secretary and Financial Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110003.

3. **Dr. Arvind Duggal** — Member
   Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110 003.
   Phone - 011-24361215
   Email -duggal.dbt@nic.in

4. **Mr. Chandra Prakash Goyal** — Member
   Joint Secretary (Admin)
   Department of Biotechnology
   Block - 2, 7th Floor, CGO Complex
   Lodhi Road, New Delhi - 110003
   Phone - 011-24362982,
   Email - cpgoyal@nic.in

5. **Prof. W.N. Gade** — Member
   Vice Chancellor,
   Savitribai Phule Pune University,
   Ganeshkhind,
   Pune - 411 007
   Phone - 020-25693868
   Email - puvc@unipune.ac.in

6. **Dr. Soumya Swaminathan** — Member
   Director General- ICMR &
   Secretary Dept. of Health Research,
   Indian Council of Medical Research,
   Ansari Nagar, Post Box 4911,
   New Delhi - 110029
   Email: soumya.s@nic.in,
   doctorsoumya@yahoo.com

11. **Prof. Ameeta Ravikumar** — Member
    Head of the Department
    Department of Biotechnology
    Savitribai Phule Pune University,
    Ganeshkhind, Pune - 411007
    Phone - 020-25694952, 25690442
    Email - directoribb@unipune.ac.in

12. **Dr. Yogesh Shouche** — Member
    Scientist 'G',
    NCCS, Pune - 411 007.
    Phone - 020 25329026
    Email - yogesh@nccs.res.in

13. **Dr. S. C. Mande** — Member Secretary
    Director, NCCS,
    Pune - 411007
    Phone - 020-25708121
    Email - director@nccs.res.in

7. **Dr. T. Mohapatra** — Member
Director General,
Indian Council of Agricultural Research
And Secretary, Dept. of Agricultural
Research & Education,
Krishi Bhavan, New Delhi - 110114.
Phone: 011-23382629, 23386711,
E-mail: dg.icar@nic.in

8. **Prof. Jaya Tyagi** — Member
Professor,
Department of Biotechnology,
All India Institute of Medical Science (AIIMS),
Ansari Nagar, New Delhi - 110029
Phone.: 011-26588491
Email:jstyagi@gmail.com,
jstyagi@aiims.ac.in

9. **Prof. Ameeta Ravikumar** — Member
Head of the Department
Department of Biotechnology
Savitribai Phule Pune University,
Ganeshkhind, Pune - 411007
Phone - 020-25694952, 25690442
Email - directoribb@unipune.ac.in

10. **Prof. V. Nagaraja** — Member
President,
Jawaharlal Nehru Centre for Advanced
Scientific Research (JNCASR),
Jakkur, Bangalore -560064
Phone : 080 - 22082752
Email: president@jncasr.ac.in,
vraj@mcbl.iisc.ernet.in

11. **Dr. Yogesh Shouche** — Member
Scientist 'G',
NCCS, Pune - 411 007.
Phone - 020 25329026
Email - yogesh@nccs.res.in

12. **Dr. Kanury Rao** — Special Invitee
Sr. Scientist & Head,
Immunology Group,
International Centre for
Genetic Engineering And Biology,
Aruna Asaf Ali Marg,
New Delhi - 110 067
Phone - 011-26715114
Email - kanury.rao@gmail.com

13. **Dr. Debashis Mitra** — Special Invitee
Scientist 'G' and Dean (Academics),
NCCS, Pune - 411007
Phone - 020-25708151
Email - dmitra@nccs.res.in

14. **Dr. S. C. Mande** — Member Secretary
Director, NCCS,
Pune - 411007
Phone - 020-25708121
Email - director@nccs.res.in

Finance Committee members

1. **Prof. K. VijayRaghavan** — Chairman
   Secretary, Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110003.
   Phone - 011- 24362950
   Email - vijay.dbt@nic.in

2. **Ms. Sumita Mukherjee** — Member
   Joint Secretary and Financial Adviser,
   Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodhi Road,
   New Delhi - 110003
   Phone - 011-26590491,26963689
   Email - fa-dst@gov.in

3. **Prof. Jaya Tyagi** — Member
   Professor, Department of Biotechnology
   All India Institute of Medical Science (AIIMS)
   Ansari Nagar, New Delhi - 110029
   Phone - 011- 26588491
   Email:jstyagi@gmail.com, jstyagi@aiims.ac.in

4. **Dr. (Mrs) Vidya Gupta** — Member
   Former Chief Scientist and Chair,
   Biochemical Sciences Division,
   National Chemical Laboratory.
   Dr. Homi Bhabha Road,
   Pune - 411008
   Phone - 020-25902647 / 2237 / 2247
   Email - vs.gupta@ncl.res.in,
   vidyagene@gmail.com

5. **Dr. Debasisa Mohanty** Ph.D. — Member
   Staff Scientist - VI,
   National Institute of Immunology,
   Aruna Asaf Ali Marg,
   New Delhi - 110067
   Phone - 011-26703749
   Email - deb@nii.res.in

6. **Dr. Arvind Duggal** — Special Invitee
   Adviser, Department of Biotechnology,
   Block No. 2, 7th - 8th Floor,
   CGO Complex, Lodi Road,
   New Delhi - 110003
   Phone - 011-24361215
   Email - duggal.dbt@nic.in

7. **Mr. Chandra Prakash Goyal** — Special Invitee
   Joint Secretary (Admin)
   Department of Biotechnology
   Block - 2, 7th Floor, CGO Complex
   Lodhi Road, New Delhi - 110003
   Phone - 011-24362982,
   Email - cpgoyal@nic.in

8. **Dr. S. C. Mande** — Member Secretary
   Director, NCCS,
   Pune - 411 007
   Phone - 020-25708121
   Email - director@nccs.res.in

Building Committee members

1 **Dr. Dinakar Salunke** — Chairman
Executive Director,
Regional Centre for Biotechnology,
180, Phase - I, Udyog Vihar,
Gurgaon - 122 016

2 **Smt. Kusum Lata Sharma** — Member
Deputy Secretary,
Department of Biotechnology
Block No. 2, 7th Floor,
CGO Complex, Lodi Road,
New Delhi - 110 003

3 **Dr. Arvind Duggal** — Member
Nodal Officer & Adviser,
Department of Biotechnology,
Block No. 2, 7th Floor,
CGO Complex, Lodi Road,
New Delhi - 110 003

4 **Shri. V. H. Rao** — Member
Consultant,
National Institute of Animal Biotechnology,
Visiting Scholars House,
Lake View Guest House,
University of Hyderabad Campus,
Prof. C. R. Rao Road, Gachibowli,
Hyderabad - 46 (A.P)

5 **Dr. Sukhanand Sopan Bhosale** — Member
Prof. & Head Department of Civil
College of Engineering (COEP)
Pune - 411005

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Dean-School of Construction Management
National Institute of Construction Management
and Research (NICMAR)
25/1, Balewadi, N.I.A. Post Office,
Pune 411 045

7 **Mr. Pushkar M. Kanvinde** — Member
Principal,
BKPS College of Architecture,
2043, Sadashiv peth,
Tilak Road,
Pune - 411 030

8 **Executive Engineer** — Member
Pune Central Circle, CPWD,
Nirman Bhavan, Mukund Nagar,
Pune 411037

9 **Dr. S. C. Mande** — Member
Director,
National Centre for Cell Science,
Ganeshkhind,
Pune - 411 007

10 **Shri. A. C. Pendhari** — Convener
Tech. Officer 'C' (Maintenance)
National Centre for Cell Science,
Ganeshkhind,
Pune - 411 007

Research Advisory Panel-Scientific Advisory Committee (RAP-SAC) Members

1. **Dr. Kanury Rao** — Chairman
   Translational Health Science and
   Technology Institute,
   NCR-Biotech Science Cluster,
   3rd Milestone,
   Faridabad-Gurgaon Expressway,
   P.O. Box No. 4,
   Faridabad- 121001

2. **Dr. Mahendra S. Rao** — Member
   Vice President for Regenerative Medicine,
   The New York Stem Cell Foundation,
   178 Columbus Avenue #237064,
   New York, NY 10023,USA.

3. **Dr. Vamsi K. Mootha** — Member
   Massachusetts General Hospital,
   Dept. Of Molecular Biology,
   185 Cambridge Street,
   6th Floor, Boston MA 02114,USA.

4. **Prof. Jaya Sivaswami Tyagi** — Member
   Department of Biotechnology,
   All India Institute of Medical Sciences,
   Ansari Nagar,New Delhi - 110 029, India

5. **Prof. Kumarvel Somasundaram** — Member
   Microbiology & Cell Biology,
   Indian Institute of Science,
   Bangalore - 560 012, India

6. **Prof. Rajiv Sarin,** MD, FRCR — Member
   Ex-Director, Advanced Centre for
   Treatment Research &
   Education in Cancer (ACTREC),
   Tata Memorial Centre,
   Kharghar, Navi Mumbai - 410210, India

7. **Dr. Alok Srivastava** — Member
   MD, FRACP, FRCPA, FRCP
   Professor of Medicine
   Head, Department of Haematology &
   Centre for Stem Cell Research
   Christian Medical College
   Vellore - 632004, India

8. **Dr. Madan Rao** — Member
   Scientist, Cellular Organization and signaling,
   National Centre For Biological Sciences (NCBS),
   Tata Institute of Fundamental Research
   GKVK, Bellary Road,
   Bangalore - 560065, India

9. **Prof. Maneesha S. Inamdar** — Member
   Jawaharlal Nehru Centre for
   Advanced Scientific Research (JNCASR)
   Jakkur P.O, Bangalore-560064, India

10. **Dr Vineeta Bal** — Member
    Ex-Scientist
    National Institute of Immunology,
    Aruna Asaf Ali Marg,
    New Delhi 110 067. India

11. **Prof. V. Nagaraja** — Member
    Microbiology & Cell Biology,
    Indian Institute of Science,
    Bangalore - 560012, India

12. **Dr. Rajan Sankaranarayanan** — Member
    Group leader,
    Structural Biology Laboratory,
    Center for Cellular and
    Molecular Biology(CCMB),
    Uppal Road, Hyderabad- 500 007, India

13. **Dr. B. Ravindran** Member
Ex-Director,
Institute of Life Sciences,
Nalco Square, Chandrasekharpur
Bhubaneshwar - 751 023, India

14. **Prof. L.S. Shashidhara** Member
Coordinator Biology,
Indian Institute of Science Education &
Research (IISER),
Sai Trinity Building, 3rd Floor,
Room No: 305, Sutarwadi,
Pashan Pune - 411021. (Maharashtra)

15. **Dr. Arvind Duggal** Member
Adviser
Department of Biotechnology
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, 2017):

Scientists	:	32
Administrative Staff	:	42
Technical Staff	:	72
Total	:	146

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. NCCS also follows the Government of India rules for Persons with disabilities (Equal Opportunities, Protection of Rights and Full Participation Act 1995). NCCS have five employees with disabilities. NCCS provides double transport allowance to these employees and the salaries of such employees are exempted from deduction of Professional Tax. Liaison officers have been nominated to ensure compliance with the reservation orders regarding benefits admissible to SC/ST/OBC/PH 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', has been nominated as the First Appellate Authority.

Security

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

Committees

The Centre has formed the following committees as required under various statutes and guidelines for smooth functioning of the institute:

1. Grievance Committee
2. Internal complaints committee (for the prevention of sexual harassment at the workplace)
3. Institutional Animal Ethics Committee (IAEC)
4. Institutional Biosafety Committee (IBSC)
5. Institutional Ethical Committee (IEC)
6. Institutional Committee for Stem Cell Research (IC-SCR)
7. Committee for recruitment of SC /ST / OBC candidates.

Disciplinary Matters

The Centre follows CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the Centre.

Vigilance Matters

Quarterly reports on vigilance-related matters at NCCS were sent to the DBT from April 2016 till September 2016. From October 2016 to March 2017, monthly reports were sent according to the new vigilance guidelines. The vigilance awareness week (31st October 2016 to 5th November 2016) was celebrated at NCCS, in accordance with the requirements of the CVC, New Delhi. The theme for this year was 'Public participation in promoting integrity and eradicating corruption.' To commemorate this week, talks by the below-mentioned invitees were organized for the staff and students of NCCS:

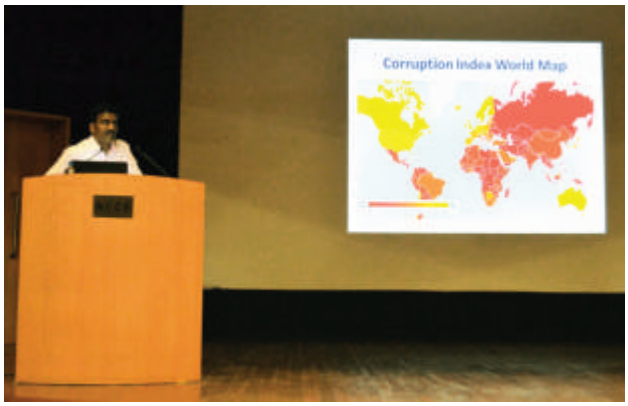
(I) Ms. Anuradha Ghodkhande (Assistant Director, National Commission for Schedule Caste, Pune region) - spoke on 3rd November, 2016.

(ii) Dr. M. R. Kadole (Suprintendent of Police and Head of Branch CBI, ACB, Pune) - spoke on 7th November 2016.

Both of them gave compelling and eye-opening talks on themes related to corruption and its prevention.

(photographs on the next page)

Ms. Anuradha Ghodkhande
Assistant Director, National Commission for
Schedule Caste, Pune region



Dr. M. R. Kadole
Suprintendent of Police and Head of Branch CBI, ACB, Pune

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 to discuss various ways of doing this.

It was decided to organise 'Hindi Fortnight' instead of 'Hindi Week', which was implemented from 2016. The Hindi fortnight was celebrated with much enthusiasm by holding various competitions, including 'Hindi essay writing', 'Hindi Advertisement Writing', 'Hindi Handwriting & Dictation' and 'Hindi Elocution', for the staff & students of NCCS. Taking into consideration last year's enthusiastic and overwhelming response, each of these competitions was organized separately for 'Hindi Bhashi' & 'Non-Hindi Bhashi' participants this year as well. Separate cash prizes and certificates were awarded to winners of each competition for the two categories. Dr. (Mrs). Swati Chaddha, Hindi Officer, National Chemical Laboratory, Pune, graced the Hindi Day function held on 21st September, 2016, as the Chief Guest. On this day, the fourth issue of 'Meemansa' (Hindi patrika) was released by Dr. (Mrs). Swati Chaddha, Dr. G. C. Mishra (former Director of NCCS), Dr. Shekhar C. Mande (Director, NCCS) and Dr. Shailza Singh (Scientist & Chief Editor-Meemansa).

We are proud of the achievements of the NCCS Team at the 'Akhil Bhartiya Rajbhasha Sammelan Evam Chintan Shivir', which was organized at Munnar, Kerala, in June, 2016, by the Rajbhasha Seva Sansthan, New Delhi. As mentioned in the 'Awards and Honours' section, NCCS received 6 awards at this conference. These were received by Mrs. Smita Khadkikar, Junior Hindi Translator, and Assistant Editor of Meemansa, who participated in this conference. These awards were handed over to the respective winners during the Hindi Day function held at NCCS on 21st September, 2016.

Chief guest, **Dr. (Mrs). Swati Chaddha** (Hindi Officer, NCL, Pune), speaking at the Hindi Day function on 21st September, 2016

Release of the **fourth issue** of '**Meemansa**' by
Dr. (Mrs). Swati Chaddha, Dr. G. C. Mishra (former Director of NCCS),
Dr. Shekhar C. Mande (Director, NCCS) and
Dr. Shailza Singh (Chief Editor-Meemansa).

National Centre for Cell Science
An autonomous institution aided by the Department of Biotechnology, Govt. of India

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Layout & Printing : United Multicolour Printers Pvt. Ltd., 264/4, Shaniwar Peth, Pune 411 030
Email: unitedprinters@rediffmail.com

National Centre for Cell Science

NCCS Complex, S. P. Pune University Campus, Ganeshkhind, Pune 411 007, India

Phone : (+91)-(20)-25708000,

Fax : (+91)-(20)-25692259

Email : infonccs@nccs.res.in

Website : www.nccs.res.in