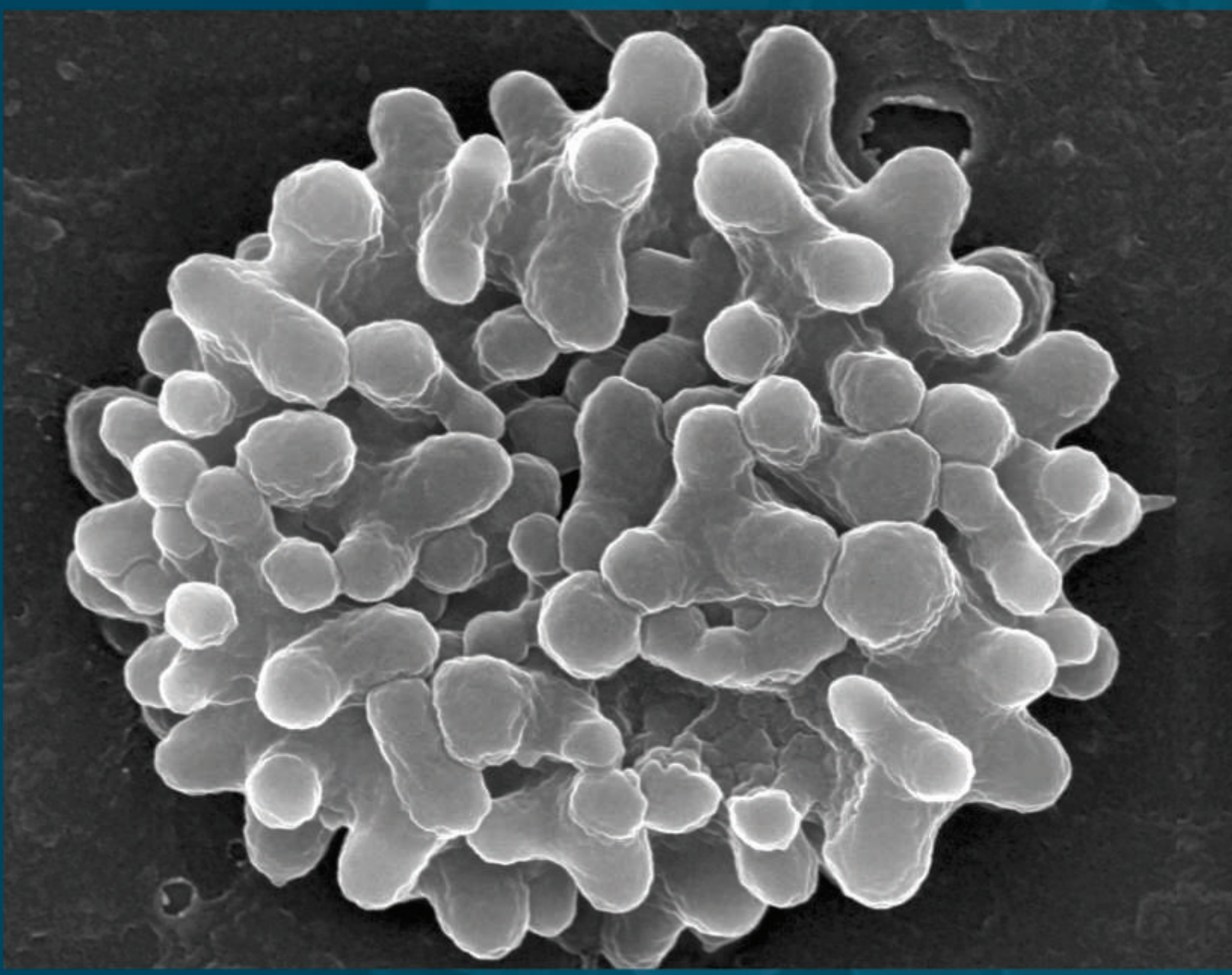
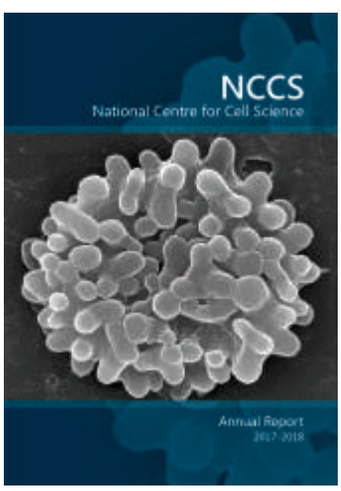
NCCS

National Centre for Cell Science



Annual Report

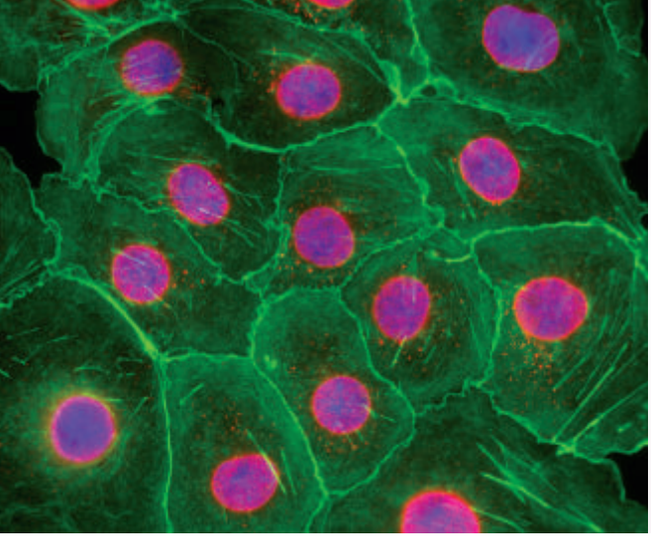
2017-2018



Cover page image
Scanning Electron Micrograph of a natural killer (NK) cell from human peripheral blood.
Magnification: 100000X
(Image courtesy of Dr. Girdhari Lal and Ms. Meenakshi Jadhav)

National Centre for Cell Science

Annual Report 2017 - 2018



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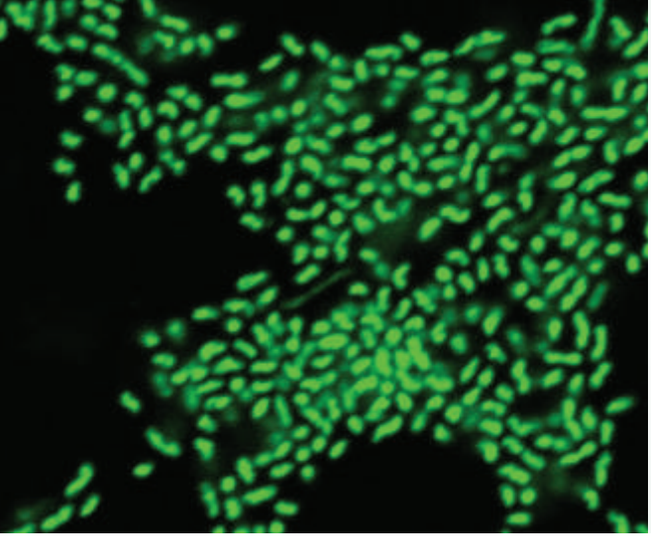


Mandate of NCCS

- 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.

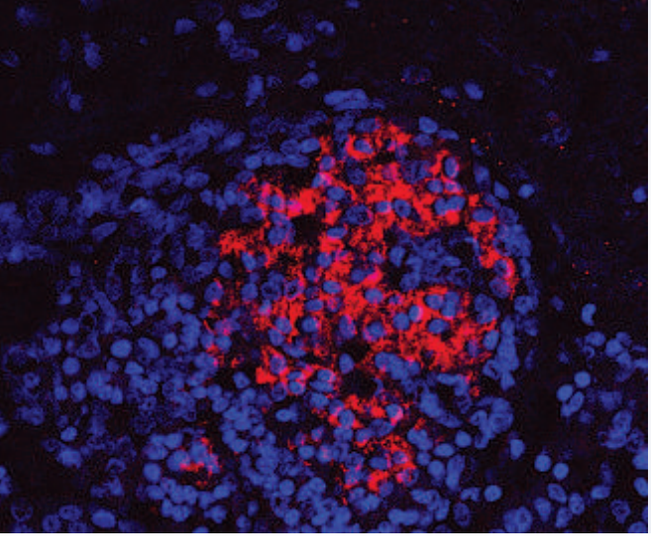
- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

- 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 structure, properties and biological activities of the cell. Cellular activities are also influenced by other determinants, including interactions between these molecules, as well as 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 National Centre for Microbial Resource (NCMR) 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 NCMR 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 microbial cultures and providing related services, such as identifying microorganisms using cutting-edge techniques. Further, NCMR 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.

In addition to carrying out research and extending services as mentioned above, NCCS also contributes immensely to capacity building of the nation and human resource development through several teaching, training & outreach activities that benefit students, researchers & academicians from various organizations across the country, as well as the general public. NCCS conducts the Ph.D. (biotechnology) coursework for students registered with the S. P. Pune University. The NCCS scientists also visit various educational organizations to deliver lectures and provide hands-on training for students in their own organizations. For example, 'Edu-Bridge', an ongoing teaching programme initiated by NCCS, enables the scientists to teach fundamental concepts of science through lectures & hands-on activities to students of the Jankidevi Bajaj College of Science (JBCS), Wardha. Students and faculty members from educational institutions across India also visit NCCS throughout the year, which provides them the opportunity to learn about cutting-edge science, techniques and instruments that they may not have exposure to at their own institutions. Furthermore, the scientists at NCCS provide valuable mentorship and training in research to Ph.D. students and other students who carry out short-term research projects at NCCS every year as summer trainees (selected from among the Indian Academy of Sciences Summer Research Fellows) and project trainees (from various academic institutions).

NCCS serves to educate the general public and students about diverse topics in science by organizing various outreach activities. This includes public talks by eminent scientists, including Nobel laureates, open day at NCCS on the National Science Day (with public talks by eminent speakers & displays), contribution of material for 'Vigyan Rail' (the science exhibition on wheels initiated by the Government of India), display of exhibits at various science exhibitions like the India International Science Festival, articles published in newspapers and magazines in English as well as Indian languages, science-themed talks & discussions broadcast through All India Radio, participation in science documentaries for telecast on national channels like the DD National channel, DD Bharati, Lok Sabha TV & Rajya Sabha TV, etc.

From the Director's Desk

It is my proud privilege to present the Annual Report of the National Centre for Cell Science (NCCS) for 2017-18. Established as a national cell repository, NCCS has been, and continues to be a fine institution, which supplies cell lines to researchers across the country, carries out research in cutting-edge areas of cell biology, and facilitates capacity building by providing training in various forms. Through 2017-18, NCCS provided over five thousand cell lines to over four hundred organizations all over India, as a part of its commitment to serve as a national cell repository. Twenty-four research scholars were admitted into the PhD programme at NCCS, with the total number of research scholars registered for a Ph.D. being one hundred and twenty-four, as on 31st March, 2018. NCCS also trained sixteen summer trainees and twenty-seven students as project trainees, towards developing high quality talent.

Being one of the pioneering institutions in India involved in stem cell research, we have come a long way in this field. Dr. Vaijayanti Kale and her team, who have been working in this area over the past several years, recently arrived at a fascinating new approach to return lost functionality to aged hematopoietic stem cells (HSCs), involving the transfer of microvesicles (MVs) derived from young mesenchymal stromal cells (MSCs) containing positive regulators of autophagy. Their findings assume special relevance given that stem cell transplantation (SCT) is the only curative therapy for various malignant as well as non-malignant disorders like leukemia, lymphoma, aplastic anaemia, etc. The efficacy of clinical transplantation critically depends on the functionality of the HSCs present in the donor graft. It is therefore essential to ensure that the donor HSCs have good engraftment ability. Modern medical science recognizes donor age to be a major concern, restricting the use of aged HSCs in bone marrow transplantation, due to their compromised ability to engraft and show altered blood formation. The severe limitations introduced by this factor in bone marrow stem cell transplantation, especially when a single HLA-matched aged donor is available, underscores the need for strategies to "rejuvenate" aged HSCs. The findings of Dr. Kale's group, which have shown that brief interactions of aged HSCs with young MSCs rejuvenates them and restores their functionality, are strongly relevant in this context. This effect was found to be achieved via the inter - cellular transfer of microvesicles (MVs) containing autophagy - related mRNAs, which resulted in reversing the signs of aging in HSCs. Importantly, the aged MSCs exhibited elevated levels of activated AKT signaling, which reduced the levels of autophagy - related mRNAs in their MVs. Also, this partitioned miR - 17 and miR - 34a, negative regulators of autophagy, into smaller membrane-derived extracellular vesicles known as exosomes, which upon transfer into HSCs, inhibited their autophagy. These studies have thus identified previously unknown mechanisms operating in niche-mediated aging of HSCs. Further, pharmacological inhibition of AKT in aged MSCs was found to rescue the system, increase the levels of autophagy - related mRNAs in rescued aged MVs, and reduce the levels of miR - 17 and miR - 34a in aged exosomes. Interestingly, transplantation experiments showed that the rejuvenating power of these "rescued" MVs was even better than that of young MVs. Collectively these findings demonstrated the critical role played by extracellular vesicles from MSCs in modulating HSC functionality, and led to a very novel approach to rejuvenating aged HSCs, which could be used to expand the donor cohort for application in stem cell transplantations.

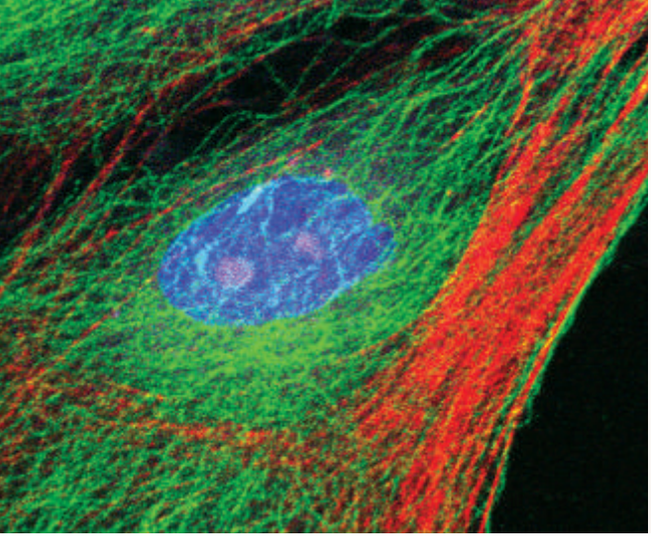
Another group at NCCS has been involved in research with relevance to regenerative medicine. Dr. Mohan Wani and his team have been working on elucidating the role of Interleukin 3(IL-3) in regulating the pathophysiology of bone remodeling. Bone remodeling comprises balanced activities between bone resorbing osteoclasts and bone-forming osteoblasts, which is

regulated by two important molecules, the receptor activator of the NF-κB ligand (RANKL), and osteoprotegerin (OPG). Unfavourable alterations in the RANKL/OPG ratio can adversely influence bone homeostasis in important skeletal diseases such as osteoporosis, osteoarthritis and rheumatoid arthritis. RANKL is found both as a transmembrane glycoprotein, and as a secreted soluble protein. In skeletal diseases, membrane RANKL decreases and soluble RANKL increases. The research findings of Dr. Wani's group have revealed that IL-3 increases membrane RANKL and decreases soluble RANKL. Interestingly, IL-3 was also found to restore RANKL expression in adult mice by enhancing bone-specific RANKL and decreasing serum RANKL. IL-3 differentially regulated two functional forms of RANKL through metalloproteases and the JAK2/STAT5 pathway, and helped in restoring the decreased RANKL/OPG ratio in adult mice. These studies have revealed a hitherto unknown novel role of IL-3 in regulating bone homeostasis in important skeletal disorders.

The research groups at NCCS also delve deeper into the molecular machinery operating inside the cells, to understand their functioning under conditions of health and disease. For example, Dr. Jomon Joseph and his group study the functions of nucleoporins, which assemble into the nuclear pore complex, the molecular gates on the nuclear envelope (NE), through which macromolecules are transported between the nucleus and cytoplasm. Messenger RNA exported from the nucleus, can have different fates in the cytoplasm, depending on the regulatory events that they are subjected to. One such event is microRNA (miRNA)-mediated translation suppression and/or degradation of specific mRNA. The Argonaute (AGO) family of proteins functions at the core of this phenomenon. AGO proteins loaded with specific miRNA act as miRNA-induced silencing complexes (miRISC), which identify and bind to their target mRNAs in the cytoplasm, based on sequence complementarity, and regulate their translation. However, it was unknown whether a sorting machinery exists for coupling the mRNA exported from the nucleus and/or present in the cytoplasm with the miRISC. The studies carried out by Dr. Joseph's group has highlighted that the nucleoporin, Nup358, which resides on the cytoplasmic side of the NE and at the cytoplasmic structure called annulated lamellae (AL), has an important role to play in this process. They observed that Nup358-positive AL structures dynamically interact with two cytoplasmic messenger ribonucleoprotein particles (mRNPs), the P bodies and stress granules (SGs). Moreover, a population of these mRNPs also associates with the NE. Interestingly, they found that Nup358 depletion caused significant and specific disruption of P bodies, a structure intimately connected with miRNA-mediated gene silencing. Moreover, reporter-based assays confirmed an essential and specific role for Nup358 in the miRNA pathway. Further studies suggested that Nup358 interacts with AGO and GW182, another component of miRISC, and mediates the association of the target mRNA with miRISC. Revelation of this hitherto unknown function of Nup358 provides a framework for future investigations into the molecular details underlying the RNA silencing process.

In addition to publishing our research findings in leading journals like PNAS, Biochemistry, Journal of Immunology, and Cell Death and Disease, the entry of NCCS into its 30th year in 2017 was also marked by several other developments, including new additions. A Project Management Cell was created to cater to the expanding vistas of research at NCCS, which will be headed by the new faculty member, Dr. Ajay Pillai. We also welcomed on board Dr. Arunkarthick, who will be in charge of the FACS and confocal imaging central facilities. New FACS and proteomics facilities were inaugurated by the Chairman of our RAP-SAC, Prof. Partha Majumdar. Continuing this trend, we look forward to further growth on all fronts, in the coming years.

Shekhar C. Mande
Director



Human Resource Development

The beneficiaries of the NCCS academic programmes during the year 2017-18 are as follows:

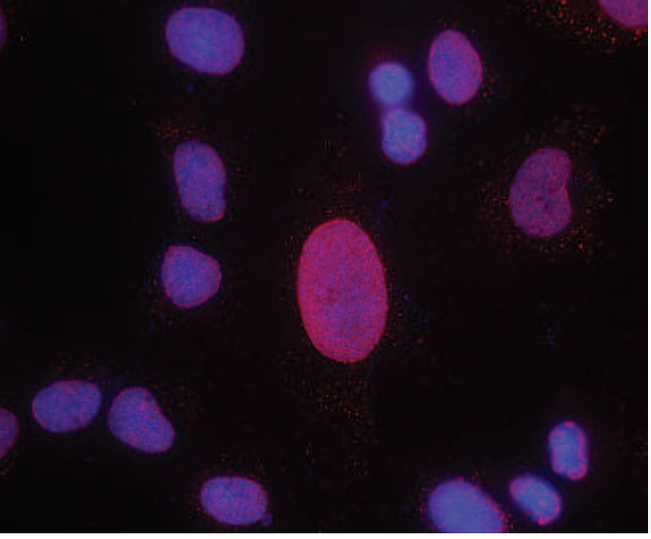
Twenty-four research Fellows joined NCCS, and twenty-four research scholars registered for a Ph.D. with the University during this year, taking the total number of registered Ph.D. students to 124, as on 31st March, 2018. Twent-three students submitted their thesis to the University for evaluation and twenty-six 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 2017-18 is as follows:

Project Trainees: 27
Summer Trainees: 16



Cell Repository

The Team

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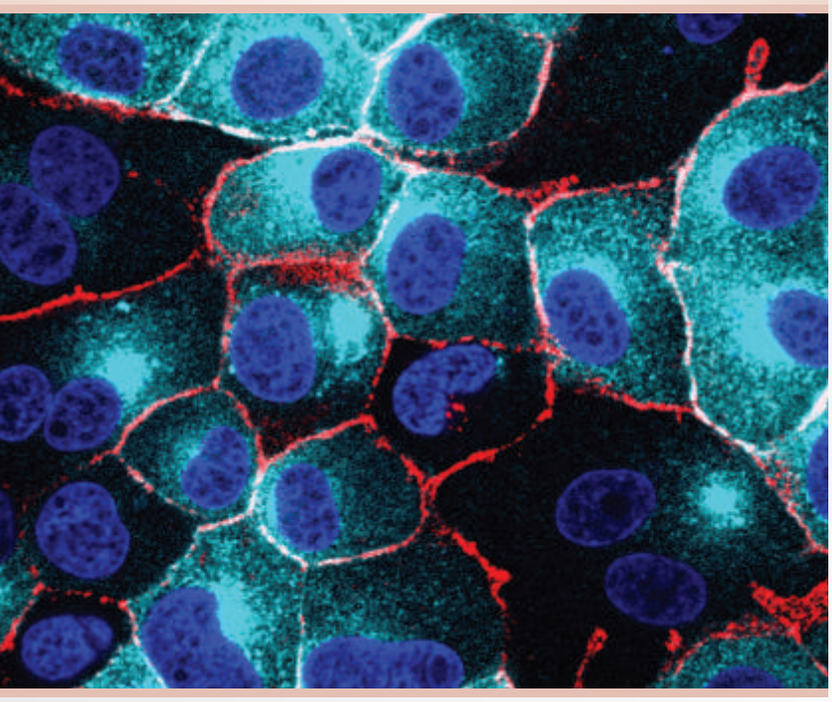
Mrs. Medha V. Gode
Mrs. Nivedita A. Bhave
Mrs. Anjali M. Patekar
Mr. Dharmendra V. Bulbule
Mr. Nitin S. Sonawane
Mr. Bhimashankar G. Utage
Mr. Vikas Mallav
Mr. Yogesh Kumbhar

NCCS has been functioning as a National Cell Repository for animal cell lines in India since its inception. 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 2017-18, five thousand nine hundred and twenty-five cell lines were supplied to four hundred and fifty organizations across the country.

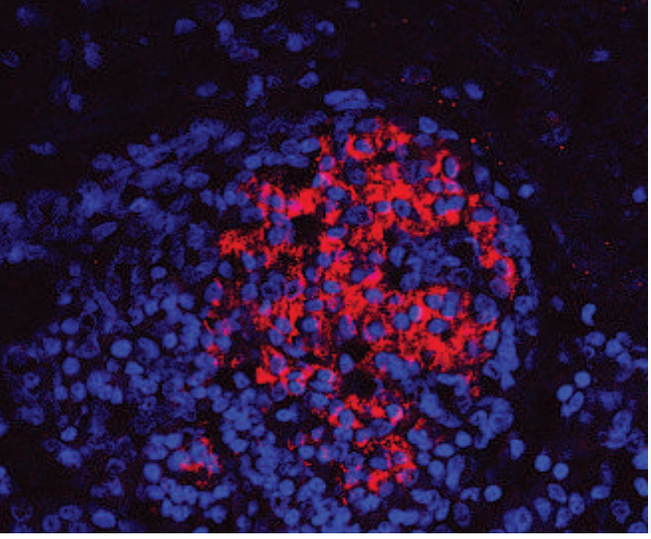
The repository organized two national hands-on training workshops on "Basic Cell Culture Technology" during May 15th-18th, 2017 and October 9th-12th, 2017. The workshops included modules for important cell culture techniques related to cell line maintenance, expansion, cryopreservation and revival. Early career researchers, including doctoral students, young faculty and technical staff from academic and non-academic institutions from all over the country, were selected and imparted training. A total of 38 participants from 33 institutes across the nation were imparted training.

The repository personnel participated in the open day at NCCS organized on the occasion of the National Science Day on February 28th, 2018. Different types of cell lines were shown to the visitors and they were informed about the importance of their use in research.

The repository has initiated services for cell line authentication using Short Tandem Repeat (STR) analysis and mycoplasma testing. Both the services are available for in-house scientists, and on chargeable basis for external users.



Research Reports



Index of Research Reports

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Quantitative Approaches towards Gauging Ovarian Cancer Plasticity

Background

Existence of intermediate cellular states during epithelial – mesenchymal transitions has expanded our comprehension of phenotypic plasticity in development and disease. Recent studies in tumor biology have reassigned some of the functional attributes earlier associated with either epithelial or mesenchymal cells, to hybrid and intermediate phenotypic states. In the present study, our previously identified phenotypic spectrum was utilized for deriving quantitative metrics towards assigning migratory modalities and biological functions across the phenotypic spectrum of high-grade serous ovarian adenocarcinoma (HGSC) cell lines, and stratifying clinical samples based on associated functionalities.

Aims and Objectives

- *In vitro* quantification of migratory modalities and biological functions across the phenotypic spectrum of high grade serous ovarian adenocarcinoma (HGSC) cell lines.
- Establishment and evaluation of an immunohistochemistry-based scoring panel that reflects phenotypic plasticity towards stratification of molecular subtypes *in situ*.

Work Done

We performed phenotypic and molecular characterization of a panel of HGSC cell lines to resolve a phenotypic spectrum exhibiting differential spheroid generation, invasive and migratory capabilities. This was based on our earlier study that identified three distinct molecular subtypes of HGSC wherein Class1 and Class2 were associated with signatures representative of collective cell

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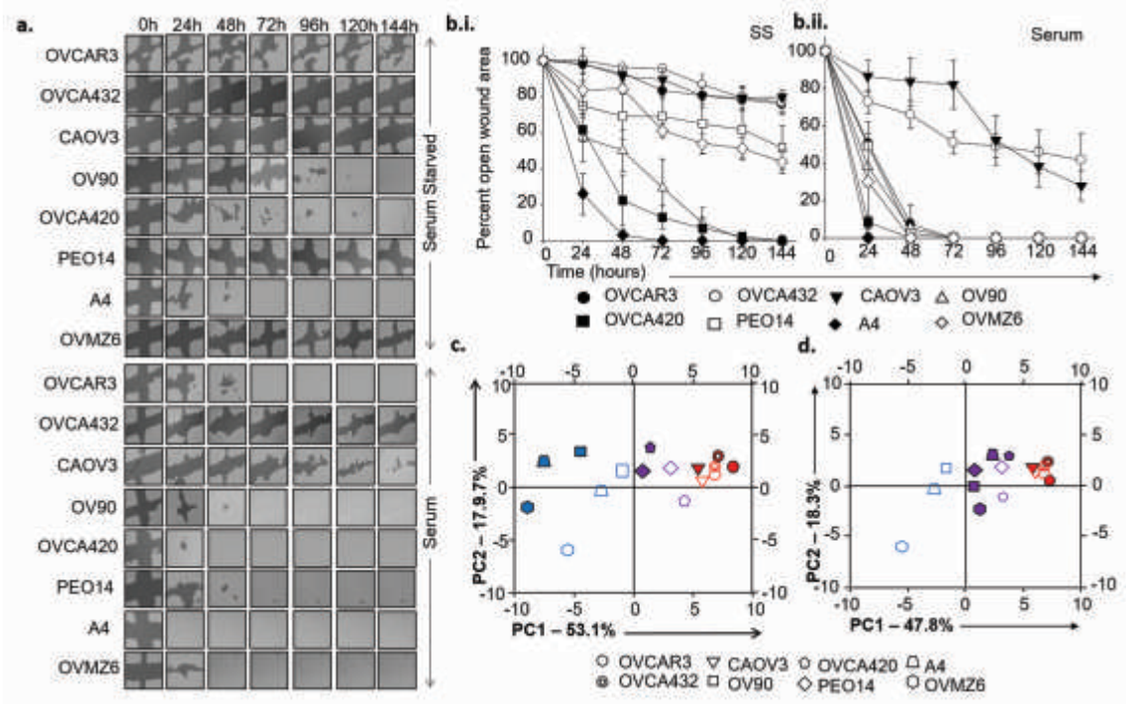

Fig. 1: Quantification of migratory modalities across a HGSC cell line panel. a. Representative images depicting wound healing capacity of HGSC cell lines in the absence and presence of serum; **b.** Percent wound closure by HGSC cell lines in absence **(b.i)** and presence **(b.ii)** of serum (n=3); **c.** Principle component (PC) analysis used to project segregation of migratory modes in -the phenotypic spectrum based on quantitative metrics - **Final 'Y'**, velocity and nearest neighbors - emerging from time-lapse based migration data. Filled shapes indicate the presence of serum and empty shapes indicate that cells were serum starved. Shape colors represent modes of migration (EMT-blue, aCCM-purple, pCCM-red); **d.** PC analysis to project segregation of migratory modes following exposure to paclitaxel (filled shapes) and DMSO (empty shapes). All data are representative of experiments performed in triplicate and are depicted as mean + SEM, *p<0.05, **p<0.01, ***p<0.001.

migration (CCM) and epithelial to mesenchymal transition (EMT) modes of migration respectively. Towards correlating functional attributes observed across the spectrum with the molecular subtypes and possibly annotating Class3, we primarily examined the migratory capacities of the cell line panel in the absence / presence of serum (Fig. 1a). Intermediate mesenchymal (iM - A4) and epithelial-mesenchymal hybrid (EM - OVCA420, PEO14) cells achieved successful wound closure in the absence of serum as opposed to epithelial (E -OVCAR3, OVCA432), intermediate epithelial (IE - CAOV3, OV90) and mesenchymal (M - OVMZ6) cells (Fig. 1b.i). Enhanced wound closure was evident across the spectrum following serum addition, despite lack of complete wound healing in OVCA432 and CAOV3 cells (Fig. 1b.ii). Thus, a distinct assignment of migratory modes was not possible from the wound closure assays. We addressed this limitation through application of time lapse microscopy of the *in vitro* scratch assay along with derivation of three quantifiable metrics – cell displacement (d), velocity (v) and number of nearest neighbours (Nn) to distinguish migratory modalities. Nn successfully distinguished EMT (low Nn) from CCM (High Nn) whereas CCM was segregated into passive CCM (pCCM - low velocity and displacement) and active CCM (aCCM - moderate / high velocity and displacement). Principal component (PC) analyses of the migratory metrics revealed pCCM in E and iE (CAOV3), aCCM in epithelial –mesenchymal hybrid (E-M), coupling of aCCM-EMT in intermediate mesenchymal (iM) and EMT in M cells (Fig. 1c). Interestingly, highest efficacy of wound healing was associated with the aCCM-EMT combination highlighting the importance of cell contact during migration. To improve applicability of the assay and further define alterations in migratory phenotypes following drug exposure, PC analysis was performed following live cell imaging of wound closure in paclitaxel treated cells. A distinct switch from EMT to aCCM mode for M and iM cells was evident after treatment, E-M and iE rigidly retained their characteristic modes of migration albeit at reduced velocities, and pCCM in E cells was unaltered (Fig. 1d). Establishment of these quantitative metrics thus identified

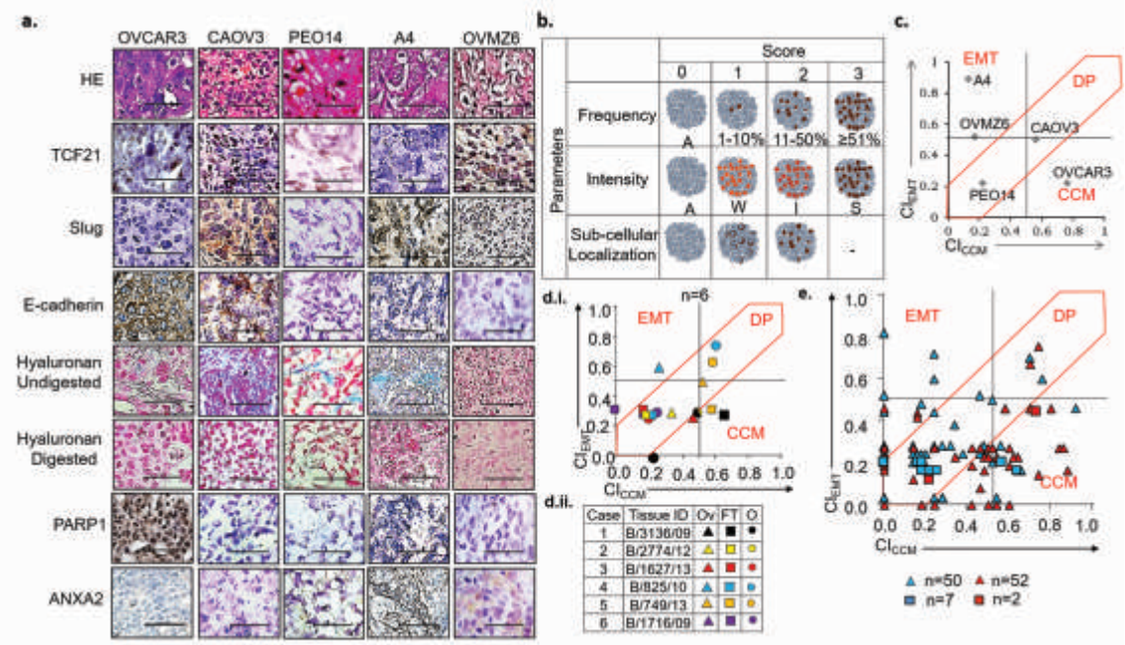

Fig. 2: IHC/HC based stratification of molecular subtypes in HGSC. **a.** Representative images of HGSC xenograft stained sections for H&E (hematoxylin and eosin), TCF21, Slug, Ecadherin, PARP1 and ANXA2 and HA fibers (untreated and hyaluronidase digested). Scale bars-50µm; **b.** Schematic of scoring guidelines for IHC based staining of nuclear markers (TCF21, PARP1, Slug), A: Absent, W: Weak, I: Intermediate, S: Strong, Mis: Mislocalised, N: normal localization. A similar approach was used for scoring of membrane markers (E-cadherin, ANXA2) except that sub-cellular location was scored either 1 (cytoplasm) or 2 (cell membrane), while extracellular expression of hyaluronan fibers (evaluated as blue color developed by Alcian blue staining that is lost on hyaluronidase) was scored 1 in distant tumor stroma, and 2 in tumor epithelial cell nests; **c.** Scatter plots of CICCM vs. CIEMT derived from xenograft scoring; **d.** Scoring of HGSC chemo-naïve cases - tumors detected in ovary (Ov), fallopian tube (FT) and omentum (O); **d.ii.** Reference case-chart for **d.i; e.** Scatter plots of CICCM *vs.* CIEMT distribution for single chemo-naïve or -treated (red and blue shapes respectively) primary tumors: ovarian Δ and fallopian tube □. All data are representative of experiments performed in triplicate.

preference for CCM mediated migration following drug exposure across the HGSC spectrum.

Development of similar quantitative measures *in situ* was deemed necessary to stratify clinical specimens and identify class switching following chemotherapeutic challenge. We established immunohistochemistry (IHC) and histochemistry (HC) based scoring systems for a panel of six markers [Tcf21, Cdh1, PARP1 (CCM/E class), Slug, hyaluronan (HA), and ANXA2 (EMT/M class)] in HGSC cell line derived xenografts (Fig. 2a). Observed patterns of marker expression were quantified via computation of scoring guidelines using the metrics of marker frequency, intensity and localization (Fig. 2b). Subjectivity of analyses was minimized by generating a normal tissue reference panel and collecting blinded scores from 5 observers. Consensus scores for each marker and xenograft were used to derive specific Biomarker indices (BI) and eventually Class-indices (CI). Applying the distribution of median CIEMT vs. CICCM values towards class stratification identified OVCAR3 (E) xenografts as CCM-class, A4 (iM) and OVMZ6 (M) as EMT-class and CAOV3 (iE) and PEO14 (E-M) as double positive (DP;Fig. 2c). Importantly, this scoring reassigned the mixed/heterogeneous Class3 as DP tumors by relative association of marker expression. Towards assessing further clinical representation, we obtained and stratified tumor samples pathologically diagnosed as HGSC from 96 patients. These included primary [ovary (T), fallopian tube (FT)] and metastatic tumors [omentum (O)]; analyses was perfomed in the following groups-

Group A. Paired chemo-naïve (CN) tumors from different sites (T *vs.* FT *vs.* O; n=6),

Group B. Unpaired tumors from CN (50-T, 7- FT) and chemo-treated (CT) cases (52-T, 2-FT),

BI-CI scores and CICCM-CIEMT values for each tumor were computed followed by evaluation of CI distribution and class assignment. Group A included representatives of all three classes (CCM, EMT, DP). While primary tumors and metastases

of case B/3136/09 predominantly expressed CCM- markers, metastases of all other cases segregated into DP class even when the primary tumor represented another class (Fig.2d). Similarly, all three classes were represented in CN T- and FT-tumors of Group B whereas increased representation of CCM-class and CCM-/DP- class was observed in CT T- and CT F-tumors respectively (Fig. 2e). Overall, DP-class was better represented in 'Group B' CN-tumors as compared to xenografts. Our analysis thus represents a quantitative module towards tumor subtyping and the assessment of molecular switching following chemotherapy.

Conclusion:

Development of quantitative approaches allowed the delineation of migratory modalities *in vitro* and stratification of tumor subtypes *in situ*. Both these approaches successfully gauged the effect of chemotherapeutic regimes on cellular phenotypes and exhibit applicability towards clinical assessment. Interestingly, the *in situ* molecular classes represent the functional migratory phenotypes observed *in vitro*. Importantly, this supports the derivation of robust clinical inferences pertaining to cell migration and metastasis.

Future Research Plans

- Elucidation of molecular players governing distinct migratory modalities.
- Application of IHC based scoring panel to study chemotherapy induced molecular changes in paired clinical samples.

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Cancer, Chemotherapy, and Metabolic Disorders

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Background

Metabolic disorders diabetes and obesity alter the risk of developing variety of cancers, and the associations are biologically plausible. 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 establishes a link between obesity and cancer. Bulk of epidemiological studies available, support the interrelation-ship between metabolic disorders and cancer, 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.

Obesity is characterized by increased accumulation of white adipose tissue in the body. White adipose tissue secretes many cytokines and hormonal factors, which are collectively referred to as adipokines. In obese state, the secretion profile of adipose tissue is altered. As a consequence, increased level of pro-inflammatory adipokines and a simultaneous decrease in anti-inflammatory adipokines is observed. Adipokines act through receptors or membrane-associated molecules, and mediate their effect by activating various cellular signaling pathways.

The altered systemic and local microenvironment that occurs as a consequence of obesity not only increases the likelihood of tumor development and progression, but also potentially creates an unfavorable state for response to chemotherapeutic regimens. Numerous key inflammatory and metabolic factors and their pathways are assumed to mediate the obesity-associated impairment of chemotherapeutic responses. The mechanistic studies on the impact of the obese phenotype towards the outcome of cancer therapy are still

lacking. There is a need to re-consider the dosing pattern of chemotherapeutic drugs with the concomitant implication of interventions which curtail adiposity.

The involvement of adipose tissue in impairing the therapeutic response in cancers has been reported. However, very little is known about the specific role of adipokines in the outcome of cancer therapy. Adipokines such as leptin and resistin are found to be elevated in the serum of obese individuals. Leptin is known to activate various signaling pathways including PI3K/Akt, JAK/STAT, and MAPK. Its role in growth and proliferation has been extensively explored in breast and prostate cancers. However, the specific role of leptin in modulating melanoma cell proliferation and the chemotherapeutic outcome is obscure. Another important adipokine, resistin is known to promote cancer growth. There is a considerable amount of experimental and epidemiological evidences which suggest that resistin may have pathophysiological effects, particularly in some cancer types in addition to its traditional roles in energy homeostasis. Resistin is also known to promote drug resistance phenotype in certain malignancies.

Previously, study from our group has shown that obesity impairs the therapeutic outcome of dacarbazine (DTIC) in melanoma and induces drug resistant phenotype by upregulating fatty acid synthase (FASN), caveolin (Cav)-1 and P-glycoprotein (P-gp). In the present study, we investigated the specific role of leptin and resistin in melanoma cell growth, proliferation and the outcome of DTIC-based chemotherapy. Using appropriate *in vivo* and *in vitro* approaches, we have shown that these adipokines not only modulate the growth and proliferation of melanoma cells, but also for responsible for impairment in the efficacy of DTIC

Aims and Objectives

- The present study is aimed at unraveling the specific role of leptin and resistin in melanoma growth and the chemotherapeutic outcome, using appropriate in vitro and in vivo approaches.

Work Done

Results

Leptin and resistin impair the efficacy of DTIC in melanoma cells

Firstly, to explore whether leptin and resistin could have any role in modulating the sensitivity of melanoma cells to DTIC, A375 cells were treated with DTIC in the presence or absence of leptin or resistin. We observed that leptin significantly impaired the response of A375 cells to DTIC as evident from the increase in IC50 of DTIC by ~5-fold (2592 μM) as compared to the control (461 μM) Similarly, in A375 cells treated with resistin IC50 for DTIC increased to 2739 μM as compared to control (421 μM).

Additionally, to verify the involvement of leptin in modulating the response of melanoma cells to DTIC therapy, B16F10 cells were cultured in the medium containing serum of genetically obese mice strains ob/ob (leptin deficient) or db/db (leptin receptor deficient). As compared to ob-WT serum, the cytotoxicity of DTIC was found to be impaired in ob/ob serum (Fig. 1C). Interestingly, the IC50 value of DTIC was increased (7720 μM) in cells grown in ob/ob serum as compared to those cultured in ob-WT serum (1630 μM). Similarly, the response of melanoma cells to DTIC was found to be reduced in the presence of ob/ob serum when compared to that of db-WT serum. Higher IC50 value of DTIC was observed in melanoma cells grown in the serum of db/db mice (8410 μM), which contains very high circulatory leptin, as compared to control (1830 μM).

Diminished circulatory level of leptin and resistin improves the efficacy of DTIC in melanoma cells

Further, to complement the role of leptin and resistin and other obesity-associated factors in cell growth, long-term cell survival assay was performed. Melanoma cells were cultured in the medium containing serum from ob/ob or db/db mice or the caloric restricted (CR), in the presence or absence of DTIC. Enhanced cell growth and proliferation was observed in B16F10 cells grown in the medium containing the serum from ob/ob or db/db mice as compared to those cultured in the medium containing serum from their WT counterparts in the long-term culture. Importantly, in the long-term culture, we observed impairment in the efficacy of DTIC in the B16F10 cells grown in the medium containing the serum from ob/ob (Fig. 1A and 1B) or db/db (Fig. 1C and 1D) mice as compared to the control. The effect of DTIC was markedly rescued upon culturing these cells in the medium containing serum of calorically restricted ob/ob (Fig. 1A and 1B) or db/db mice (Fig. 1C and 1D).

To further check whether modulation of chemotherapeutic outcome by obesity-associated factors is dependent on leptin or resistin, B16F10 and B16F1 cells were cultured in the medium containing HFD-C57BL/6J serum which was immuno-depleted of leptin or resistin by respective antibodies either alone or

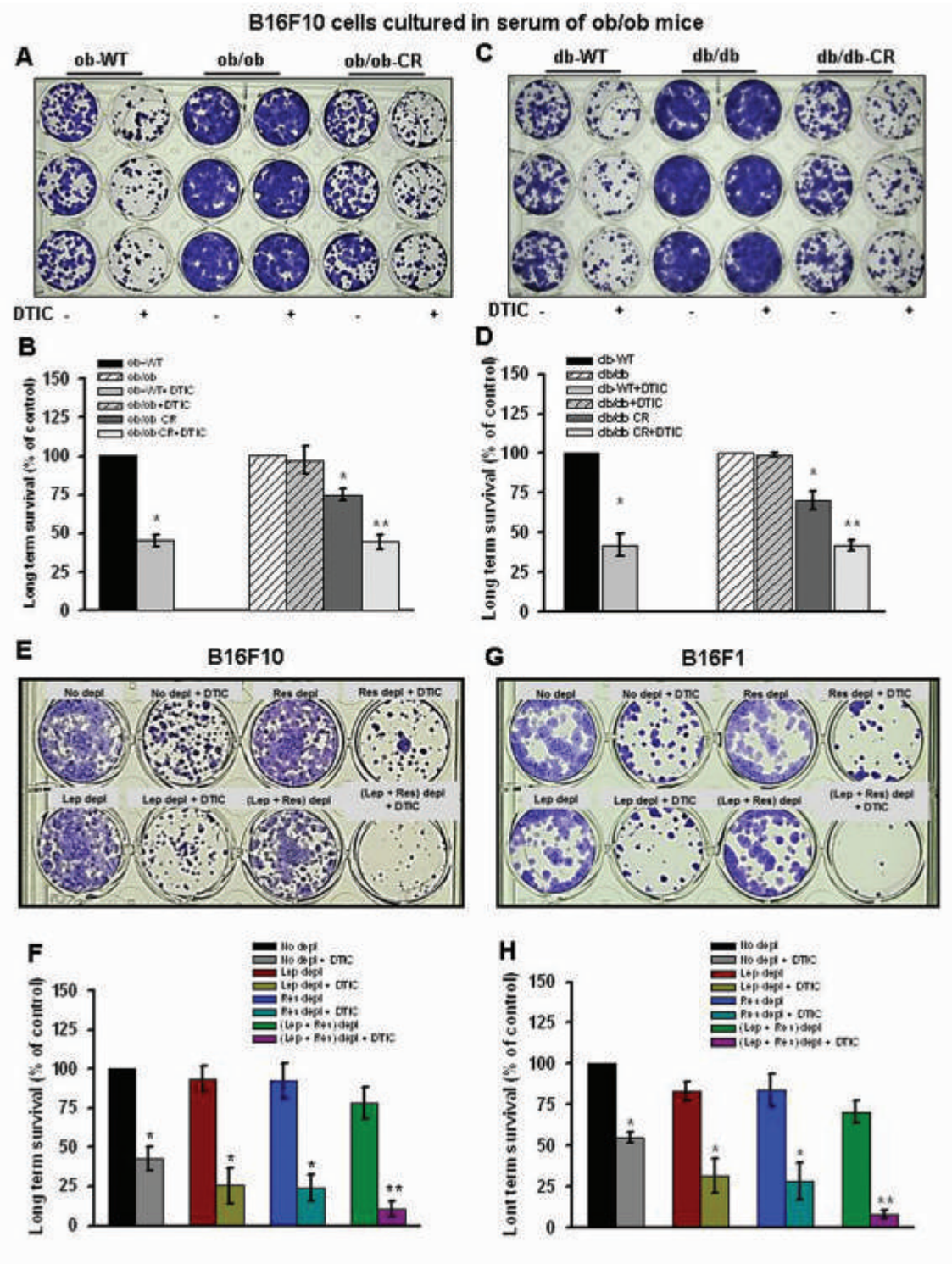

Fig. 1: Effect of obesity-associated serum factors on the long term survival of B16F10 and B16F1 cells treated with DTIC.

(A and B) B16F10 cells were chronically grown in the medium containing 5% serum collected from experimental ob/ob mice for 15 days. Thereafter, these cells were subjected to DTIC treatment, for 48 h. Then, the medium was changed and fresh medium was added. The medium was changed every 2-3 days. (A) Representative image showing the long term survival of B16F10 cells. (B) Bar graph showing the quantitation of number of surviving population from the image shown in (A). (C and D) Similar experiment was performed in B16F10 cells chronically grown in the serum from db/db mice. After 10 days, the cells were stained with 0.05% crystal violet, and images were taken using Olympus digital camera. (E-H) B16F10 and B16F1 cells were cultured in serum (collected from C57BL/6J mice) which was immuno-depleted of leptin and or resistin for 48 h. Then, DTIC treatment was given, and cells were incubated for 48 h. Next, the medium was changed and fresh medium was added. (E) Representative image showing the long term survival of B16F10 cells. (F) Bar graph showing the quantitation of number of surviving population from the image shown in (E). (G) Representative image showing the long term survival of B16F1 cells. (H) Bar graph showing the quantitation of number of surviving population from (G). The data are representative of experiments performed two times at least in triplicates.

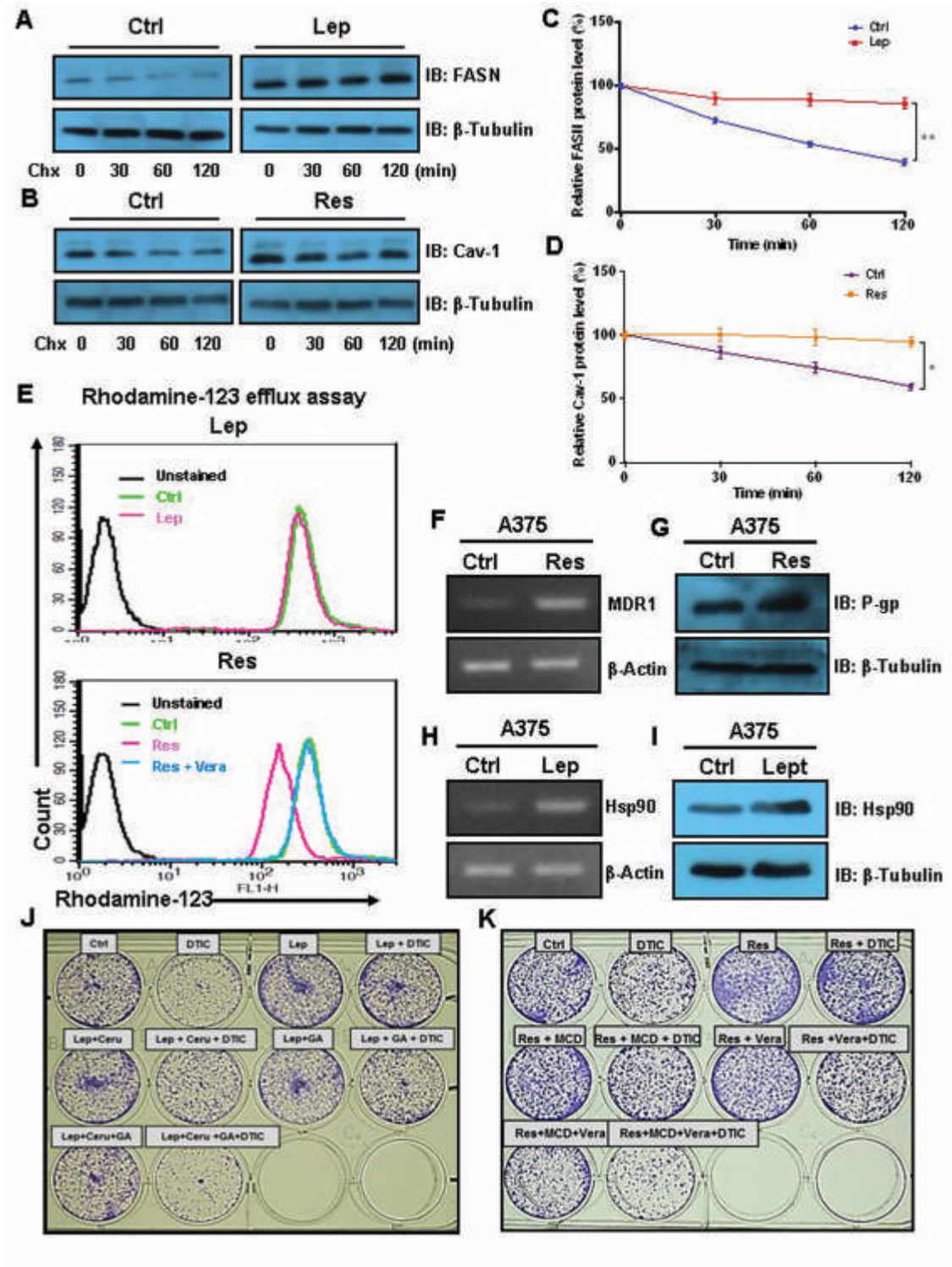

Fig. 2: Molecular events associated with leptin and resistin induced impaired outcome of DTIC therapy in melanoma cells.

(A and B) A375 cells were treated with leptin or resistin at a concentration of 100 ng/ml in DMEM containing 1% FBS for 48 h as described in Materials and Methods. Thereafter, cycloheximide (100 µg/ml) treatment was given for the indicated time points. Representative immunoblot of FASN (A) and Cav-1 (B) in A375 cells treated with leptin or resistin respectively. (C and D) Bar graph showing the quantitation of band intensity of FASN and Cav-1 immunoblots. (E) Rhodamine-123 efflux assay in A375 cells treated with leptin (upper panel) or resistin (lower panel). A375 (human melanoma) cells were plated in 12-well plates. After 24 h, cells were treated with 100 ng/ml of recombinant leptin in DMEM containing 1% FBS for 48 h. Thereafter, these cells were subjected to Rh-123 efflux assay via flow cytometry. (F and G) RT-PCR (F) and Immunoblotting (G) analysis of MDR and P-gp respectively in A375 cells treated with resistin. (H and I) RT-PCR (H) and Immunoblotting (I) analysis of HSP90 in A375 cells treated with leptin. (J and K). Representative image showing the long term survival of A375 cells grown in the presence or absence of leptin (J) or resistin (K) together with inhibitors. Ctrl- control, Lep- leptin, Res- resistin, Chx- Cycloheximide, Ceru or C - cerulenin; GA or G - Geldanamycin.

together. Interestingly, we observed that leptin depletion significantly improved the efficacy of DTIC as evident by decrease in the number of colonies in long-term survival assay as compared to the cells grown in the medium containing the control serum [(B16F10: Fig 1E and 1F) and (B16F1: Fig. 1G and 1H)]. Similarly, resistin depletion resulted in improved efficacy of DTIC in melanoma cells as compared to the cells grown in the medium containing the control serum [(B16F10: Fig 1E and 1F) and (B16F1: Fig. 1G and 1H)]. Interestingly, upon simultaneous immuno-depletion of both leptin and resistin, the effect of DTIC was prominently improved [(B16F10: Fig 1E and 1F) and (B16F1: Fig. 1G and 1H)].

Leptin- and resistin-induced impaired response of melanoma cells to DTIC is mediated by FASN/Hsp90 and Cav-1/P-gp respectively

To get insights into the role of leptin and resistin in causing attenuation in the response to DTIC, we intended to analyze the status of FASN and Cav-1 which are upregulated in melanoma in the obese (HFD) mice, and are known to be involved in resistance to cancer chemotherapy Firstly, we checked the protein levels of FASN and Cav-1 in A375 cells upon treatment with leptin or resistin. An increased level of FASN was detected in A375 cells upon leptin treatment (Fig. 2A), while resistin treatment caused increase in the level of Cav-1 in A375 cells (Fig. 2B). Previously, it has been reported that leptin and resistin do not affect the mRNA expression of FASN and Cav-1 at transcription levels; however, they increase protein level of these molecules. Therefore, these findings suggested that both leptin and resistin modulate the protein levels of FASN and Cav-1 respectively, likely by increasing the stability of these proteins. To verify this, cycloheximide chase experiment was performed in A375 cells. We observed that leptin promoted stabilization of FASN, whereas resistin caused Cav-1 stabilization (Fig. 2C and 2D respectively) in these cells.

Another important molecule primarily responsible for pumping out the anticancer drugs from cancer cells thereby rendering them resistant to chemotherapy is P-gp. To confirm the involvement of P-gp in decreasing the response to DTIC in the presence of leptin or resistin, Rh-123 efflux assay was performed. We noticed that leptin did not affect Rh-123 efflux in A375 cells (Fig. 2E, upper panel). On the other hand, resistin did increase the efflux of Rh-123 in A375 cells, which was reversed upon treatment of verapamil, an inhibitor of P-gp (Fig. 2E, lower panel). Also, it was observed that resistin increased P-gp mRNA as well as protein levels (Fig. 2F and 2G), suggesting that resistin plays a role in chemotherapeutic outcome in melanoma, in part, via increasing the expression of P-gp, while leptin-mediated impaired response of cancer cells to DTIC is independent of P-gp activity. This led us to explore another possible mechanism by which leptin contributes to the impaired DTIC action on melanoma cells. It has been reported that leptin modulates the levels of heat shock proteins (Hsps). Hsp90 is one of the major heat shock proteins known to contribute to drug resistant phenotype. Therefore, we analyzed the involvement of Hsp90 and checked the expression of Hsp90 in melanoma cells upon treatment with leptin. We found that leptin treatment indeed increased transcript and protein level of Hsp90 in A375 cells (Fig. 2H and 2I).

To confirm whether FASN and Hsp90 are involved in leptin-induced impairment in the response of melanoma cells to DTIC, we used their respective inhibitors. Inhibition of FASN and Hsp90 individually by cerulenin and geldanamycin respectively increased the sensitivity of A375 cells to DTIC even in the presence of leptin (Fig. 2J). Moreover, combined inhibition of FASN and Hsp90 enhanced the effect of DTIC in A375 cells compared to single inhibitor treatment (Fig. 2J). Similarly, to verify the role of Cav-1 and P-gp in impairing the response to DTIC by resistin, their inhibitors MCD and verapamil respectively were used. Inhibition of these molecules resulted in increased sensitivity of A375 cells to DTIC (Fig. 2K). Simultaneous inhibition of both of these molecules profoundly improved the anticancer effect of DTIC (Fig. 3K).

Elevated serum level of leptin and resistin is correlated with enhanced melanoma growth and impaired efficacy of DTIC *in vivo*

To corroborate the involvement of leptin in melanoma growth and in the outcome of DTIC treatment, we employed ob/ob and db/db mice. Leptin is an important adipocyte-secreted factor involved in controlling appetite. Due to lack of functional leptin and leptin receptor respectively, ob/ob and db/db mice are morbidly obese (despite fed on a normal fat diet) exhibiting higher level of fat accumulation. Thus, these mice serve as good models for the leptin-related studies.

To understand the involvement of leptin in melanoma progression, and the outcome of chemotherapy, ob/ob, and db/db mice, as well as their WT counterparts, were ectopically isografted with B16F10 cells as shown in Fig. 3A. After the appearance of palpable tumors, DTIC was administered for 5

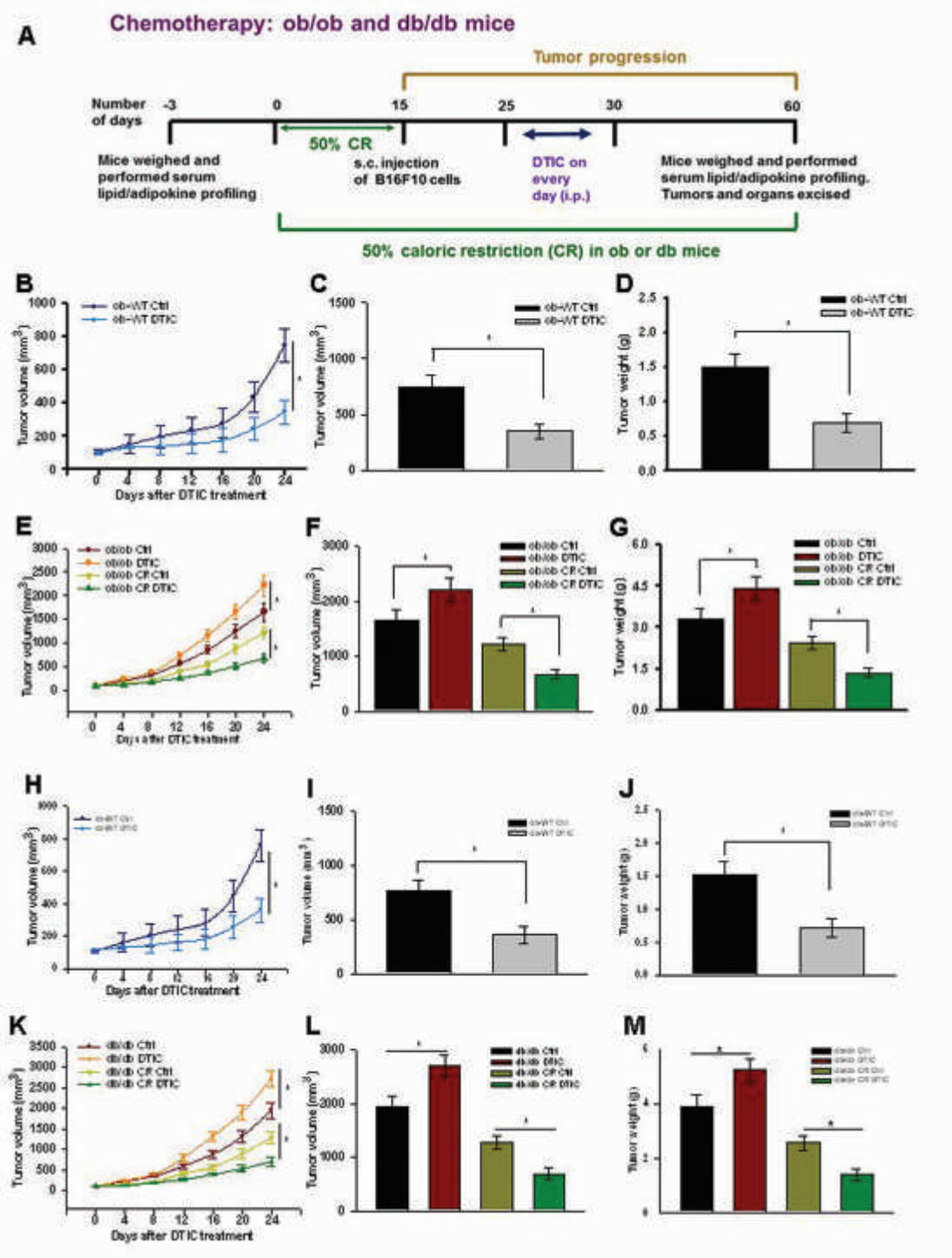

Fig 3: Impact of leptin on melanoma progression and on the outcome of DTIC therapy in ob/ob and db/db mice.

(A) layout of the in vivo experiments. (B-D) ob-WT mice were injected with B16F10 cells (2×10^5 cells/mouse in 100 µl PBS). After the tumor formation, vehicle or DTIC treatment (N = 6 per each group) was given as per the experimental layout shown in (A). (B) Tumor progression, (C) Tumor volume, and (D) Tumor weight. (E-G) ob/ob mice were divided into two major groups. One group was fed ad libitum on normal diet. In the second group, caloric intake was restricted to 50% by providing half the quantity of feed in normal before inoculating B16F10 cells. After 15 days, mice of all groups were injected subcutaneously with B16F10 cells (2×10^5 cells/mouse in 100 µl PBS). After tumor formation, vehicle or DTIC treatment (N = 6 per each group) was given as per the experimental layout shown in (A). (E) Trend of tumor progression, (F) Tumor volume, and (G) Tumor weight. (H-J) db-WT mice were injected with B16F10 cells (2×10^5 cells/mouse in 100 µl PBS). After tumor formation, vehicle or DTIC treatment (N = 6 per each group) was given as per the experimental layout shown in (A). (H) Trend of tumor progression, (I) Tumor volume, and (J) Tumor weight. (K-M) db/db mice were divided into two major groups. One group was fed ad libitum on normal diet. In the second group, caloric intake was restricted to 50% by providing half the quantity of feed in normal before inoculating B16F10 cells. After 15 days, mice of all groups were injected subcutaneously with B16F10 cells (2×10^5 cells/mouse in 100 µl PBS). After tumor formation, vehicle or DTIC treatment (N = 6 per each group) was given as per the experimental layout shown in (A). At the end of the experiment, mice were sacrificed and tumors were collected. (K) Trend of tumor progression, (L) Tumor volume, and (M) Tumor weight. The results are given as means ± standard error of the mean for 4E and 4K).

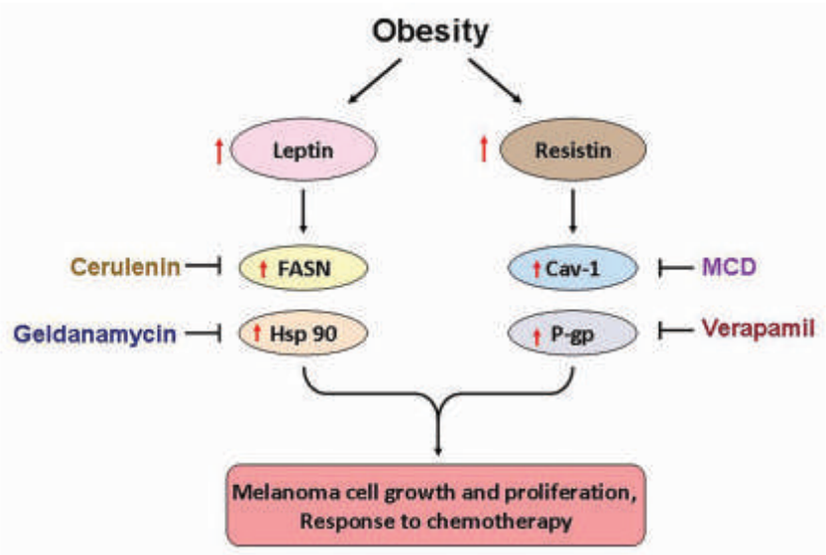

Fig 4: Proposed model of study on impact of leptin on melanoma growth and the outcome of dacarbazine therapy.

Leptin modulates FASN and Hsp90 levels while resistin modulates Cav-1 and P-gp thereby enhancing melanoma growth. Collectively, these events are responsible in part for impaired outcome of DTIC therapy. Inhibition of these molecules restricts melanoma growth and improves the outcome of chemotherapy.

consecutive days and tumor progression was followed up until the termination of the experiment. We observed that DTIC treatment did not affect the serum levels of obesity-associated factors in ob/ob and db/db mice as well as in their WT littermates. As expected, DTIC significantly retarded tumor progression in respective WT counterparts, as evident by reduced tumor volume and weight (Fig. 3B-D and (Fig. 3H-J). Surprisingly, therapeutic efficacy of DTIC was impaired in both ob/ob and db/db mice as is evident by increase in tumor progression as compared to respective untreated controls (Fig. 3E-G and Fig. 3K-M).

In previous study, we have shown that shifting mice from HFD to ND has a profound impact on melanoma progression, which correlates with normalization in the body weight and in the levels of obesity-associated factors. Therefore, to determine whether caloric restriction (CR) could improve the effect of DTIC in ob/ob and db/db mice, we subjected these mice to CR by reducing their feed by 50% as shown in Fig. 3A. In the present study, it was observed that CR itself reduced tumor progression in both ob/ob and db/db mice which correlated with the limited normalization in the levels of obesity-associated factors. More importantly, CR significantly improved the outcome of DTIC therapy in these mice (Fig. 3E-G and Fig. 3K-M).

Collectively, these data suggest that both leptin and resistin partly play a crucial role in melanoma growth, and adversely affect the chemotherapeutic outcome by modulating the molecules which are involved in tumor growth and drug resistance. In a nutshell, this study highlights the role of leptin and resistin in melanoma growth, and impairment in the chemotherapeutic outcome (Fig. 4)

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. 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 and or adipokines induced alterations at molecular level and in signaling cascades, in solid tumors cells.
- The interrelation-ship between metabolic abnormalities and cancer; Influence on growth and therapy.

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Understanding the Novel Functions of MAR Binding Protein, SMAR1

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Background

The nuclear matrix is an intricate yet dynamic organelle of the cell that provides structural integrity to the nucleus and also tethers several proteins which are important for various different processes like replication, transcription, splicing, DNA damage repair and recombination. The nuclear chromatin is organized in loops by the nuclear matrix, thus modulating the chromatin architecture. Among different factors involved in compaction of chromatin, Scaffold/Matrix binding proteins (MARBPs) play crucial role. SMAR1 (Scaffold/Matrix attachment region 1) is one such nuclear matrix-binding protein which belongs to a family of BEN domain proteins. This BEN domain is crucial for the DNA as well as protein binding function of these proteins. SMAR1 was first identified from double positive mouse thymocytes. It binds to a putative MAR (MARβ) which is a DNase I hypersensitivity site located 400 bp upstream of the transcriptional enhancer (Eβ) at the T cell receptor β locus (Chattopadhyay et. al. 2000 Genomics). Earlier studies from our lab have shown that SMAR1 is a chromatin modifier which recruits HDAC1 to the promoter and causes the changes in the adjacent chromatin. These changes in the chromatin results in the modulation of the activity of various promoters like Cyclin D1. SMAR1 was also reported to regulate apoptosis and survival by regulating the expression of Bax and Puma in response to genotoxic stress. Our lab has already reported the role of SMAR1 as a stress response protein, wherein SMAR1 was reported to modulate the acetylation status of Ku70 by interacting with HDAC6 (Chaudhary et. al. 2014 *Cell Death and Disease*). In addition, SMAR1 was reported to negatively regulate alternative splicing by modulating the acetylation status of Sam68 by recruiting HDAC6 (Nakka et. al. 2015 *PNAS*). 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*). We have also reported that the switch between effector T cells and regulatory T cells is governed by 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*). It is a well-established fact that SMAR1 is highly downregulated in higher grades of cancer, but the reason was unknown. Recently we reported the mechanism which leads to diminished expression of SMAR1 in higher grades of colorectal cancer. Upon Wnt/β-Catenin reactivation, there is enhanced proteosomal degradation of SMAR1 through the D-boxes (Taye et. al. 2018 *Oncotarget*). The proteosomal machinery that is involved in degradation of SMAR1 involves CDC20, which is an E3 Ubiquitin Ligase that mediates this degradation. (Paul et. al. 2017 *Cell Death and Disease*)

Aims and Objectives

- Studies on the regulation of antigen processing and presentation by SMAR1 and its implication in tumorigenesis.
- Determination of the role of a nuclear matrix binding protein SMAR1 in vertebrate embryogenesis.
- Understanding the role of SMAR1 in inhibiting stem cell traits in colorectal cancer cells.

Work Done

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 antigen processing machinery affecting the major histocompatibility complex (MHC) I pathway. Proteins with chaperone activity like calnexin and calreticulin play a pivotal role in 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) by 2D gel electrophoresis has been done. In this screen, one of the target proteins of SMAR1 was calnexin. Further detailed regulation of calnexin expression by SMAR1 was studied. Our experiments confirmed that SMAR1 regulates calnexin gene expression. To understand its biological significance in context to antigen processing and presentation, an immunoprecipitation experiment was carried out to check the interaction between MHC1 and calnexin in HCT116 $p53^{+/+}$ cells (Figure 1A and 1B). Flow cytometry analysis also revealed that SMAR1 overexpression increases MHC1 expression in cancer cells (Figure 1C). TSA (an HDAC inhibitor) largely reduced the SMAR1 mediated induction of MHC I, clearly indicating the involvement of HDACs (Figure 1D). We already had shown that SMAR1 suppresses calnexin expression through HDAC1. Wang et al (2013) reported for the first time that p53 regulates MHC1 in cancer and infection by up regulating ERAP1 (ER aminopeptidase1). Our lab had already established the fact that SMAR1 stabilizes p53, so we checked the correlation between SMAR1 and ERAP1 to further strengthen our results. Overexpression of SMAR1 in HCT 116 $p53^{+/+}$ cells resulted in increased expression of ERAP1 whereas SMAR1 overexpression in HCT 116 $p53^{-/-}$ cells does not affect p53 expression (Figure 1E and 1F). From here we came to the conclusion that SMAR1 stabilizes p53 and in turn increases ERAP1 expression. All these results led us to predict two tier regulation of MHC I by SMAR1 in both p53 dependent and independent manner. MHC I surface expression is drastically reduced in cancer cells leading to lower survival of patients. Also it has been reported that calnexin expression increases with cancer progression. To extrapolate and confirm our findings, we utilized the Kaplan Meier plotter for breast cancer model to look at the calnexin expression profile and their survival. The Kaplan-Meier plotter is a tool capable of assessing the effect of 22,277 genes on survival using 10,188 cancer samples, of which 4,142 are breast cancer samples with a mean follow-up of 69 months (Gyorffy *et al.*, 2013). We performed the survival analysis on 1975 distant metastasis free survival (DMFS) breast cancer patient data with respect to calnexin expression levels. The KM plot for calnexin established that higher expression levels of calnexin correlated with poor prognosis and survival (Figure 1G). Therefore, we conclude that SMAR1 expression positively correlates with disease free survival while calnexin is a predictor for poor prognosis and disease outcome.

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 a nuclear matrix binding protein SMAR1 in vertebrate embryogenesis

SMAR1 has been shown to have a multifaceted role. It acts as a

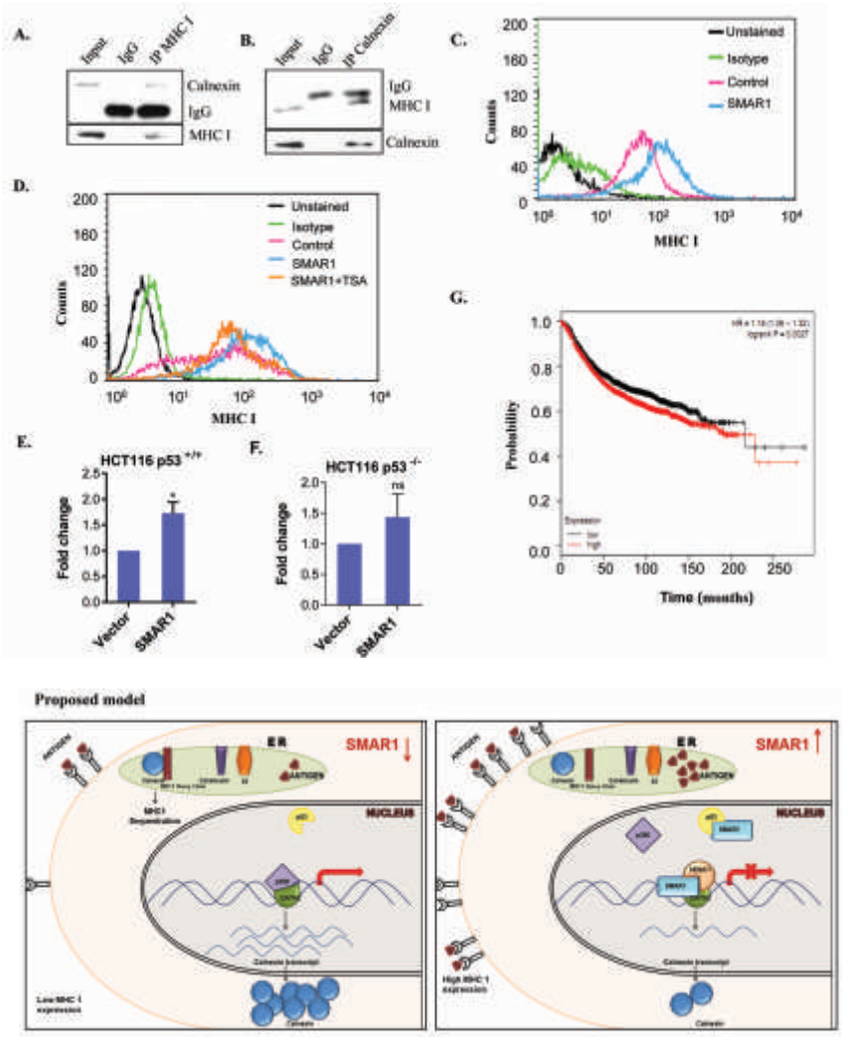

Fig. 1: SMAR1 increases MHC I expression on cancer cells. A. & B. HCT116 p53$^{+/+}$ cells were lysed and immunoprecipitation studies were carried out to check the interaction between calnexin and MHC I. IP was performed with α-calnexin and α-HLA ABC antibodies. Western blot analysis was done and blots were probed with α-calnexin and α-HLA ABC antibodies. **C.** HCT116 p53$^{+/+}$ cells were transfected with flag-vector and flag-SMAR1 plasmid constructs. Cells were harvested after 48h and stained with PE-HLA ABC (MHC I) antibody. Flow cytometry acquisition was done with FACS calibur. **D.** HCT116 p53$^{+/+}$ cells were transfected with flag-vector or flag-SMAR1 plasmid constructs and treated with 100nM trichostatin A. Cells were harvested after 48h and stained with PE-HLA ABC (MHC I) antibody. Flow cytometry acquisition was done with FACS calibur and data was analyzed with cell quest software. **E. & F.** HCT116 p53 $^{+/+}$ and HCT116 p53$^{-/-}$ cells were transfected with flag-vector and flag-SMAR1-an overexpression construct and kept for 48 h. Cells were harvested and RNA was isolated followed by cDNA preparation. Real-time PCR was done to check the relative gene expression of ERAP1. GAPDH was used as endogenous control. **G.** Kaplan-Meier distant metastasis free survival analysis for calnexin gene in 1975 breast cancer patients. Higher expression levels of calnexin are correlated with poor survival (p value=0.0027).

tumor suppressor by virtue of its interactions with p53, in modulation of cell cycle and 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% identity with 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 hematopoietic part. Cloning of the ORF and protein expression, followed by its purification by affinity chromatography have been achieved. This purified protein was confirmed as SMAR1 from zebrafish using MALDI.

Morpholino antisense oligonucleotide against SMAR1 transcript was used to block the translation of SMAR1 and knockdown in the embryos was achieved. Knock-down of SMAR1 was marked by embryonic malformations - smaller

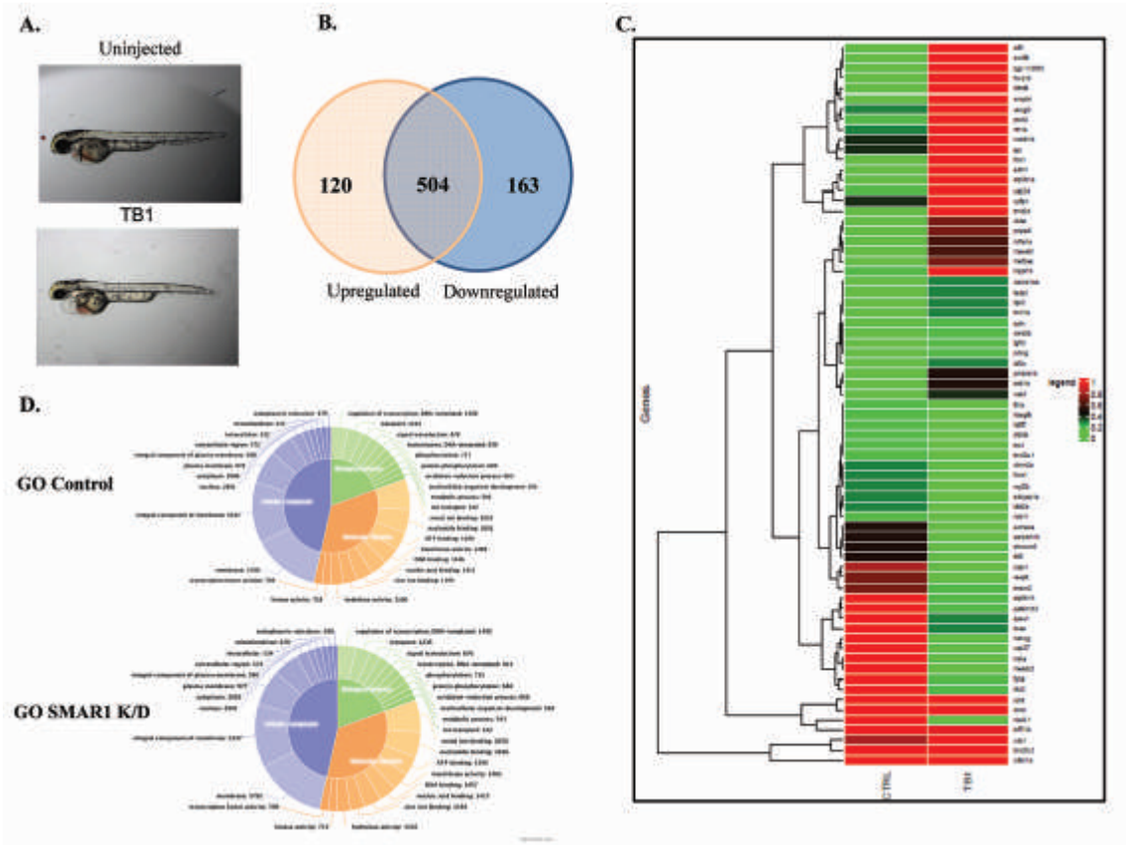

Fig. 2: Differential whole transcriptome sequencing under SMAR1 knockdown condition in zebrafish embryos. A. Phenotypic changes observed at 48 HPF upon Morpholino mediated knock down of SMAR1. B. Venn diagram representation of genes showing significant up- or down-regulation upon SMAR1 knockdown. C. The heatmap representation of differentially regulated genes combined with clustering method grouping genes together based on the similarity of their gene expression pattern. Rows represent individual genes and columns signify each sample in replicate. D. Gene Ontology (GO) analysis of whole transcriptome sequencing data in the form of pie chart showing GO annotation results from control set and SMAR1 knockdown condition.

head size, pericardial edema and a linear heart tube phenotype (Fig 2A). 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 taken and differential whole transcriptome analysis was performed. This revealed several of the transcripts showing variations in the absence of SMAR1. List of significant genes was extracted and heat map was generated based on their FPKM distribution. Heat map revealed several of the genes to be differentially regulated (either over expressed or down regulated) on knocking down SMAR1 (Fig 2D). Gene Ontology (GO) analysis of whole transcriptome sequencing data was also done which indicated GO annotation results from control set and SMAR1 knockdown condition (Fig 2C). The GO database is a relational database comprised of the GO ontologies as well as the annotations of genes and gene products to terms in those ontologies. Housing both the ontologies and the annotations in a single database allows powerful queries of the annotations using the ontology. It classifies functions along three aspects: molecular function (molecular activities of gene products); cellular component (where gene products are active); biological process (pathways and larger processes made up of the activities of multiple gene products). Genes with different molecular functions involved in various biological pathways, markedly cell cycle regulation, mRNA surveillance and FoxOsignaling, were altered. The molecular mechanism underlying such malformations might be interesting to study.

Role of SMAR1 in inhibiting stem cell traits in colorectal cancer cells.

One of the hallmarks of cancer is general loss of cell cycle regulation leading to indefinite proliferation and immortalization. One of the mechanisms by which immortalization of a cell is achieved is by reactivation of enzyme telomerase (hTERT). hTERT is active in germ cells, stem cells but most somatic cells express hTERT transiently in S-phase as it is

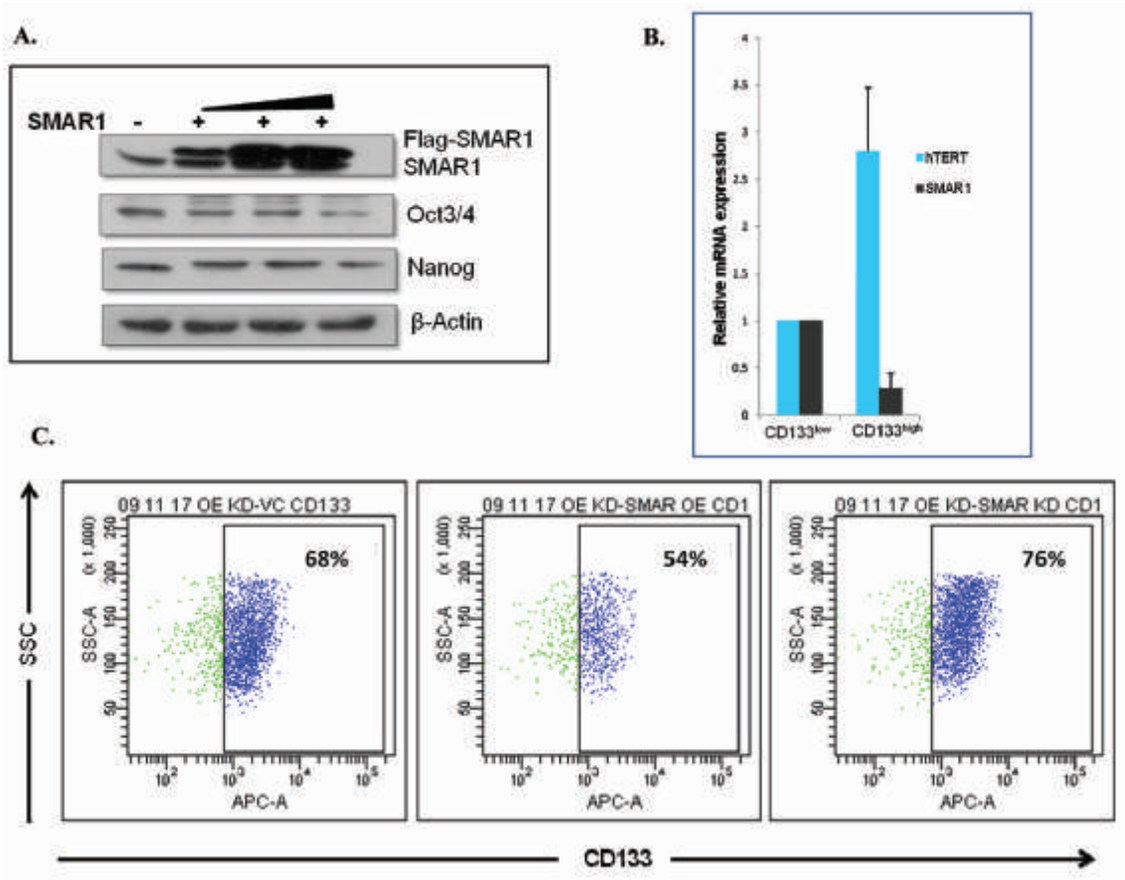

Fig. 3: SMAR1 inhibits stem cell traits in colorectal cancer cells. A. SMAR1 over-expression in HCT116 cells leads to down regulation of stem cell markers Oct3/4, Nanog. **B.** RT-PCR for the levels of SMAR1 and hTERT in FACS sorted $CD133^{high}$ and $CD133^{low}$ cells. **C.** SMAR1 over-expression leads to decrease in the CD133+ population and SMAR1 knock-down leads to increase in the CD133+ population.

involved in telomeric end replication. Most solid tumors reactivate hTERT as it confers various advantages to the cancer cells like protection against DNA damage, maintenance of stem cell pool, increased angiogenesis, etc.

Our study identified a tumor suppressor, SMAR1 as a repressor of *hTERT*. SMAR1 is reported to be highly downregulated in higher grades of cancer. We find that SMAR1 interacts with HDAC1/mSin3a complex at the hTERT promoter and brings about transcriptional repression. Interestingly we find that occupancy of HDAC1 on hTERT promoter is SMAR1-dependent. The recruitment of repressor complex at hTERT promoter results in deacetylation of histones that maintains a repressive environment. Major cause of cancer relapse is persisting cancer stem cells (CSC's). We find that SMAR1 over-expression inhibits the expression of CSC markers (Fig 3A) and $CD133^{+}$ colorectal CSC's express low levels of SMAR1 (Fig 3B). We also find that knock-down of SMAR1 promotes CD133+ CSC's and SMAR1 over-expression inhibits CD133+ CSC's (Fig 3C). We also identify certain plant derivatives which stabilize SMAR1 and repress hTERT, leading to decrease in the stem cell pool. Our study thus provides insights into CSC's regulatory mechanism and thus suggests a potential therapeutic target in treating colorectal cancer.

Future Research Plans

- 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.

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Structural and Functional Studies on Components of the Nuclear Pore Complex

Background

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

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

Aims and Objectives

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

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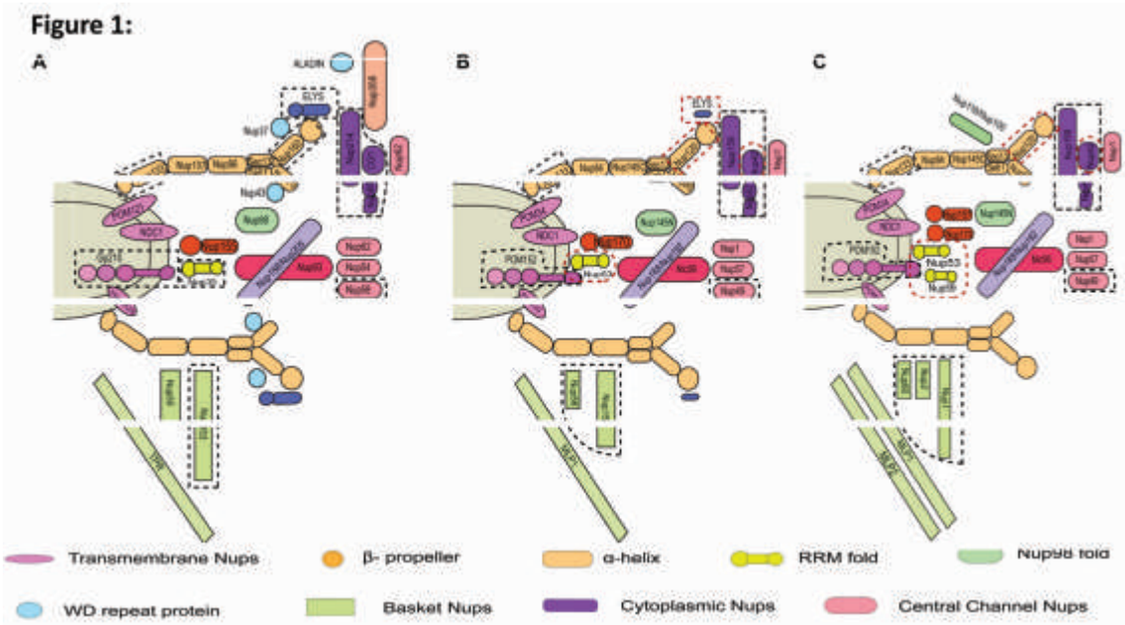

Fig. 1: Schematic representation of the compositional differences in human and yeast NPC.
Two-dimensional representation of the NPC composition based on homology searches and fold prediction for A. *H. sapiens*, B. *C. thermophilum* and C. *S. cerevisiae*. These differences indicate species specific composition of the nuclear pore complexes from different organisms. Differences between the metazoan and fungal species are marked with black dotted lines and those between the fungal species are marked with red dotted line.

- X-ray crystallographic studies on reconstituted minimal complexes of Nups.
- Analysis of the Nups in regulating transport activity and various cellular functions

Work Done

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

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

Our recent *in-silico* study on entire NPC in various species, highlighted the differences amongst the nucleoporins from different phyla and proposes divergent evolution of NPC from fungi to metazoans

The comprehensive phylogenetic and supertree analysis guided from structure-based alignments of all the nucleoporins from fungi to human demonstrate divergent evolution of the NPC across species. We compiled the major differences between yeast and Human NPC at sequence, secondary structure and domain organization level to demonstrate that these species has distinct NPC assembly (Figure 1). For example, Nup58 of the central channel is shown to be specific to the metazoans where it harbors additional domain and its interaction with other central channel proteins is also specific in nature. We also validate the species specificity of central channel protein using in-vitro pull down analysis, which demonstrate that fungal Nup49 cannot mimic the interaction interface of its metazoan ortholog Nup58. These findings provide for the first time comprehensive picture of evolution of NPC from fungi to metazoans particularly in a structural perspective. Overall, we propose that the protein-protein interactions in such large multimeric assemblies are species specific in nature and hence their structure and function should also be studied in an organism specific manner.

Future Research Plans

- Mapping interactions of Nups based on immuno precipation based assays and in-silico approach to predict interactions among the Nups in entire NPC.

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Regulation of Microridges in Developing Zebrafish Peridermal Cells by Nup358

Background

Cell polarization is a fundamental process involved in growth, development and homeostasis of multicellular organisms. The establishment and maintenance of polarity across different organisms and cell types are regulated by players that are conserved across evolution. Par polarity complex, consisting of Par3, Par6 and atypical protein kinase C (aPKC), represents one such module that has well-established roles in polarity during epithelia formation, directed cell migration, asymmetric division of one-cell *C. elegans* embryo and axon-dendrite differentiation of neurons.

The kinase component of Par polarity complex aPKC phosphorylates multiple substrates such as Lgl, Numb, Par1 to achieve spatio-temporal regulation of processes that are involved in the establishment and maintenance of cell polarity. This requires a tight regulation of aPKC activity. The aPKC molecule is inhibited by intra-molecular interaction involving the pseudosubstrate domain present in the N-terminal region and the C-terminal catalytic domain. Phosphorylation of specific residues on aPKC, for example, PDK1 dependent phosphorylation of T410 (human PKC zeta isoform) and TORC2 mediated phosphorylation of T585, is known to activate the kinase. Another mechanism of aPKC activation involves binding of Cdc42-GTP to Par6-aPKC. However, whether other mechanisms of activation of aPKC also exist is not well understood.

We recently reported that aPKC can be activated by Nup358-dependent SUMOylation. SUMO is a small peptide that gets covalently conjugated to specific lysine (K) residues in the target protein, which alters its activity, protein interaction partners and / or intra-cellular localization. SUMOylation of the

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target molecule takes place with a specific set of E1 (SAE1/SAE2 heterodimer), E2 (Ubc9) and E3 ligases. The SUMO E3 ligases include members of the protein inhibitor of activated STAT (PIAS) family, Pc2 and the nucleoporin Nup358. Nup358 is shown to act as an E3 ligase for SUMOylation of topisomerase2, borealin and RanGTPase.

Nup358 (also called RanBP2) is a nucleoporin present on the cytoplasmic side of the nuclear pore complex, and in the cytoplasm as a component of annulate lamellae (AL). Although Nup358 is involved in cargo-specific and receptor-specific nucleo-cytoplasmic transport, it appears to be dispensable for general transport. Many non-traditional roles for Nup358 have been reported. Studies from our laboratory revealed a role for this nucleoporin in different contexts of cell polarity. Nup358 is shown to be involved in microtubule dynamics, localization of the microtubule plus-end-binding protein APC to the leading edge and regulation of polarized cell migration. It also interacts with Par polarity complex and plays a role in the determination of axon-dendrite polarity during differentiation of isolated rat hippocampal neurons. Although it is evident that Nup358 plays a role in cell polarization, the mechanism by which it functions in this process is unclear.

Zebrafish is an attractive model organism for studying polarization events during early embryonic development. One of the polarized actin-rich structures present on the apical membrane of peridermal zebrafish embryo is microridges. They are thought to be important for mucous retention and spread, abrasion resistance and membrane storage. These polarized apical structures are recently shown to be regulated by aPKC and lethal giant larvae (Lgl). It has been reported that aPKC negatively regulates microridge length by phosphorylating Lgl and thereby preventing its localization to the apical membrane (9).

We sought to investigate the possible in vivo function of Nup358 in cell polarity using zebrafish as a model system. As our previous study had demonstrated the activation of aPKC by Nup358-mediated SUMOylation, we hypothesized that Nup358 plays an upstream role in regulating the microridge length in zebrafish epidermal cells through aPKC and Lgl.

Aims and Objectives

- Studying the role of Nup358 in early zebrafish development.
- Understanding the mechanism by which Nup358 functions in microridge formation in zebrafish peridermal cells.

Work Done

Knockdown of Nup358 by morpholino leads to developmental defects in zebrafish

Sequence analysis suggested that Nup358 protein is highly conserved between human and zebrafish (Fig.1a). To investigate the role of Nup358 in the regulation of polarity in zebrafish, knockdown of Nup358 was achieved using a splice-blocking morpholino (MO) targeting the intron2-exon3 junction of Nup358 pre-mRNA (Nup358-MO). Nup358-specific morpholino was injected in one-cell stage zebrafish embryo, and a five base-pair mismatch morpholino was used as control (Control-MO). The reduction in Nup358 protein level was confirmed by western blotting (Fig. 1b). Reverse transcription-polymerase chain reaction and sequencing of the defectively spliced products showed that the intron between the Exon2 and Exon3 was retained in at least in ~50 % of Nup358 mRNA at 9 hour post-fertilization (hpf) (Fig. 1c). Due to the inclusion of this intron a premature stop codon was introduced in the ORF after the 47th codon, leading to possible production of a very short N-terminal peptide of Nup358.

The most prominent feature of Nup358 morphants was hydrocephaly, seen distinctly at and after 30 hpf (Fig. 1d). Almost 80% of the Nup358-MO injected embryos showed the hydrocephaly phenotype as against none in the Control-MO injected embryos. To rule out any off-target effects of Nup358-MO, we attempted to rescue the hydrocephaly phenotype in Nup358 morphant by ectopic expression of human Nup358 (hNup358). *In vitro* synthesized RNA encoding green fluorescent protein (GFP)-tagged version of hNup358 was co-injected with Control-MO or Nup358-MO in one-cell stage embryos. Exogenous expression of hNup358 reverted the hydrocephaly phenotype caused by Nup358 knockdown, confirming the specificity of Nup358-MO used. Moreover, the fact that human homolog rescued the hydrocephaly phenotype in zebrafish Nup358 morphants indicated the functional conservation of this protein across evolution.

Nup358 knockdown stabilizes microridges in zebrafish peridermal cells

As described earlier, the F-actin-rich microridges present on the apical membrane of zebrafish periderm cells are regulated by aPKC and Lgl. To test if Nup358 functions in the regulation of these polarized microridge structures in zebrafish, we injected Nup358-MO or Control-MO into one-cell embryos, and examined the microridges. As compared to Control-MO

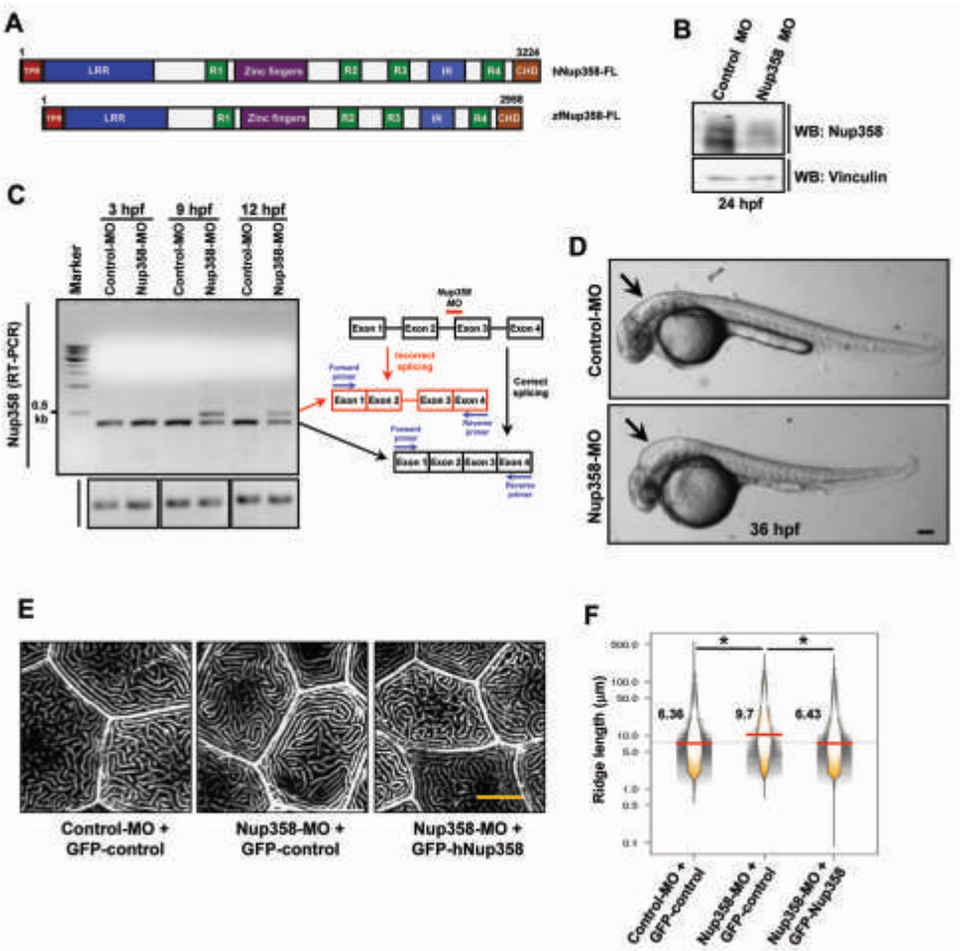

Fig. 1: Nup358 knockdown phenotype in developing zebrafish embryos: (A) schematic diagram showing domain architecture of Nup358 homologues in humans as well as zebrafish. Validation of Nup358 knockdown by (B) Western blotting performed at 24 hpf stage, and (C) RT-PCR. (D) DIC images of control MO and Nup358 MO injected 36 hpf embryos showing a prominent hydrocephaly developed in Nup358 morphants (arrow). Scale bar = 100 µM (E) Rescue of the long ridge phenotype of Nup358 knockdown by GFP hNup358 overexpression, where GFP RNA is used as control and control MO was used as morpholino control. Scale bar = 10 µM. (F) Frequency distribution of microridge length and median (red horizontal line) represented in the form of bean plot (plotted using R software). Quantitation of microridge lengths was done using ImageJ. Ridge length data of all the groups was analyzed using Kruskal-Wallis test and pairwise comparison between two groups was made by the Dunn's method.

injected cells, the length of microridges increased significantly in Nup358 depleted cells (Fig. 1e, f), which was rescued by ectopic expression of hNup358 (Fig. 1f). Taken together, the results indicated that Nup358 is a major determinant of microridge length in zebrafish peridermal cells.

Nup358-mediated regulation of microridges is Lgl-dependent

The tumor suppressor protein Lgl is known to be a strong binding partner of non-muscle myosin 2, and it has been shown that Lgl stabilizes microridges through non-muscle myosin II. Zebrafish has two functionally redundant isoforms, Lgl1 and Lgl2. To find out if Nup358 mediated regulation of microridges is dependent on Lgl, Nup358 depletion was performed in *Lgl2 (penner)* mutant embryos. In these embryos, almost complete depletion of Lgl2 isoform was achieved by 30 hpf. Detailed analysis suggested that Nup358 morphant, in penner mutant background, showed significantly reduced microridge length as compared to control siblings. These data support the conclusion that Nup358-mediated regulation of microridges is Lgl-dependent.

Nup358 functions in microridge regulation via aPKC

Previous studies indicated an important role for aPKCλ isoform in microridge formation in developing zebrafish peridermal cells. As we had earlier shown that Nup358 activates aPKC via

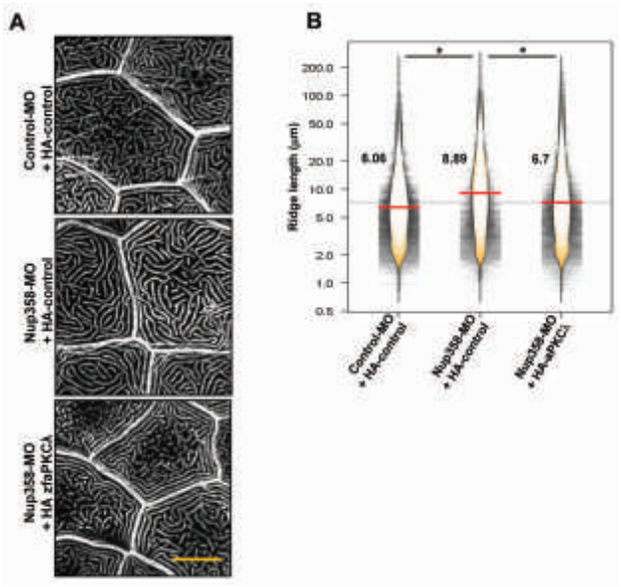

Fig. 2: **Nup358-mediated regulation of microridges is aPKC dependent:** (A) Apical confocal sections of control MO + GFP-HA, Nup358-MO + GFP-HA and Nup358 MO + HA-zf-aPKCλ injected embryos at 33hpf stained with phalloidin. (B) Bean plots representing the distribution of microridge lengths in the respective groups. Quantitation of microridge lengths was done using ImageJ. Ridge length data of all the groups is analyzed using Kruskal-Wallis test and pairwise comparison between two groups is made by the Dunn's method. Scale bar = 10 µM.

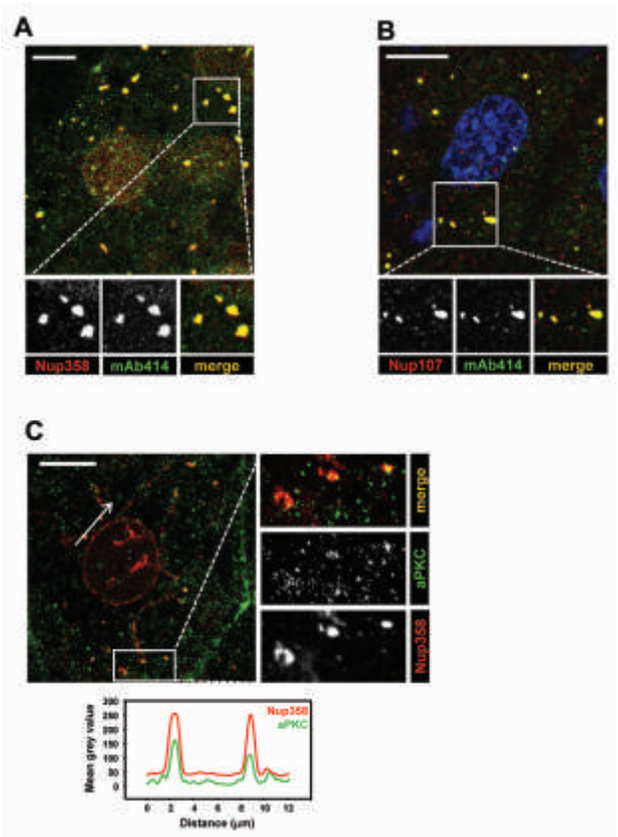

Fig. 3: **Nup358 may regulate aPKC at the annulate lamellae structures.** (A) Co-immunostaining with anti-Nup358 antibody and anti mAB414 antibody in 9hpf zebrafish embryos. (B) Co-immunostaining with anti-Nup107 antibody and anti-mAb414 antibody (recognizes FG-containing nucleoporins) in 9 hpf zebrafish embryos. (C) Upper panel: Co-immunostaining with anti-Nup358 antibody and anti-aPKC antibody in 9hpf in zebrafish embryos. Scale bar = 10 µM. Lower panel: Mean grey value intensity plot for aPKC and Nup358 for the region represented in arrow. Scale bar = 10 µM.

SUMOylation, we wished to test if Nup358 functions through aPKC in the regulation of microridge length. If so, we reasoned that overexpression of aPKC could rescue the microridge phenotype in Nup358 morphants. Earlier studied had indicated that aPKCλ plays a prominent role in regulating Lgl-dependent microridge formation in zebrafish peridermal cells. Our experimental results showed that the long-ridge phenotype seen in Nup358 knockdown embryos was partially reversed by the ectopic expression of zebrafish aPKC (Fig 2a, b), indicating that Nup358 functions through aPKC.

Nup358 may regulate aPKC at the annulate lamellae structures

How does Nup358 regulate SUMOylation and activation of aPKC in cellsΔThis is particularly interesting, given that Nup358 primarily resides in the nuclear membrane as a part of NPC and aPKC is mostly present at the apical junctions of epithelial cells. In many cell types including oocytes, Nup358, along with a subset of nucleoporins, is also found in the cytoplasm as a part of AL structures. To analyze the distribution of Nup358 and other nucleoporins in zebrafish, we resorted to fluorescence microscopy using specific antibodies. Immunostaining with Nup358, Nup107 and mAb414 (recognizes FG-containing nucleoporins including Nup358) antibodies, showed that the cytoplasmic structures are positive for Nup358, Nup107 and possibly other nucleoporins (Fig. 3a, b), thereby representing the AL. Further, co-staining of Nup358 and aPKC in zebrafish cells indicated that ~ 44 % of Nup358-positive AL puncta in the cytoplasm was associated with aPKC (Fig. 3c). Taken together, the data indicate that AL might act as platforms for Nup358 to SUMOylate and activate aPKC in the cytoplasm. Based on these findings, we propose that Nup358 at the AL structure associate with, SUMOylates and activates aPKC, which phosphorylates and inhibits Lgl and restricts the microridge formation.

Future Research Plans

- To test if SUMOylation of aPKC is important for aPKC mediated regulation of microridges.

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Curcumin Reverses Diabetes-Induced Endothelial Progenitor Cell Dysfunction Enhancing MnSOD Expression and Activity in vitro and in vivo.

Background

Out of the 2 different ongoing projects from the Lab., the project on Endothelial progenitor cells (EPC) is described in detail below:

Diabetes mellitus (DM)-induced hyperglycemia is known to cause multiple secondary complications, including, but not limited to, nephropathy, retinopathy, neuropathy, non-healing ulcers and impaired wound healing. Endothelial progenitor cells (EPCs), known to migrate towards and contribute to neovascularization by getting incorporated into wound tissue, are key players in wound healing process. Any impairment in these abilities leads to Endothelial Progenitor Cell Dysfunction (EPCD). A major contributory factor in DM-EPCD is the constitutive exacerbation of overall ROS levels and simultaneous depletion of cellular free radical scavenging ability. This exacerbated ROS negatively impacts their ability to migrate towards the site of injury and participate in the process of revascularization. It is evident that reduction in ROS levels would possibly reverse EPC dysfunction, suggesting that antioxidant therapies may improve diabetic wound healing. In recent times, EPC therapy has emerged as an alternative to current therapeutic and surgical treatments of wound healing. However, in patients with chronic ischemic cardiomyopathy, autologous cell therapy did not work in diabetic patients. Since EPC function is impaired in diabetics, we speculated that if the dysfunction of EPCs from diabetic patients could be reversed, the efficacy of autologous cell therapy could improve significantly.

In non-diseased scenarios, EPCs are resistant to oxidative stress as they contain intrinsically high levels of antioxidant enzymes including manganese superoxide dismutase (MnSOD), which plays a key role in EPC resistance to oxidative stress via scavenging ROS. In contrast, EPCs in DM patients express

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significantly lower levels of MnSOD, which contributes to their dysfunction. Collectively, these reports suggest that MnSOD might be a promising target in the rescue of hyperglycaemia-induced dysfunction of EPCs. Earlier studies have employed genetic approach of over-expressing MnSOD in the diabetic EPCs to augment their in vivo functions. Although this strategy has shown promising results, there are some limitations to such genetic approach. Hence, there is a need to develop an alternate strategy to reverse EPCD.

Curcumin, the active ingredient of *Curcuma longa*, is well known for its potent anti-oxidative, anti-inflammatory, and free radical-scavenging activities. Its efficacy in preventing and reversing DM-induced secondary complications, including wound healing, has also been reported. Curcumin supplementation has been shown to reduce oxidative stress in some of the disease models viz. cardiovascular diseases, diabetes, Alzheimer's disease and multiple sclerosis. Importantly, curcumin has been shown to enhance the activity of MnSOD in MIN6 pancreatic beta-cells and Het-1A cells.Taking these points into consideration, we hypothesized that curcumin might rescue DM-EPCD by inducing expression of MnSOD in them. Although, several reports are available on curcumin and wound healing, the precise mechanism involved in the curcumin-mediated prevention of high glucose (HG)-/hyperglycaemia-induced EPCD has not been demonstrated. Especially, its application in autologous cellular therapy for diabetic wound healing has not been explored.

Here, we evaluated the potential of curcumin in the rescue of high glucose (HG)-/hyperglycaemia-induced EPCD both, in-vivo and in-vitro model systems. We demonstrate here that curcumin treatment reverses dysfunction of D-EPCs in vitro as well as in vivo by restoring the levels and activity of MnSOD in them. This study specifically underscores the potential application of curcumin in autologous cell therapy for treatment of non-healing diabetic wounds and ulcers.

Aims and Objectives

- To determine whether in vitro treatment with curcumin would reverse EPC dysfunction in vitro.
- To determine whether treatment with curcumin would retard DM-induced damage to murine EPCs in vivo and enhance the rate of wound healing in experimental diabetic animals.
- Determine the mechanism involved in the rescue of EPCs from DM-induced dysfunction in vitro and in vivo.

Work Done

In-vivo curcumin treatment accelerates wound healing

It was evident from our in-vitro data that curcumin reverses DM-EPCD (Data not Shown). We further examined whether such rescue is also seen in-vivo. For this purpose, mice were injected with curcumin (i.p.,7.5 mg/kg/day) for 7 d. Post-curcumin administration, full-thickness wounds were created on the flanks of mice (diabetic and non-diabetic) and EPCD rescue was evaluated by standard wound healing assay.

As expected, delayed wound healing was observed in the Diab mice, as compared to the control mice (Figure 1a). Curcumin treatment improved the rate of wound healing in the Diab+CUR mice and a near-complete wound closure was observed by d10

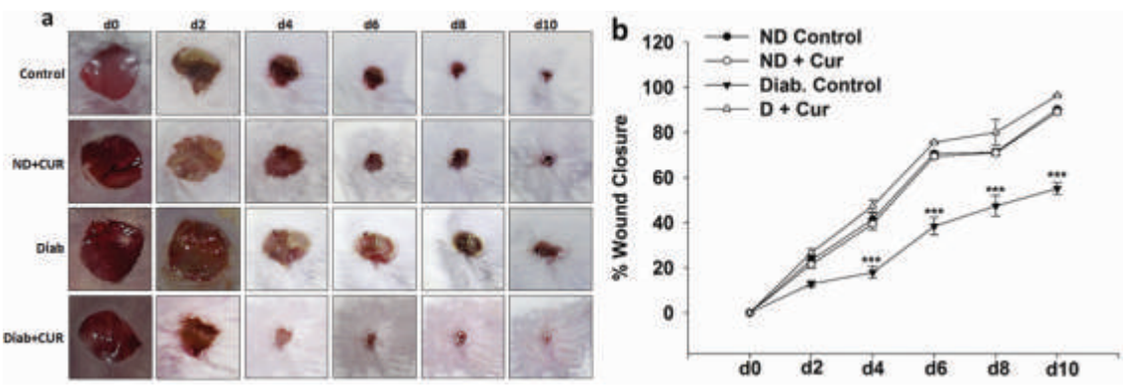

Fig. 1: Curcumin accelerates wound healing process in Diabetic mice. Non-diabetic (ND) and diabetic (D) mice were injected with vehicle or curcumin (i.p.,7.5 mg/kg/day) for 7 d. Full thickness wounds were created on their flanks and the % wound closure was determined by image analysis of the pictures taken every alternate day.(a) Representative images of wounds in Vehicle treated Non-diabetic (Control), curcumin treated Non-diabetic mice (ND+C), diabetic mice (D) and curcumin treated diabetic mice (D+C) from d 0 to d 10 post-wounding are shown. (b) Graphical representation of percent wound closure in the above-mentioned groups. Percent wound closure is significantly higher in D+C mice, as compared to untreated D mice. The data are represented as mean ± SEM. N=4, n=3. ***$p \leq 0.001$

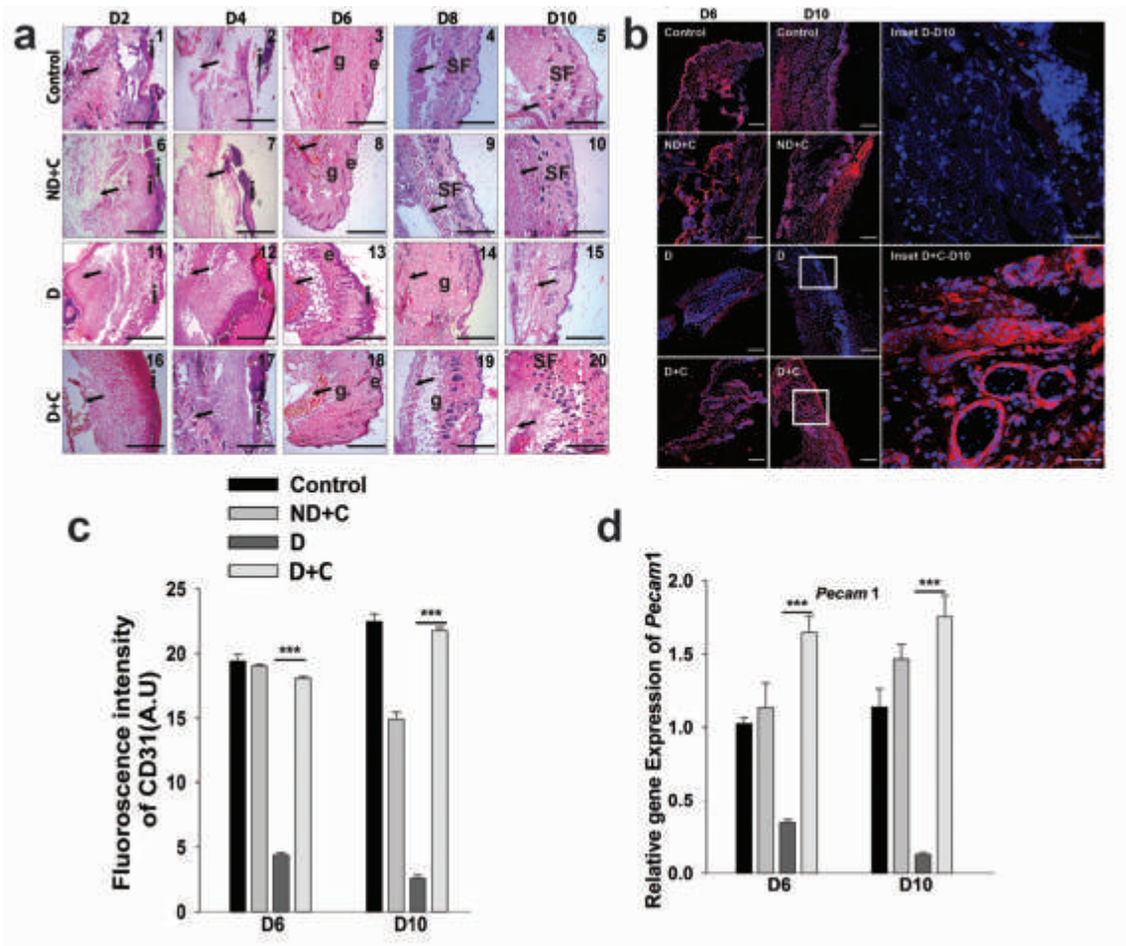

Fig. 2. Curcumin accelerates wound healing process in Diabetic mice. Histo-pathology images of wound healing process are illustrated. Vehicle treated Control (Figure 2a; images 1-5), Non-diabetic + curcumin treated (ND+ C) (Figure 2a; images 6-10), Untreated diabetic control (Figure 2a; images 11-15) and Curcumin treated diabetic mice (D+C) (Figure 2a; images 16-20) Scale bar is 200 µm. i = site of inflammation and macrophage infiltration, g = granulation, e = epithelialization, SF = secondary follicles. Black arrows indicate panniculous carnosus. Data are representative of 4 independent experiments with at least 3 mice per time point. **Curcumin rescues diabetes-induced EPCD in-vivo by increasing CD31 expression.** Non-diabetic (Control) and diabetic (D) mice were injected with vehicle or curcumin (i.p., 7.5mg/kg/day) for 7 d and full thickness wounds were created on their flanks. At d6 and d10 post-wounding, wound biopsies were collected and cDNA prepared from them were subjected to qRT-PCR analysis. (b) Representative images of cryo-sections of the wounds collected from Control, ND+C, D and D+C mice collected at D6 and D10 post-wounding and immuno-stained with an antibody to CD31. DAPI was used to demarcate the nuclei. Bar represents 50µm for low magnification and 10µm for inset images. (c) Graphical representation of fluorescence intensity of CD31 measured in the confocal microscopy images of the wound sections. A.U. = Arbitrary Units. The data are represented as mean ± SEM. N=4, n=3.***p≤ 0.001. (d) Graphical representation of qRT-PCR analysis of CD31 (Pecam1) in the wound biopsies collected at D6 and D10 post-wounding. The data were normalised with β-actin.

(Figure 1a, last row). Percentage wound closure analysis revealed that Diab+CUR mice showed a significant improvement in wound healing from d6 itself (75.4%), as opposed to the diabetic mice, which showed only 39.6% closure. By day 10, the Control, ND+CUR and Diab+CUR mice showed 91.2% , 91.5% , 97.3% wound closure, respectively (Differences not significant), suggesting that wounds in curcumin treated diabetic mice follow the same wound healing dynamics as normal wounds (Figures 1a and 1b). Diab mice, as expected, did not achieve complete wound closure even by day 10 (55.5 %).

However, it was important to examine whether the cellular profile of the wounds in curcumin-treated diabetic mice was comparable with the normal wound healing. Therefore, we performed histo-pathological analyses of the wound sections. At day 2, all wounds showed inflammation and macrophage infiltration. More inflammation was observed in Control, ND+C treated and D wounds (Figure 2 images- 1, 6, 11), as compared to curcumin treated diabetic (D+C) wounds (Figure 2 image-16). Although, curcumin treated ND wounds showed initial inflammatory response and macrophage infiltration by day 4, all wounds, except those of untreated diabetic mice (Figure 2

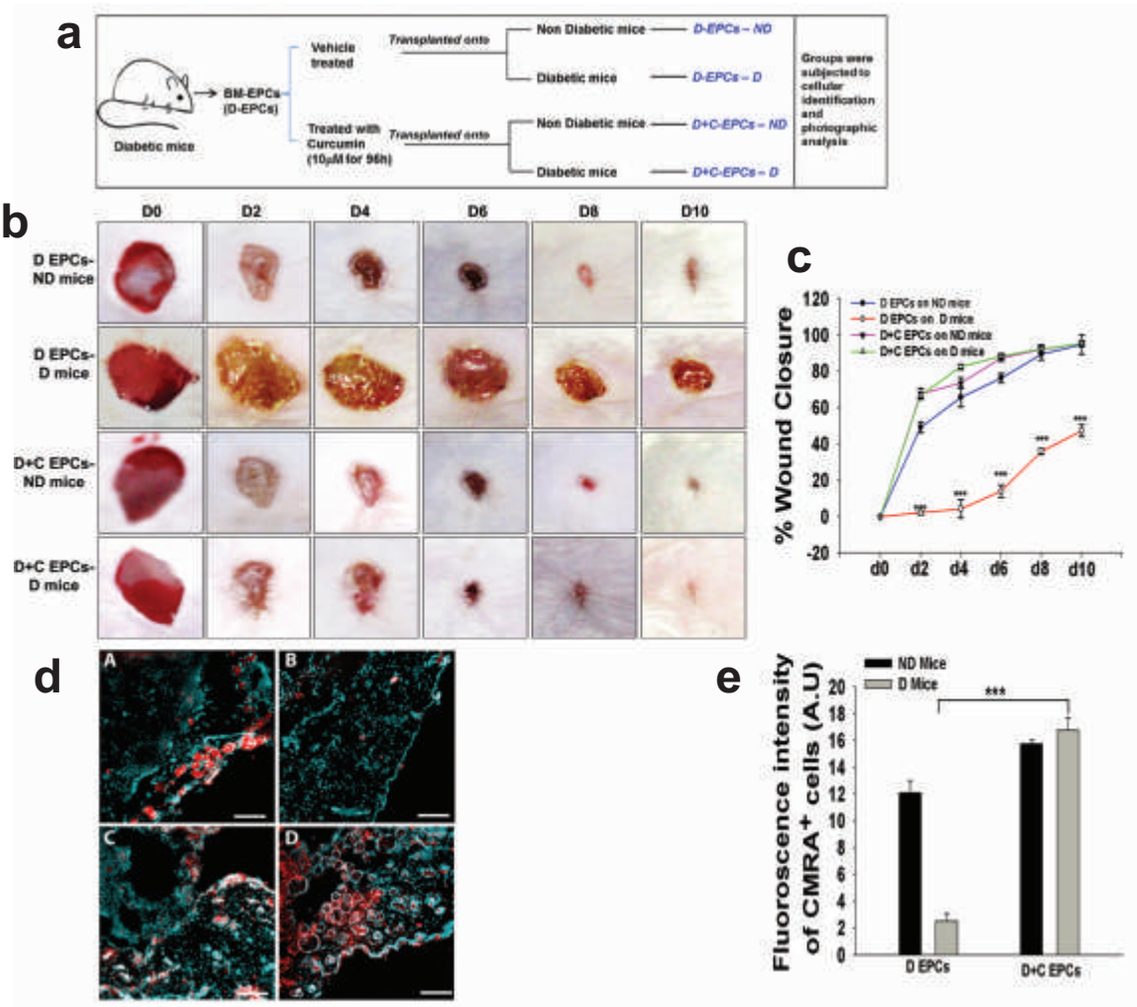

Fig. 3. Transplantation of curcumin rescued D-EPCs enhances the rate of wound healing in murine model of experimental diabetes. (a) Panel depicts the study design. (b) Photographic evidence of wound healing process in all four groups at various time points post-wounding. At least 3 mice were analysed for each time point. (c) Graphical representation of percent wound closure in various groups; (d and e) D-EPCs and D+C-EPCs were pre-stained with cell tracker-Orange (CMRA) and were delivered at d 0 onto non-diabetic and diabetic wounds for 72h.Cryosections were made, counterstained with DAPI and analysed on a confocal microscope to determine the incorporation of pre-stained EPCs into wound beds. Representative images of wound sections showing migration of labelled EPCs (d). Fluorescence intensity of CMRA positive cells in the wound beds as determined using Image J software is graphically illustrated. (e) A= D EPCs-ND mice, B=D EPCs-D mice, C=D+C EPCs-ND mice, D=D+C EPCs-D mice (e). Scale bar is 100 μm. The data are represented as mean ± SEM. N=4, n=3.***$p \leq 0.001$.

images- 2, 7, 17), showed a marked reduction in inflammation and macrophage infiltration. Initiation of granulation with reduced inflammation was seen in all wounds by day 6, except diabetic untreated wounds where inflammatory response persisted till day 8 (Figure 2a images- 3, 8, 13, 18 and images- 4, 9, 14, 19). Curcumin treated ND and D wounds showed a marked decrease in inflammatory response and formation of secondary structures by day 6, indicating accelerated wound healing (Figure 2a images- 8 and 18). Both groups showed secondary structures and epithelial formation. Although diabetic control wounds showed reduced inflammation and initial epithelialization, they showed very little secondary structure formation by day 6 (Figure 2a image-13). At day 8, wounds from ND, ND+C and D+C groups showed comparable epithelialization and secondary structure formation (Figure 2a images-4, 9, 19). Epithelialization, but no secondary structures were observed in control diabetic wounds (Figure 4a images-14). By day 10, curcumin treated diabetic wounds showed mature hair follicle formation similar to normal ND control (Figure 2a images-5, 10, and 20), whereas untreated diabetic mice showed poor and delayed wound healing (Figure 2a image-15).

Collectively, the data showed that the wounds of curcumin treated diabetic mice displayed normal wound healing dynamics, whereas those from untreated diabetic mice showed delayed healing response

In vivo Curcumin treatment maintains higher levels of CD31 in the wounds of mice

Cd31 is an endothelial specific marker and, it is an indicator of angiogenesis which is important for progressive wound healing. Thus, it can be used to identify the well-formed blood vessels in wound histological sections to evaluate the degree of angiogenesis in wounds. Since we observed that curcumin significantly restored the levels of Ang1 in diabetic wounds (Data not Shown), we believed that this would lead to higher expression of CD31 protein in diabetic wounds. Tissue biopsies collected from wounds were lysed and cDNA were prepared to assess expression of CD31-specific mRNA *(Pecam1)*. Similarly, frozen sections of the wound biopsies were stained with antibody to CD31. As expected, there was a time-dependent decrease in mRNA and protein levels of CD31 in the wounds of diabetic mice (Figure 2b-d).We observed that curcumin treatment significantly increased the expression of CD31-specific mRNA (Pecam1) and protein in diabetic wounds as compared to diabetic control wounds (Figure 2b-d). These data suggest that curcumin enhances the angiogenesis process in diabetic wounds.

Transplantation of curcumin-treated D-EPCs accelerates diabetic wound healing

The results obtained so far clearly demonstrated that curcumin treatment leads to the rescue of EPCD in both, in-vitro and in-vivo systems. To determine whether the D-EPCs treated in vitro with curcumin could accelerate wound healing in an in-vivo model system, the "rescued EPCs" were transplanted on the wounds created on the flanks of diabetic mice and the kinetics of wound healing was measured (Figure 3a). We found that transplantation of rescued D-EPCs significantly augmented the rate of wound healing in diabetic mice, as compared to the untreated diabetic EPCs (Figures 3b and 3c). Since the wound closure was similar to that obtained with Control-EPCs, the data clearly demonstrate that curcumin reverses the dysfunction of D-EPCs.

We speculated that this could be due to a better incorporation of the rescued EPCs into the wound beds, as compared to the untreated diabetic EPCs. To examine this possibility, EPCs, rescued or otherwise, loaded with a vital fluorescent dye (CellTracker™ Orange CMRA Dye) were transplanted on the wounds of diabetic mice as described previously (Kanitkar, *et al.*, 2013). The mice were then sacrificed after 72 hr, as maximal EPC incorporation into the wounds was expected to occur by this time point. We observed an elevated number of CMRL positive cells (red coloured) in the cryo-sections of wounds of diabetic mice transplanted with rescued D-EPCs (D+C EPCs), as compared to the sections of wounds from diabetic mice transplanted with untreated D-EPCs (Figures 3d and 3e).

These data demonstrate that D-EPCs treated in-vitro with curcumin, not only migrate better, but also get incorporated into the diabetic wound beds as compared to the untreated D-EPCs

Future Research Plans

- We plan to identify the aging-induced epigenetic changes causing hematopoietic stem cell dysfunction: rescue using in vitro niche technology.

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Natural Resistance of Mycobacerial Strains to Capreomycin: Do bacteria defect?

Background

Emergence of drug resistant strains of mycobacterial species is a serious problem, which has been highlighted by world health organization. Although many aspects have come to light regarding this problem, the major reasons attributed to drug resistance could be due to evolution of mutant forms. Among the number of possibilities that can contribute to drug resistance, the following are distinct *viz.* 1. Drug is not experienced by the bacterium i.e. its failure to penetrate the bacterium or effectively pumped out of the bacterium. 2. The drug does not find its target. 3. Processing of pre- or pro- form of the drug fails. 4. Emergence of mutations that prevent the drug binding either in nucleic acid sequences or its product proteins. Based on these broad categories one can easily see many sub- categories except for the first possibility for which requires specialized efflux pumps which are yet to be delineated at molecular level. It remains to be explored whether they can pump all drugs that are currently in use. However, the possibilities 2-4 have an underlying phenomenon i.e. whether or not heterogeneity can contribute to drug resistance. For example, a given drug may not find its target if a single bacterium has evolved in such a way that it did not express or partition a particular protein during cell-division as pictorially depicted in Fig. 1. In such a scenario, the bacterium that retained the bulk of the protein will be susceptible while the bacterium that did not receive or make the same protein will be resistant. In this possibility, it is important to note that the genome of both bacteria is same while one is susceptible and the other resistant. Hence, the question whether such bacteria in principle exist or not and can be experimentally explored.

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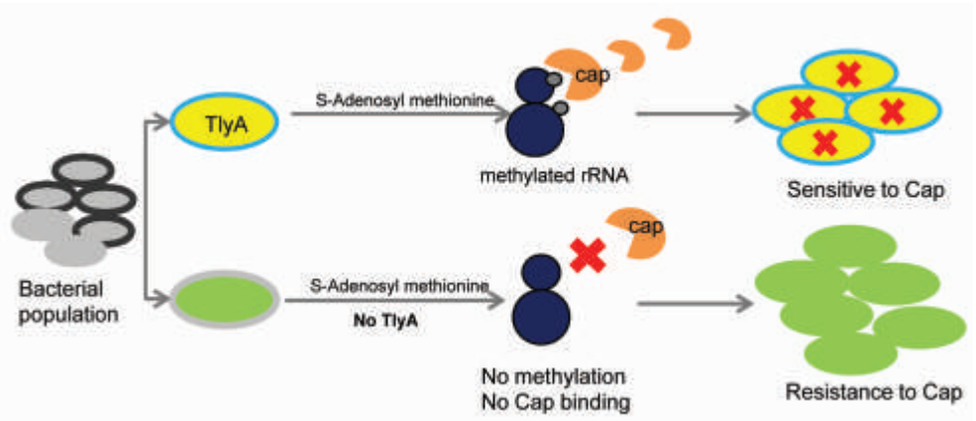

Fig. 1: Illustration of TlyA expression dependent susceptibility. The bacteria that express the TlyA protein are susceptible to Capreomycin due to methylation of 16S and 23S rRNA, while the bacteria that do not express the protein will be resistant due to absence of methylation. It is known in literature that the TlyA is not essential for *in vitro* growth of mycobacteria (based on transposon mutagenesis) and hence possibility of lack of its expression.

Aims and Objectives

- To investigate the role of bacterial TlyA in evolution of resistance to second generation antibiotics.

Work Done

The genus *Mycobacterium* has many members that are pathogenic to variety of species and in humans, it is known by the tuberculosis disease. The *Mycobacterium marinum* causes systemic tuberculosis like disease in fish and frogs and also localized skin suppurations' in immunocompromised humans. Both the human and animal infections are marked by the presence of a granulomatous host response, the hallmark of *M. tuberculosis*. *M. marinum* is genetically closely related to *M. tuberculosis* and has been used increasingly as a model for understanding the pathogenesis of tuberculosis. *M. tuberculosis* and share 99.4% sequence homology based on 16S rRNA sequences. The virulence of non-pathogenic strains of *mycobacterial species* can be restored with the counter-parts of the *Mycobacterium marinum* which is well demonstrated in the literature. Detailed study of *M. marinum* can greatly help in developing screening methods for anti-mycobacterial agents since: (i) it has phylogenetically close relationship to *M. tuberculosis* (ii) it has a relatively rapid doubling time (iii) shows similar drug susceptibilities to *M. tuberculosis* (iii) less expensive bio-safety level environment and its evolutionary aspects can be studied safely.

The strains designated with M1, M2 and M3 respectively represent the transposon insertion strains of *M. marinum* that disrupt Mh3866, Mh3867 and Mh3868 which are homologous to Rv3866, Rv3867 and Rv3868 of *M. tuberculosis* respectively. Based on the literature these genes are necessary for hemolysis exhibited by the *M. marinum*. We have earlier shown that the *M. marinum* expresses the *tlyA* gene product which has been independently shown to possess both the activities i.e. hemolysis as well as S-adenosyl methionine dependent rRNA methylation activities. Susceptibility to Capreomycin by a bacterium is dependent upon the expression of *tlyA* gene product which methylates the nucleotides C1409 and C1920 of 16S and 23S rRNA respectively. Methylation of rRNA results in reduced translational ability, as the methylation of the ribosomes facilitates the binding of Capreomycin. Hence, bacteria that do not carry the *tlyA* gene are naturally resistant to Capreomycin for example, *E. coli* has no natural homolog and is resistant to Capreomycin which upon expression of the *tlyA* gene shows susceptibility.

In view of the above observations, all the four strains were examined for growth in the presence of Ampicillin, HygromycinB and Capreomycin as shown in Fig. 2. The growth curves of all the four strains in the absence of the antibiotic show rapid growth while in the presence of these three antibiotics only retarded in for about 18 hours after which the growth has dominated that of the wild type bacteria. Consistent with this observation, the antibiotic disc diffusion assay also showed no inhibition of growth in which we could see some inhibition only in Ampicillin and mild inhibition in case of HygromycinB while we could not see any kind of inhibition in case of Capreomycin. It is relevant to mention here that the whole cell lysates of these strains did not show the presence of TlyA protein.

M. tuberculosis strain H37Ra, commonly used in studies with the virulent H37Rv was originally derived from virulent strain H37.

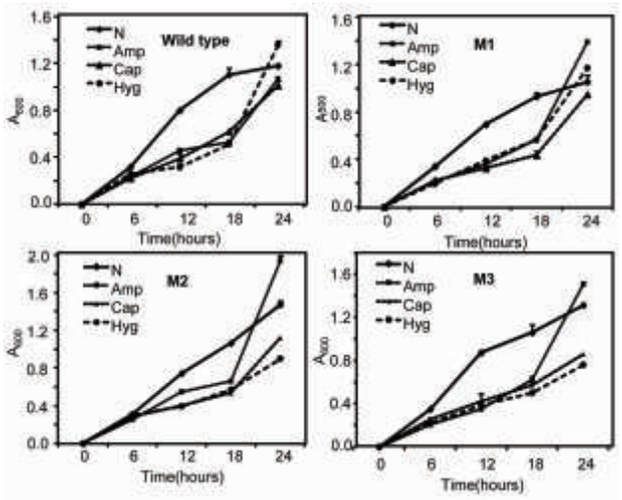

Fig. 2: *In vitro* culture of *M. marinum* wild-type, M1, M2 and M3 in presence of indicated antibiotics: All the strains were cultured as described in methods section in the presence of Ampicillin (100µg/ml), HygromycinB (50µg/ml) and Capreomycin (10, 50 and 100 µg/ml) and their growth was monitored for 24 hours.

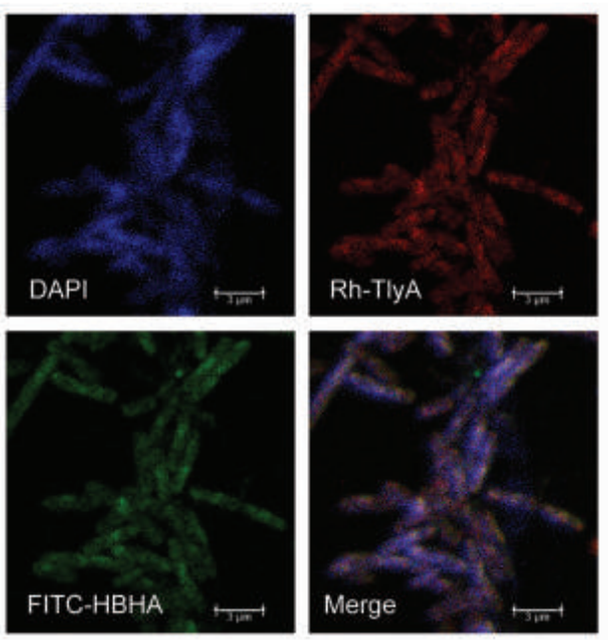

Fig. 3: Confocal visualization of H37Ra for TlyA expression: Surface staining of H37Ra was carried as described earlier for *M. marinum*. The panels labelled with DAPI, Rhodamine, FITC respectively represent staining for DNA, TlyA staining with Rhodamine-anti-Rabbit-IgG and HBHA detected with FITC-anti-mouse-IgG.

Several studies have attempted to determine the genomic and proteomic differences leading to the basis of virulence attenuation of H37Ra which appears to possess insertions, deletions and mutations in some transcription factors. However, detailed studies on H37Ra can have implications both in understanding the pathogenesis of virulent counterpart and the development of new vaccines and therapeutic agents. We, therefore, sought to examine H37Ra and its susceptibility to Capreomycin in context of *tlyA*. In contrast to the observations described above, the H37Ra did not grow at all in the presence of various concentrations of the Capreomycin in liquid media while the agar plate showed dramatic loss of growth in the presence of Capreomycin. We have also examined for the presence of TlyA protein on the surface of H37Ra which showed an unambiguous presence whereas, the expression of TlyA in M1, M2 and M3 is unobservable while the wild-type is noisy. As seen in Fig.3, the confocal microscopic visualization of H37Ra also showed positive surface staining for TlyA (Rh-Rhodamine channel) and HBHA a well-known surface protein of H37Rv (FITC-HBHA).

It is interesting to note here that the wild-type and mutant strains of *M. marinum* are resistant to Capreomycin for lowest to highest usable concentrations while the H37Ra is susceptible to it. Based on Fig. 1, it is important to understand the expression profile of TlyA for evolution of possible heterogeneity or noise in mycobacterial species in *in vitro* culture conditions and its significance for intra-cellular survival. In addition, the high prevalence of TlyA on the surface of H37Ra is very important for further studies as the H37Ra has the ability to survive in humans and mice but does not cause the classical disease and also has not been shown to form granuloma. It relevant to mention here that TlyA expression can aid to intracellular survival of even non-pathogenic versions upon expression of the same. The H37Ra has been shown to contain mutations in PhoP regulon and it is necessary to focus the future studies on PhoP regulon on its role for evolution of susceptibility or resistance to second generation antibiotic such as Capreomycin, which is not often used for treatment of tuberculosis, to enable its usage. It is also not surprising that the possibility discussed in Fig. 1 is found to be true as many clinical isolates with wild-type *rrs* and *tlyA* genes having MIC values well above 0.5mg/ml to 2mg/ml. Hence, it is very important to study the evolution of this phenotype for combating the drug-resistance problem posed by tuberculosis disease and such studies are currently underway.

Future Research Plans

- Defection, a non-cooperation approach, is to avoid incurring cost as shown in Fig. 1. How the cost can be definedΔ Is cost only a metabolic or a phenotypic natureΔ Which of the forms contribute to the fitness of mycobacteriaΔ These are some of the questions we wish to address in future.

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

Background

Ionotropic glutamate receptors (iGluRs) mediate fast excitatory neurotransmission and contribute to high cognitive processes such as learning and memory. They are essential for basic nervous system functions, including learning and development, and are involved in a remarkable range of neuronal diseases. Despite their physiological importance, our understanding of these receptors is hampered by a lack of insight into their complex structures and working mechanisms. Localized within the postsynaptic density of glutamatergic spines, iGluRs are composed of heterotetrameric receptor assemblies associated with auxiliary subunits. The association of auxiliary proteins with iGluRs modulates receptor trafficking and the kinetics of receptor gating and pharmacology. Till-date several of these transmembrane auxiliary subunits have been discovered, which regulate the native iGluRs gating properties, pharmacology, distribution and trafficking to synapses. However, there has been very little progress towards understanding the structural basis of how this expanding family of glutamate receptor auxiliary membrane protein modulates receptor function apart from a recently reported structure of GluA2-TARPγ-2.

Our research goals include structure-function analysis of the iGluR auxiliary subunits targeting both the isolated extracellular domains as well as intact full-length proteins 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.

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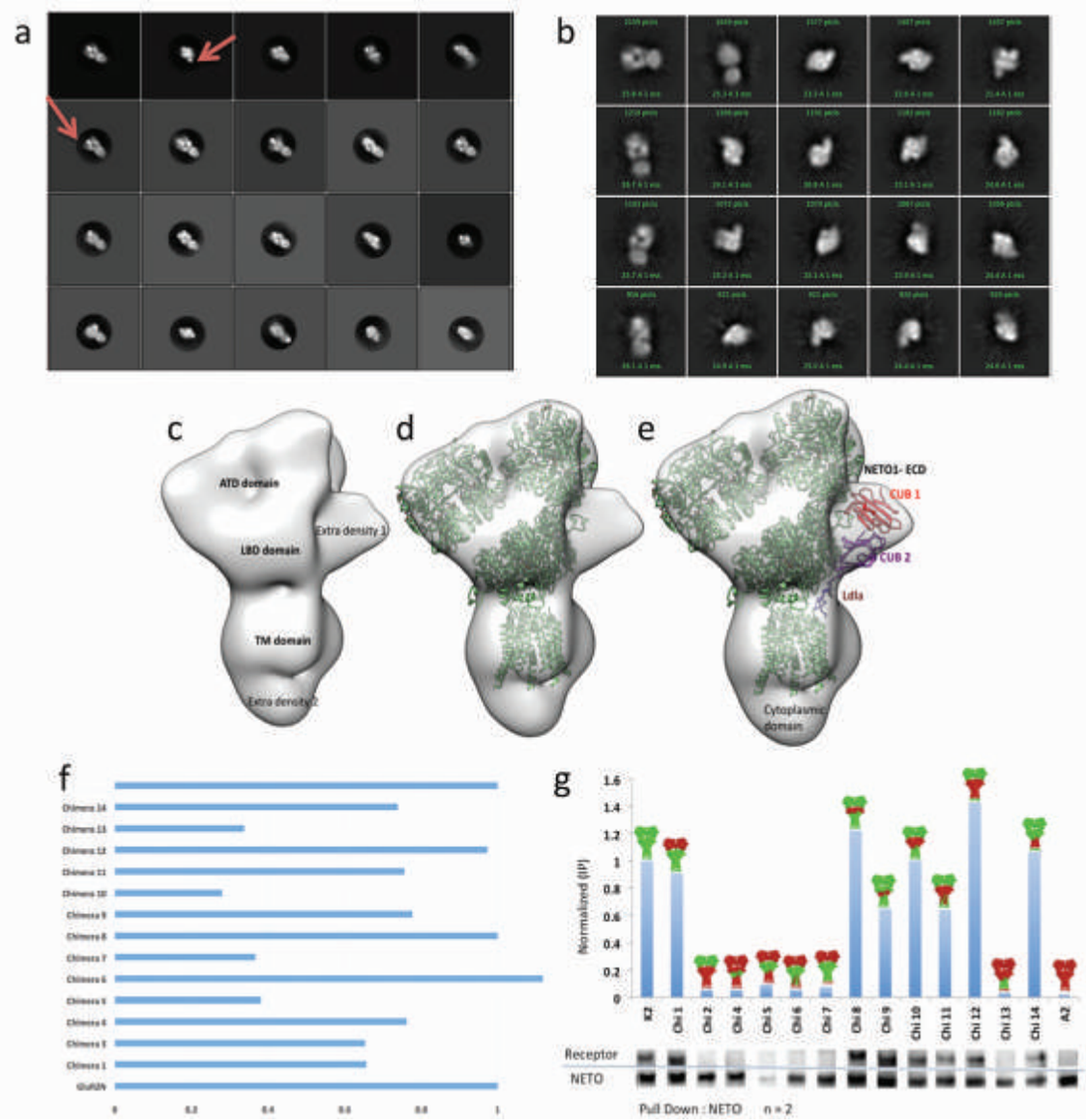

Fig. 1: GluK2-rNETO1-ECD complex Negative stain and cryo-EM data collection and processing (a) Representative 2D class averages from the negative stain dataset ~150,000 particles into 100 classes after many round of 2D classification (b) 2D class averages from the cryo-EM dataset for the GluK2-Neto1. Arrows indicate the extra density observed for NETO1-ECD in top or side views. 3D model reconstruction and model fitting from negative stain datset. (c) 3D reconstruction of GluK2-rNETO1-ECD complex from 53000 particles showing two extra density 1 and 2, (d) Antagonist bound GluK2-rNETO1-ECD closed state structure in isosurface representation, fitted with ATD dimer, LBD dimer and TM domain with C-ter unstructured region, (e) rNETO1-ECD model derived from PHYRE2 fitted nicely in extra density 1. (f-g) GluK2/GluA2 receptor chimeras surface expression confirmation by biotinylation, (f) surface expression levels of the various chimeras with respect to wild type GluA2 and GluK2 receptors elucidated by surface biotinylation experiments (n=2), (g) results of the pull-down assays performed using antibody against Neto1, receptor bands where detected by either using GluA2 or GluK2 monoclonal antibodies. The Western blots were analyzed by using Image J (n=2).

- Determination of binding site and interaction hotspots of an iGluR-auxiliary subunit complex using electrophysiological and biochemical assays.

Work Done

Using an extensive fluorescence-based screening and manipulation of constructs (7), we have optimized overexpression and purification of NETO1 and CKAMP44; the auxiliary proteins for kainate and AMPA receptors respectively. Immunoprecipitation and colocalization studies with a range of receptor chimeras generated by swapping GluK2 and GluA2 domains identify interaction hotspots. We have tested surface expression and functionality of all the chimeras via electrophysiology. We are currently validating interactions via electrophysiological recordings.

Apart from crystallization trials of auxiliary subunits alone, we are pursuing structure determination of iGluR-auxiliary protein complexes via single-particle cryo-electron microscopy. We have overexpressed and purified two kainate receptors namely; GluK2, GluK3 and an AMPA receptor GluA2 in milligram quantities from baculovirus infected mammalian cells. Complex reconstitution between GluK2 and NETO1 was found to be state and concentration dependent and required use of specific ligands for stabilization. We have solved the structure of GluK2-NETO1 via negative stain cryo-electron microscopy. As an offshoot, we have also solved first EM structures for GluK3 receptors trapped in different states of activity. Low-resolution structure of orphan delta receptor GluD1 has also been elucidated for the first time. More data collection is ongoing for high-resolution structure determination.

GluK2-Neto1 complex structure determination using negative stain-EM and single particle Cryo-EM

GluK2 receptors and its cognate auxiliary protein Neto1 were overexpressed and purified from baculovirus infected mammalian cells. The conditions for complex formation between GluK2 and Neto1 were screened via fluorescence size exclusion chromatography (FSEC) and were found to be state dependent. GluK2-NETO1-ECD complex and GluK2 alone was subjected to negative stain electron microscopy experiments. Urnayl acetate stained protein grids were analyzed at 120 KV microscope equipped with 4K X 4K camera. Multiple negative stain datasets were collected for the complex and receptor protein alone. The EM datasets were processed using a combination of Relion and Cryosparc. Both the softwares using reference free 2D and 3D classification gave similar 3D reconstructions. Some of the 2D classes clearly show extra density at the GluK2 ECD region (Fig. 1 a). The negative stain 3D structure has been refined to ~ 20Å resolution and resulted in a 3D model having extra density to accommodate one or two molecules of rNeto1-ECD (Fig. 1c-e). The interaction is mainly at ATD, LBD dimer interface and at the linker region which is in consistent with electrophysiology-based predictions for interactions as reported from other groups.

Simultaneously, we also collected a large cryo-EM data set comprising of ~4500 micrographs for the GluK2-NETO1-ECD complex. Initial 2D classification using Relion and cryosparc again show extra density for the NETO-1ECD at the extracellular regions of GluK2 receptors (Fig. 1 b). Further anaylsis of the cryo-EM data set, 3D reconstruction, 3D refinement is ongoing. However, preliminary analysis shows that ~8-10 Å resolution cryo-EM structure may be possible. After this an extensive structural comparison and analysis will be undertaken to understand key interaction hotspots and conformational changes in receptor on complex formation with NETO1.

3D classification, Refinement and model fitting

Before autopicking from complete dataset, parameters were optimized on 10 selected micrographs to reduce particle picking from background. A total of 150,000 particles were picked and than many rounds of iterative 2D classification was performed to get a clean set of class averages from the complete data set. Finally, ~ 53000 particles were classified into fifty 2D classes that were subsequently used for 3D classification and refinement. We obtained 3 different classes from the initial 3D classification. Among the three classes, one class showed extra density for a single rNETO1-ECD domain, another class showed density for 2-NETO1-ECD molecules. All the three classes showed the extra density at the C-ter region that has never been observed in any of the reported glutamate receptor structures (Fig. 1 c-e). This is because the C-terminus is disordered, however, in our case it might be contributing to density due to stabilization via glutaraldehyde crosslinking. Crystal structures of the GluK2–ATD and LBD in antagonist bound form were used to fit into 3D EM map. However, since no structure was available for rat Neto1 or any CUB domain containing auxiliary protein and at this resolution *de novo* structure determination is not possible. So, PHYRE2 program was used to generate homology model for rNETO1-ECD using reference structure available in PDB for different domains (CUB1, CUB2 and LDLa). Various domains of ATD, LBD, and 3D model of rNETO1-ECD fit well into the constraints defined by low-resolution 3D map (Fig. 1 e).

GluK2/GluA2 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. We designed fourteen (14) receptor chimeras between kainate receptor GluK2 and AMPA receptor GluA2 to be used as tools for pull-down, immunoprecipitation and electrophysiology assays (details shown in previous report). We evaluated the surface expression of the chimeras by surface biotinylation assays. Briefly, expression was carried out in transiently transfected HEK293 cells. Surface biotinylation followed by affinity

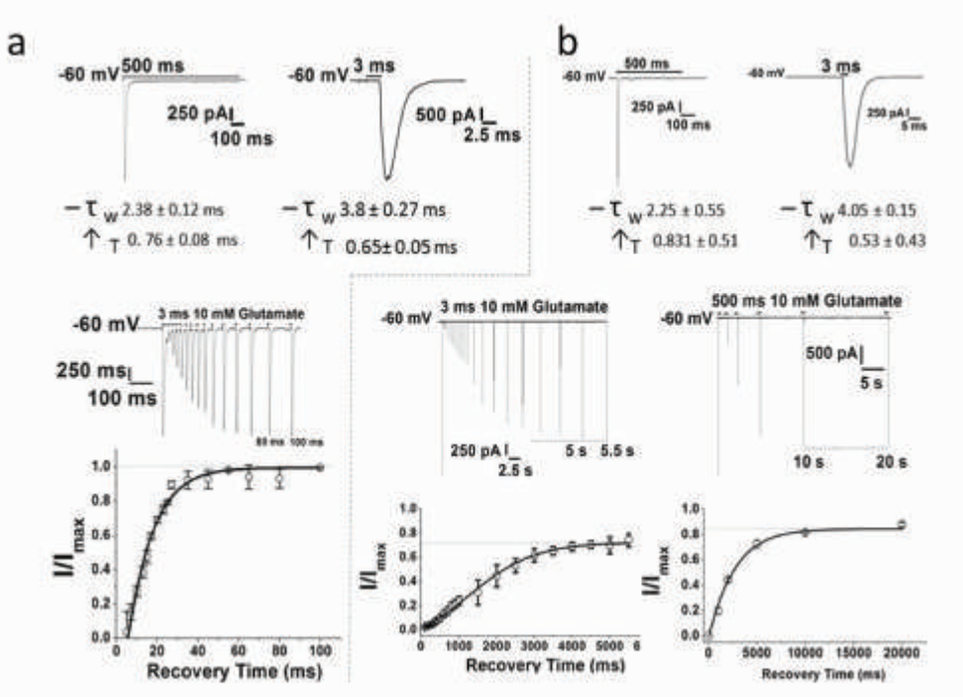

Fig. 2: Recordings of GluA2 alone and GluA2 co expressed with CKAMP44 showing channel kinetics. a & b. Sample traces of current transients in whole cell patches which were recorded from HEK293 cells (WT) 42 h after transfection with 1 µg/ µl of GluK2 cDNA. 10 mM Glutamate was applied for 3 ms and 500 ms for both deactivation and desensitization recordings. Corresponding recovery from desensitization and deactivation kinetics for the same traces have been shown in the bottom of both panels. All these whole cell recordings were repeated thrice from 2 individual transfections. The graphs were plotted by using Origin licensed software and the standard deviation (± SE) was used, to make error bars for each individual point.

purification and separation on SDS-PAGE. This was followed by Western blot with monoclonal GluK2 and GluA2 antibodies. Total expression was compared with surface expression by densitometry of developed blots using ImageJ. All the chimeras reach the cell surface, however Chimeras 5, 7, 10 and 13 show poor expression when compared to rest (Fig. 1f). We have also carried out functional validation of all the chimeras using whole-cell patch clamp and find that all the chimeras except no. 10 are functional (data not shown). Electrophysiological assays with all the chimeras co-expressed in presence of NETO1 and NETO2 is being carried out to narrow down on the interacting domains and to understand roles of various receptor regions.

In order to identify the GluK2 domain mediating interactions with NETO1, we co-expressed rNeto1-TM-mRuby construct in combination with all the 14 chimeras and native GluK2 and GluA2 receptors. Co-immunoprecipitation experiments were carried out. The Western blots after the pull-down assays were analyzed by ImageJ software. This experiment has been repeated twice with consistent results. Based on our analysis, GluK2 ATD, TM3 and C-terminal domain seem to play a critical role in interaction with rNeto1-TM interaction (Fig. 1 g).

Its also interesting to note that the pull-down experiments are consistent with our structural findings where we observe the ATD domain interactions with CUB1 domain of NETO1-ECD (Fig. 1 e). Further, the role of ATD-LBD linker and the ligand binding domain in mediating interactions with NETO1 is also consistent and verified by both the structure and pull-down assays with receptor chimeras. The cryo-EM structure which is likely to reach ~8Å resolution is likely to give better insights into these interactions which will be verified by electrophysiological recordings.

Elucidation of CKAMP44-AMPA receptor interactions using the GluK2/GluA2 chimeras

To characterize the functional consequences of the interaction between the CKAMP44 and AMPARs, electrophysiological experiments were performed employing HEK 293T cells. To investigate how gating properties are modulated by the novel CKAMPs, fast perfusion patch-clamp recordings were performed on whole cell patches from HEK293T cells expressing either GluA2 or chimera alone, or in combination with CKAMP44 (1:3 w/w).

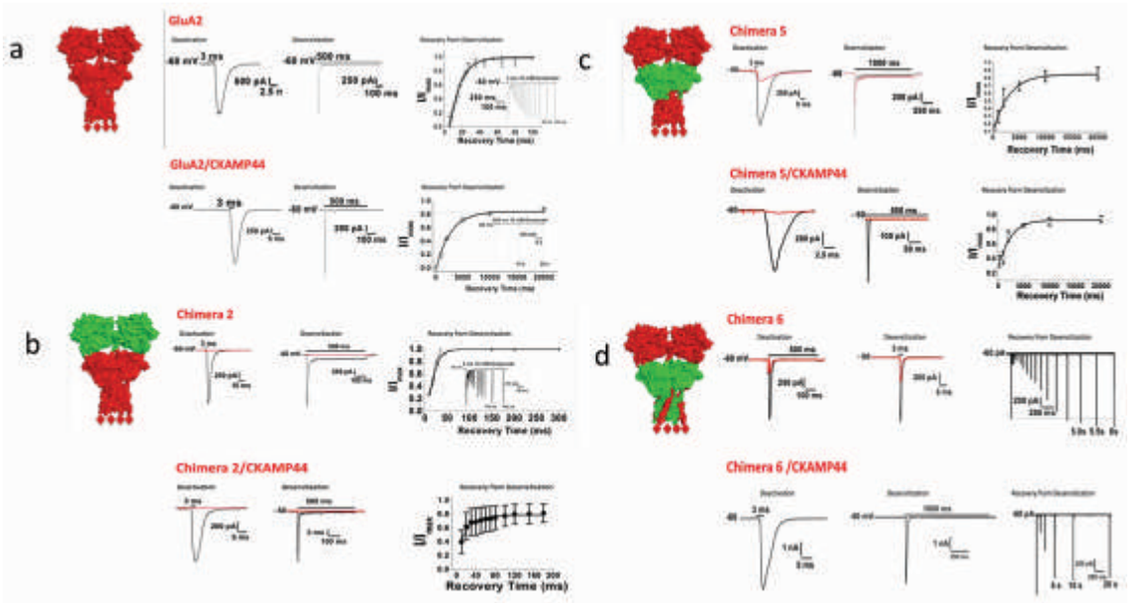

Fig.3: (a-b) Whole-cell recordings for GluA2/chimera 2 expressed with or without CKAMP44. Sample traces of current transients in whole cell patches which were recorded from HEK293 cells (WT) 42 h after transfection with 1 µg/ µl of GluA2 cDNA. 10 mM Glutamate was applied for 3 ms and 500 ms for both deactivation and desensitization functional studies. Corresponding recovery from desensitization and deactivation kinetics for the same traces is also shown. (c-d) Chimera 5/6 expressed with or without CKAMP44. Sample traces of current transients in whole cell patches which were recorded from HEK293 cells (WT) 42 h after transfection with 1 µg/ µl of each cDNA. 10 mM Glutamate was applied for 3 ms and 500 ms for both deactivation and desensitization functional studies. Corresponding recovery from desensitization and deactivation kinetics for the same traces were shown in bottom of both panels.

The electrophysiological data show that CKAMP44 slows down recovery from desensitization in native GluR2 receptors (Fig 2) which was in sync with previously reported results (13). CKAMP44 slowed down the deactivation and desensitization kinetics of Chimera 2 which has the ATD domain of GluA2 replaced with that of GluK2. The swapping of the LBD of GluA2 with that of GluK2 in chimera 5 led to an increase in rate of deactivation, desensitization and rise time. However, there was no effect on rate of recovery from desensitization. The swapping of the LBD and TM 1 and 3 of GluA2 with that of GluK2 in chimera 6 slows the desensitization rate as well as rise time (Fig 3) The electrophysiological data suggest that CKAMP44 interacts with the receptor at the amino terminal domain and the TMD. Swapping the ATD accelerates recovery from desensitization which otherwise is decelerated in GluA2. Also, swapping the LBD in chimera 5 does not bring about any such changes, however as soon the TM 1 and 3 are swapped alongwith LBD in chimera 6 the desensitization rate is slowed down. It would then be noteworthy that probably the ECD of CKAMP44 may be instrumental in bringing about the interaction with the ATD of receptor which can only be elucidated upon if we have the structure of the complex of CKAMP44 with GluA2 as well as the crystal structure of ECD of CKAMP44.

Future Research Plans

- *GluA2-CKAMP44 complex reconstitution, stabilization and structure determination via cryo-EM:* We have optimized constructs of AMPA receptor auxiliary subunits CKAMP44 (reported earlier). We intend to optimize the GluA2 (AMPA)-CKAMP44 complexe to subject them to structure determination via single particle cryo-EM.
- *Structure determination of GluK2-Neto1 complex via single particle cryo-electron microscopy:* One of the immediate goals is to solve the high resolution structure of GluK2-Neto1 complex for which we have low resolution negative stain reconstruction.
- *Functional assays via electrophysiological recordings:* We have completed testing and control experiments of our GluA2/GluK2 chimeric receptor constructs and have begun our electrophysiological recordings with native receptors and chimeras in presence of auxiliary subunits to elucidate the interaction hotspots of iGluR-auxiliary protein interactions.

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Hypoxic Environment Modulates Specific microRNAs Involved in Breast Tumor Progression and Angiogenesis

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Background

Cancer is a complicated disease that involves series of genetic and epigenetic changes in normal cells over a period of time which in association with inherited genetic susceptibilities leads to manifestation of the lethal, transformed phenotype. Research over the last decade has effectively shown the profound influence of microRNAs in various biological processes and has established them as novel regulators of gene expression. MicroRNAs are 18-25 nucleotides long non-coding RNAs which regulate gene expression at post-transcriptional level either by causing target gene specific mRNA degradation or suppressing its translation. Various studies have shown that microRNAs are differentially expressed in various human cancers and are involved in the initiation and progression of tumors in addition to their involvement in the acquisition of the various hallmarks of cancer.

Hypoxia is a fundamental patho-physiological feature of all solid tumors including that of breast and plays a pivotal role in regulation of tumor angiogenesis- a pre-requisite for tumor growth and metastasis. Various studies have established hypoxia as a vital regulator of tumor progression acting mostly via HIF (hypoxia inducible factor) family of transcription factors. These factors regulate various genes involved in angiogenesis, proliferation, cancer stem cell (CSC) selection, immune escape and metastasis. The presence of hypoxia in solid tumors hinders radiotherapy, as oxidation in aerobic conditions is required for sensitivity to irradiation (DNA damage). Besides altered pH, enhanced proliferation of hypoxic cells also hinders the distribution of anticancer drugs. Hence, many strategies have been developed to target tumor angiogenesis but the negative consequences of anti-angiogenic therapy in promoting the survival of highly resistant cancer stem cells are still not well defined. As an

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alternative strategy, vessel normalization or normalizing angiogenesis is being considered as a promising approach for cancer treatment as it has the potential of dealing with tumor resistant to drug or chemotherapy. Based on the above background information, we propose to identify and functionally characterize specific miRNAs that regulate breast tumor growth and angiogenesis in response to hypoxia.

Aims and Objectives

- To determine differentially expressed microRNAs under hypoxic conditions in breast cancer cells and validate the expression of screened microRNAs and their target genes selected based on Gene Ontology Annotation.
- To determine the role of screened microRNAs in promoting breast cancer cell-mediated angiogenesis and the mechanism underlying this process.
- To study the role of specific microRNAs in regulating breast tumor growth in pre-clinical animal model and its correlation with breast cancer progression in humans.

Work Done

Hypoxia within tumor micro-environment is a critical regulator of angiogenesis and an essential player involved in tumor metastasis. In order to determine differentially expressed miRNAs which are potentially involved in the regulation of breast cancer angiogenesis, microarray based expression profiling of MDA-MB-231 cells under hypoxic (1% O_2) as compared with normoxic conditions was performed with full coverage of human miRNAs included in miRBase release version 17 as schematically represented in Fig. 1A. The data revealed that these cells show differential expression profile of miRNAs as demonstrated by the volcano plot and heat map image of the hypoxia as compared with normoxia treated data sets (Fig. 1B & C). Out of the 587 differentially expressed miRs (p value <0.05), 334 miRs showed more than 1.4 log fold change with 205 miRs and 129 miRs being up- and down-regulated respectively under hypoxic as compared to that of normoxic conditions. Based on the differential expression profiling data, two upregulated miRNAs (viz. hsa-miR-424-5p and hsa-miR-2115-5p) and one downregulated miRNA (hsa-miR-505-3p) in microarray studies were selected for further validation by real time qPCR. The results showed that hsa-miR-424-5p is unanimously upregulated in MDA-MB-231 and MCF-7 cells respectively under hypoxic conditions as compared to normoxia (Fig. 1D, i). hsa-miR-2115-5p is upregulated under hypoxia (Fig. 1D, ii) while hsa-miR-505-3p is down-regulated under hypoxia

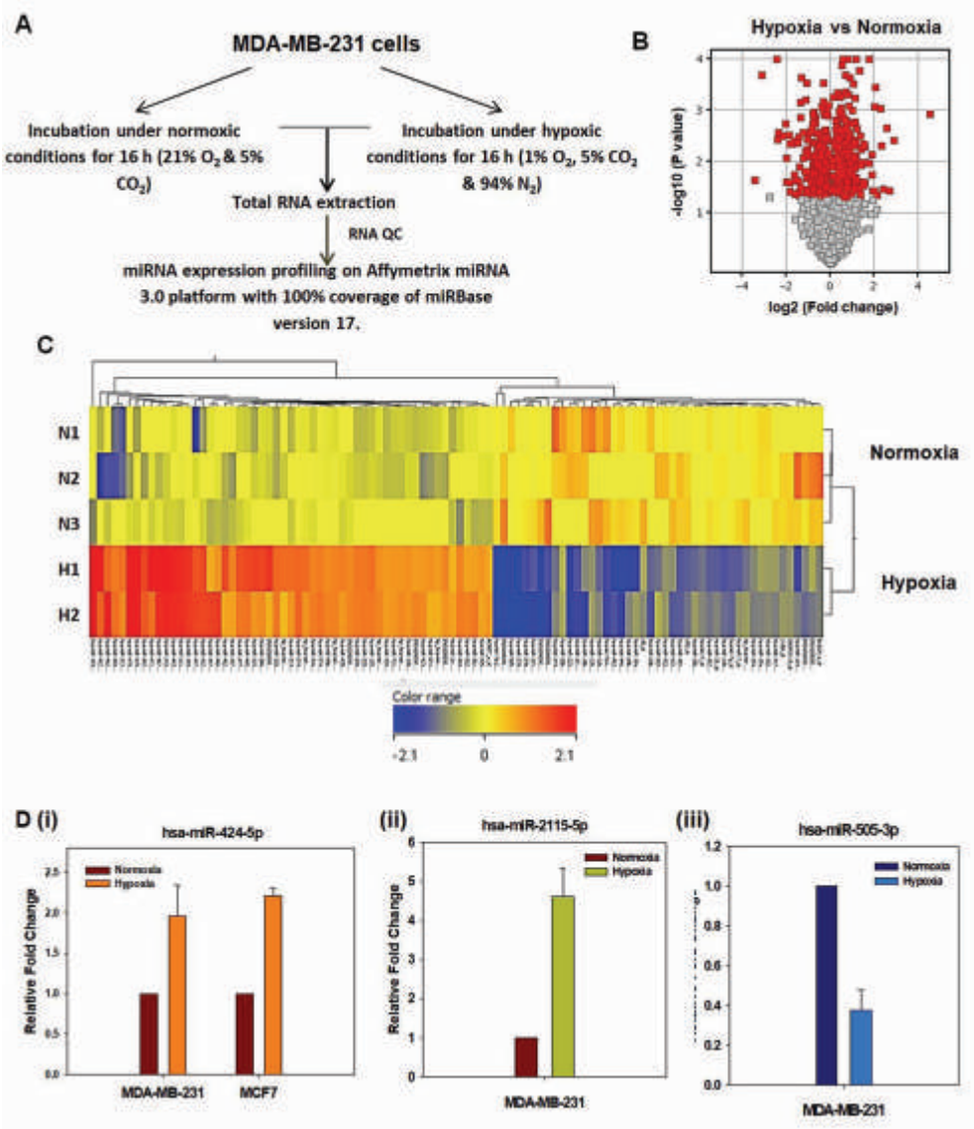

Fig.1. Differential expression profile of miRNAs under hypoxic conditions in MDA-MB-231 cells. A. Schematic representation of the experimental design for miRNA expression profiling. **B.** Volcano plot showing the negative log10 of p-value (Y-axis) vs. log (base2.0) (X-axis) of fold change representing the statistically significant miRNAs (p-value $\leq$ 0.05) and log2 (fold change $\geq$ 2.0) in red color tinted square boxes. **C.** Heat map image for combined hierarchical clustering on all samples showing most significantly differentially expressed miRNA associated with hypoxia and normoxia treated MDA-MB-231 cells. **D (i).** qPCR analysis of hsa-miR-424-5p expression in MDA-MB-231 and MCF-7 cells under hypoxia for 16h. **D (ii-iii).** qPCR analyses of hsa-miR-2115-5p and hsa-miR-505-3p expression in MDA-MB-231 cells exposed to hypoxia for 16h. Data represents relative normalized fold change. U6 snRNA was used as an endogenous control for normalization for miRNAs expression. Error bars depict standard error.

as compared with normoxia (Fig. 1D, iii) in MDA-MB-231 cells. Thus, qPCR based validation for differential expression of hsa-miR-424-5p, hsa-miR-2115-5p and hsa-miR-505-3p correlated well with our miRNA microarray data.

Many publicly available algorithms exist for the prediction of miRNA targets. While some tools like miRanda and Target Scan use the principle of sequence and seed complementarities for prediction of target genes, others like DIANA microT CDS, mirSVR are additionally based on thermodynamic considerations, statistical modeling and SVM (Support Vector Machine) based learning respectively. Thus, to reduce the likelihood of false positive and false negative target predictions plausible due to usage of a single prediction algorithm, we have used the strategy of considering only those miRNA targets for the most significantly up and down-regulated miRNAs which are commonly predicted atleast by two of the above mentioned algorithms. The targets thus predicted were further filtered based on GO (Gene Ontology) analysis to include only those genes which are involved in the regulation of tumor cell adhesion, cell motility and angiogenesis. Our *in silico* miRNA-target prediction studies revealed that there exists an

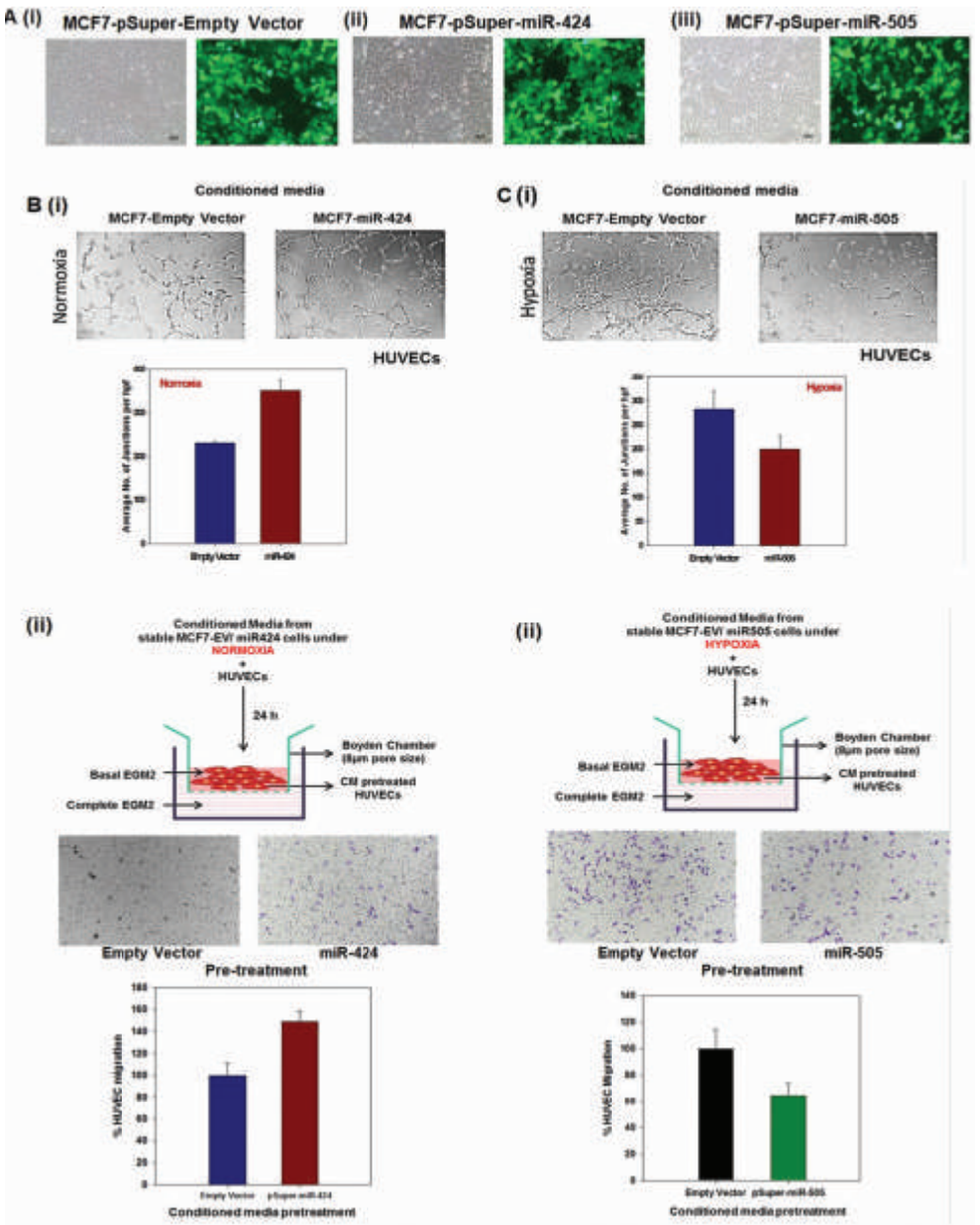

Fig. 2 miR-424 promotes while miR-505 inhibits breast cancer cell-mediated *in vitro* angiogenesis and endothelial cell migration. **A (i-iii).** Representative phase-contrast and fluorescent images of pSuper-GFP Empty Vector, pSuper-miR-424-5p-GFP and pSuper-miR-505-5p-GFP stably transfected in MCF-7 cells. **B (i-ii).** Representative images of HUVEC tube formation and cell migration in presence of conditioned media derived from normoxia treated MCF-7-pSuper-Empty vector and MCF7-pSuper-miR-424-5p cells for 24h; quantitation of the same using AngioTool v 0.5a software and Image-J and represented as fold change in no. of junctions per high power field (hpf) and % HUVEC migration respectively. Error bars represent standard error. **C (i-ii).** Representative images of HUVEC tube formation and cell migration in presence of conditioned media derived from hypoxia treated MCF-7-pSuper-Empty vector and MCF7-pSuper-miR-505-5p cells for 24h; quantitation of the same using AngioTool v 0.5a software and Image-J and representation as fold change in no. of junctions per high power field (hpf) and % HUVEC migration respectively. Error bars represent standard error.

evolutionarily conserved and thermodynamically stable interaction between the 3'UTR regions of SEMA3A and HMGB1 transcripts with miR-424-5p and miR-505-3p respectively. Respective down- and up-regulation of SEMA3A and HMGB1 under hypoxia was also observed in breast cancer cells (data not shown). These observations indicate that there might be a potential regulation of these genes through miRNAs not disregarding the epigenetic factors that might play significant role as well.

Since miR-424-5p is upregulated and miR-505-3p is downregulated under hypoxic conditions, these miRs were independently subcloned into pSuper-Neo-GFP vector and stably transfected in MCF-7 cells. The transfection efficiencies

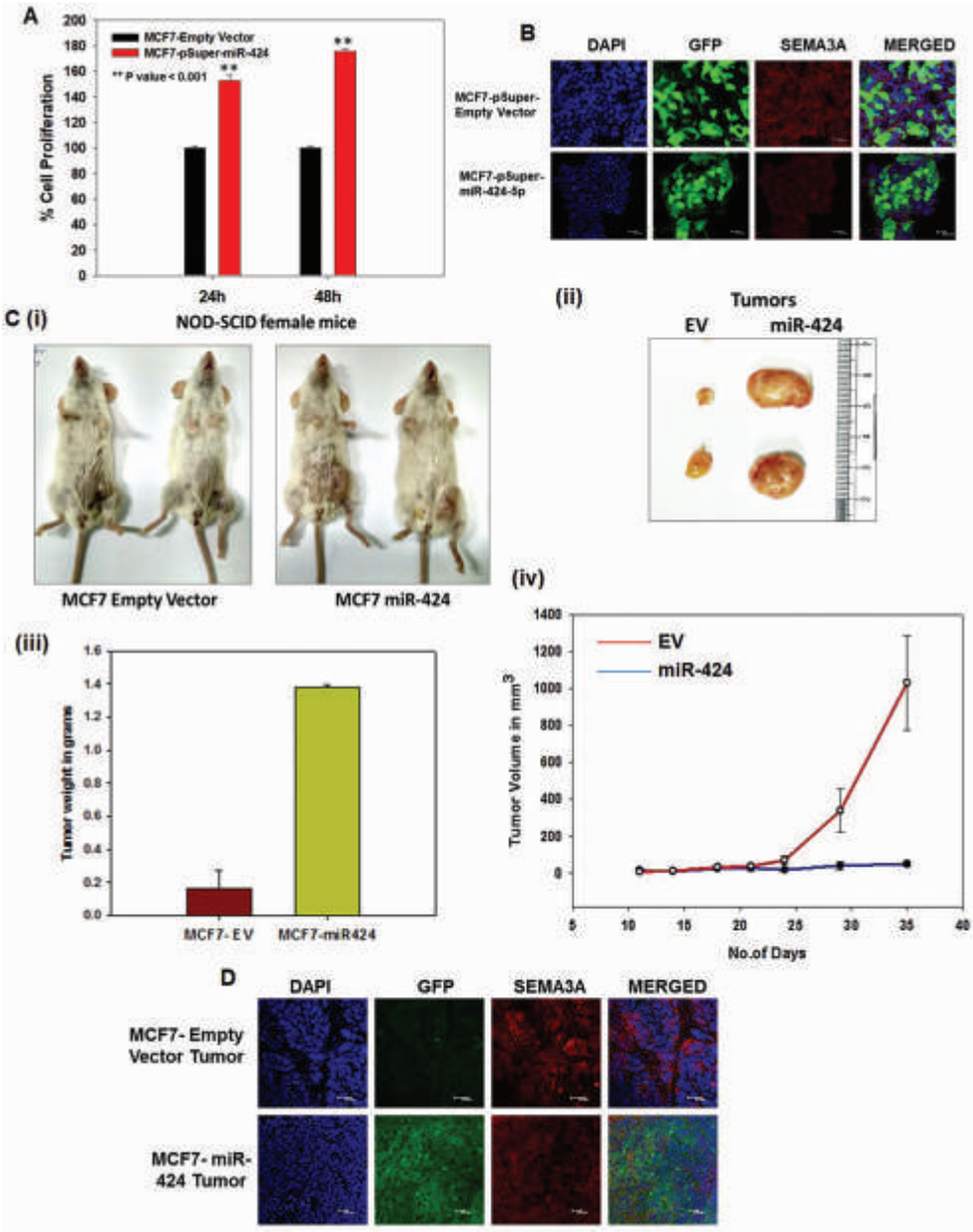

Fig.3 Ectopic expression of miR-424 enhances *in vitro* cell proliferation and *in vivo* tumorigenicity in breast cancer orthotopic xenograft NOD-SCID mice model. **A.** Relative cell proliferation of MCF-7-pSuper-Empty vector and MCF-7-pSuper-miR-424-5p cells at 24h and 48h by MTT assay. Bars represent % cell proliferation and error bars represent standard error. ** P value < 0.001. **B.** Immunofluorescence studies with MCF-7-pSuper-Empty Vector and MCF7-pSuper-miR-424 cells for assessing the expression of SEMA3A. DAPI was used as nuclear stain. **C (i-ii).** Female NOD-SCID mice (n = 4) bearing orthotopic breast tumors generated on mammary fat pad upon injection with 5 x 10^6 MCF-7-pSuper-Empty vector or MCF7-pSuper-miR-424-5p cells and respective excised tumors were shown. **(iii-iv).** Final tumor weight and tumor volume in mm^3 over the course of tumor development respectively in female NOD-SCID mice injected with indicated cells. **D.** Fluorescence based immunohistochemical analysis of SEMA3A in tumor sections derived from indicated mice tumors. DAPI was used as nuclear stain. GFP indicated stably transfected MCF7 cells in the tumor. Lines and bars represent indicated mean tumor volume and weight respectively whereas error bars represent standard error.

were shown in the form of GFP expression in these cells (Fig. 2A, i-iii). To examine the effect of miR-424 and miR-505 on *in vitro* angiogenesis, tube formation and migration assays were performed. Accordingly, HUVECs were treated with conditioned media collected from miR-424 and miR-505 expressing MCF-7 cells under normoxic and hypoxic conditions, respectively. The results revealed that over-expression of miR-424-5p significantly enhanced the tube forming capability and migration of HUVECs under normoxia as compared to empty vector harboring control MCF7 cells (Fig. 2B, i & ii). However, ectopic expression of miR-505-3p showed drastic reduction in tube formation and migration in HUVECs as compared to empty vector transfected MCF7 cells under hypoxia (Fig. 2C, i & ii). These results suggest that miR-424 and miR-505

differentially regulate breast cancer cell-mediated angiogenesis and that further corroborates with our micro-array and qPCR data.

To further examine whether miR-424 has any effect on proliferation, MCF-7 cells were stably transfected with pSuper-miR-424-5p and MTT assay was performed. The results revealed that ectopic expression of miR-424-5p enhances cell proliferation as compared to mock tranfected MCF7 cells (Fig. 3A). To further confirm the *in vitro* data, miR-424 overexpressing MCF-7 cells were injected orthotopically to NOD-SCID mice and tumorigenicity studies were performed. The results indicated that over-expression of miR-424-5p in MCF-7 cells enhances their tumorigenic potential as compared to mock transfected cells (Fig. 3C, i-iv). Immunofluorescence studies revealed that overexpression of miR-424 in MCF-7 cells attenuates SEMA3A expression, an important anti-angiogenic and tumor suppressor protein, under *in vitro* as well as *in vivo* models (Fig. 3B and D). These results demonstrate a positive correlation between miR-424 expression and breast tumor progression.

Future Research Plans

- To delineate the mechanism by which miR-424 and miR-505 regulate breast cancer angiogenesis.
- To study the transcriptional regulation of miR-424 and miR-505 by promoter analyses.
- To corelate the *in vitro* and *in vivo* findings with human breast cancer clinical specimen analyses.

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Cellular and Molecular Mechanisms of Chemokine Receptor Signaling During Inflammation and Tolerance

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Background

Inflammation is the complex set of reactions involves a set of cytokines, chemokines and adhesion molecules. Joint ventures of pro- and anti-inflammatory functions are initiated together by infiltrating leukocytes, tissue fibroblasts, epithelial and endothelial cells. Chemokine receptors and cell adhesion molecules present on the cell surface are known to 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 characterized.

Most of the chemokines and some of the adhesion molecules are G-protein coupled receptors (GPCRs). G-proteins are heterotrimer consist of α-, β-, and γ-subunits and transduce signals from surface receptors to intracellular effectors. Upon receptor activation, G-protein complex 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, Ca^{2+}, 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, cell activation, differentiation, and functions.

CCR6 is a GPCR, expresses on various immune cells and interacts with its specific chemokine CCL20. CCR6 play an important role in various diseases such as experimental autoimmune encephalitis (EAE), inflammatory bowel disease, psoriasis, chronic hepatitis, rheumatoid arthritis, chronic pulmonary

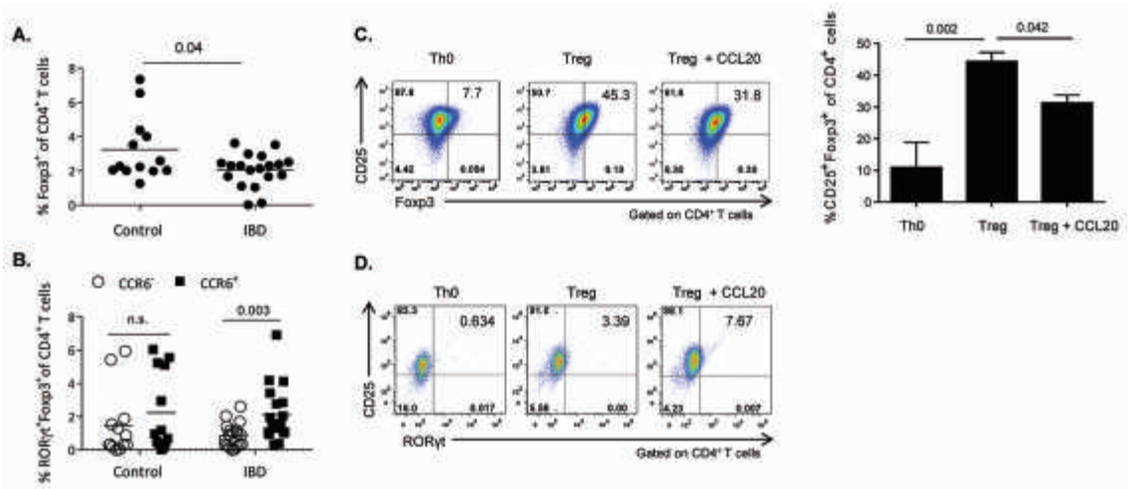

Fig. 1: CCL20 inhibits human iTreg differentiation. (A-B) The PBMCs from ulcerative colitis (UC) patients and healthy individuals were isolated and stained for indicated molecules. Each dot represents data from each individual and analyzed using flow cytometry. (A) Mean percentage of Foxp3$^+$ cells in the PBMCs was analyzed after gating on CD4$^+$ T cells and shown. (B) Mean percentage of RORγt$^+$Foxp3$^+$ cells was analyzed after gating on CD4$^+$CCR6$^-$ and CD4$^+$CCR6$^+$ cells. (C) CD4$^+$CD8$^-$CD25$^-$CD45RA$^+$ (naïve CD4$^+$ T cells) cells were purified from healthy individuals PBMCs using flow cytometry sorting. CD4$^+$CD8$^-$CD25$^-$CD45RA$^+$ (5 x l0^4 cells/well) cells were cultured with irradiated CD4$^-$CD8$^-$ PBMCs (5 x l0^4 cells/well) in Th0, Treg or Treg with CCL20 conditions for 4 days. After 4 days, cells were stained for indicated molecules and analyzed using flow cytometry. The percentage of CD25$^+$Foxp3$^+$ cells was analyzed after gating on CD4$^+$ T cells (left). The statistical analysis of 3 independent experiments is shown as bar graph (right). The error bar represents ± SEM. (D) Percentages of CD25$^+$RORγt$^+$ cells were analyzed after gating on CD4$^+$ T cells. Student t-test (A-C). Each symbol represents data from the individual human (A, B).

sarcoidosis, cancer metastasis and graft-versus-host disease. In autoimmune 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 was found to attenuate trinitrobenzene sulfonate (TNBs)-induced colitis. Most of these studies were focused on the migration function of CCR6 in the 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 does chemokine activation together with co-stimulatory molecules affect the differentiation and function of CD4 T cells.
- How does chemokine receptor signaling perturbs the epigenetic marker in regulatory elements of the genes.

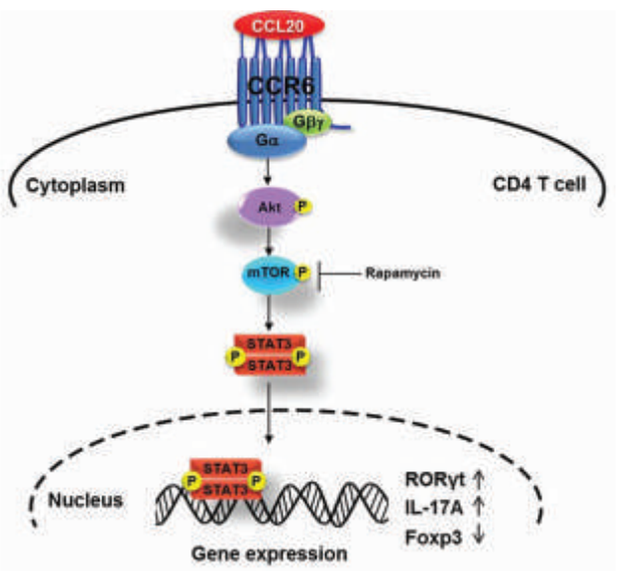

Fig. 2: Schematic representation of CCL20-CCR6 signaling in CD4 T cells.

Work Done

Recently, we showed that CCL20-CCR6 signaling that higher concentration of CCL20 in the gut during inflammation prevents the differentiation and function of induced regulatory Foxp3$^+$ CD4 T cells and drive the differentiation into inflammatory Th17 cells (Figure 1). We further showed that CCR6$^+$ Th17 cells had

higher expression ROR γ t and T-bet expression, and produced IL-17A and IFN- γ in a CCR6-dependent manner. Further, the polarization of naïve $CD4^+$ T cells into Th17 in presence of CCL20 promotes the differentiation of Th1-like Th17 cells that expressed higher IFN- γ, IL-21, IL-22, IL-23, IL-23R, aryl hydrocarbon receptor (Ahr) and GM-CSF molecules. This effect of CCL20 was due to CCR6-mediated phosphorylation of Akt, mTOR and STAT3 molecules in T cells (Figure 2). The deficiency of CCR6 in CD4 T cells prevented the differentiation of CCL20-induced pathogenic Th17 and reduced the severity of the disease. We further showed that ulcerative colitis patients had a significantly higher frequency of ROR γt^+T-bet^+ Th17 cells in the peripheral blood as compared to healthy individuals. Together, these results suggest that CCR6 signaling during gut inflammation and autoimmunity promotes the differentiation inflammatory Th17 cells, and suggests that intervention of CCR6-CCL20 signaling could potentially provide a therapeutic benefit to IBD patients.

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.

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Metabolic Status and Neural Fate Choice

Background

Neurogenesis in developing embryo as well as in adult is regulated by a variety of intrinsic and extrinsic cues, including energy intake and nutrient availability. A number of reports do suggest the critical influence of cellular metabolic status in defining the functional properties of NSCs and neurons. Imbalance in mitochondrial oxidative phosphorylation (OXPHOS) and oxidative stress do lead to a wide range of neurodegenerative disorders including that of Parkinsonism where DA neurons undergo progressive degeneration. The generation of reactive oxygen species (ROS) within mitochondria is closely associated with oxidative metabolism and ATP synthesis. Either over-production of ROS or altered antioxidant defence system leads to generation of oxidative stress culminating in disturbed cellular homeostasis. NSCs in adult maintain a high level of ROS compared to other stem cells and are highly responsive to ROS stimulation. ROS also acts as secondary messenger, which signals directly to proteins via amino acid oxidative modifications thereby causing functional changes in different proteins. In fact, neurons rely on OXPHOS to meet energy demands, while neural progenitor cells use a process called aerobic glycolysis. This switch in metabolic pathways is essential for neuronal differentiation, as neurons that fail to switch to oxidative phosphorylation (OXPHOS) die *in vitro*. Interestingly, there exists a functional link between signalling pathways such as canonical Wnt/β-catenin, Notch, ROS and cellular metabolic status. A detailed understanding on their regulation of metabolic circuitry in neurogenic lineage could support the development of novel strategies to modulate OXPHOS mediated metabolic status in NSCs to promote neurogenesis and prevent/revert OXPHOS deficit associated neurodegeneration and even cancer. Accordingly, we were interested in studying whether by altering metabolic status in cells we could modulate the differentiation status of ESCs and CSCs.

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Aims and Objectives

- Elucidation of metabolic changes associated with neurogenesis and its modulation to prevent/reverse neurodegeneration and cancer, and influence of Wnt signalling.

Work Done

Our earlier study has delineated the time window for neural progenitor generation and subsequent differentiation from embryonic stem cells (ESCs). We have also demonstrated the plausible role of Wnt as instructive signalling cue underlying neural maturation. Considering the critical influence of cellular redox state on various cellular processes ranging growth and differentiation, we were interested in investigating the correlation between ESCs metabolic status and their maintenance/differentiation. Using Th-EGFP stable ESC clone we could profile the ROS level that showed stage specific alteration. While ESCs during maintenance exhibited very high ROS level, it showed decline during neural progenitor specification. Further during neural differentiation and maturation, the level fluctuated either with its level retention similar to that of neural progenitors or showing an upward trend. This suggested the requirement for a tight balance and coordination between the redox state and ESCs maintenance as well as neural cell fate specification (Fig. 1). Taking clue from our earlier study on cell density dependent paracrine influence of Wnt during this developmental process, we activated canonical Wnt signalling by using BIO, the inhibitor of GSK3β. Our data has suggested a temporal influence of Wnt during neurogenic proceedings and DA neuronal differentiation. We are further looking into the involvement of Wnt signalling and its influence on metabolic status alteration, if any, and modulation of cell fate.

Since undifferentiated ESCs share many signatures with that of cancer cells and also Wnt is known to be hyperactive in most of the cancers including glioblastoma, it was imperative to determine the correlation of redox status and Wnt signalling in both, and compare how ESCs being precisely regulated differ from the deregulated GBM cells and also their response to oxidative stress and antioxidants. Moreover, phytochemicals are known to have antioxidant properties that exert anti-inflammatory and anti-cancerous effects through modulation of multiple signalling pathways and intracellular targets. Hence, we were interested in studying in parallel whether by altering metabolic status in cells we could modulate the differentiation status of ESCs and CSCs, and the influence of phytochemicals during the same. The ongoing studies using GBM cells (U87)

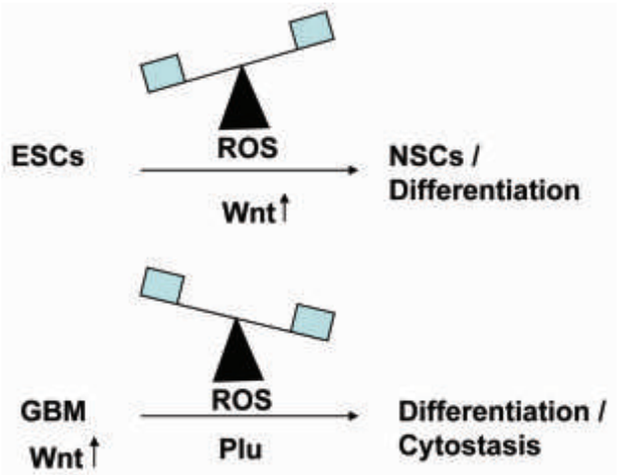

Fig. 1: Schematic prediction of redox state and its influence on ESCs maintenance and differentiation (Upper Panel) and also Plu altering the redox state in GBM cells (Lower panel).

revealed that, the treatment with Plu, the phytochemical, can lead to increase in the levels of $O2^{-}$, H2O2 and $ONOO^{-}$, and mitochondrial depolarization, and hence suggesting the stated phytochemical to have induced apoptosis in GBM cells via ROS and RNS generation and mitochondrial death pathways. The apoptotic effects of Plu seemed to involve the intrinsic pathway, as confirmed by the loss of mitochondrial membrane potential ($\Delta\psi m$) together with activation of caspase-3 and decrease in GSH. Hence, there was an induction of classical apoptotic pathway through targeting of mitochondria, which is usually deregulated in malignant cells. Plu was also found to interact directly with tubulin, induce arrest of G2/M check point of cell cycle and apoptosis in tumour cells. Importantly, there was a direct correlation of Plu treatment with reduced CSCs and NSCs population in U87 cells, as revealed by quantifying the $ALDH^{+}$-, $CD44^{+}$- and $Nestin^{+}$ cells respectively. It also induced glial differentiation as observed by increase in $GFAP^{+}$ cells following Plu treatment. Further studies will be undertaken to delineate the underlying mechanistic basis and the associated molecular targets. Collectively our findings could shed light on the critical influence of redox status on cellular homoeostasis in affecting neural cell physiology, growth and differentiation (Fig. 1) and thus paving way for its further exploration in reversing neurodegeneration and cancer.

Future Research Plans

- We would further like to delineate the underlying mechanism of action and molecular targets modulating cellular metabolic state and the differentiation potential in case of both ESCs and CSCs.

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Studies on Expansion, Cryopreservation and Differentiation of Hematopoietic, Mesenchymal and Induced Pluripotent Stem Cells Isolated from Umbilical Cord Tissues.

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Background

Out of the 3 different ongoing projects from the lab., the project on Dendritic cells is described in detail below:

Hematological malignancies are major cause of morbidity and mortality in young as well as old age. All standard treatments such as radiation, chemotherapy and allogeneic hematopoietic stem cell transplantation have their own side effects and fail to achieve and maintain remissions in many patients. Use of cancer immunotherapy in haematological malignancies has potential to completely eliminate the cancer with the help of active TAA specific CTLs. It has been proven that, DC based cellular vaccines are safe. They could generate effective antitumor immune response. Though clinical trial data is very encouraging, efficacy of the DC vaccine still needs improvements.

Multiple myeloma (MM) is a type of haematological malignancy associated with B cells or plasma cells. Though many alternatives are available for the treatment of MM, eventually it relapses, in majority of cases. Dendritic cell-based vaccine holds promise for such types of cancers. We hypothesized that DCs generated by our method of expansion and then differentiation will give rise to homogenous and immunogenic population from patients' samples.

In this study, apheresis samples from healthy donors (HD) as well as patients suffering from multiple myeloma (MM) were used for DC generation. Initially DCs were generated using direct differentiation of monocytes (Mo-DCs) obtained from these samples. Mo-DCs from MM patients (MM-Mo-DCs) were compared for their morphology, phenotype and functionality with Mo-DCs from healthy donors (HD-Mo-DCs). MM-Mo-DCs were found to be compromised in nature. We next generated DCs from the two sources of

samples by using our lab developed two step method (referred to as HD-SC-DCs and MM-SC-DCs). Their comparison showed that they were equivalent in nature.

Aims and Objectives

- Comparison of DCs generated from monocytes of healthy apheresis samples with MM samples.
- Comparison of DCs generated from stem cells of healthy apheresis samples with MM samples.

Work Done

We report that when DCs were generated from monocytes of MM samples they were found to be equivalent to DCs generated from monocytes of Healthy donors in terms of morphology (Fig.1a) and phenotype (Fig.1b) but were compromised in their in vitro (Fig. 1c) and in vivo migration ability (Fig.1d). This defect was attributed mainly to higher secretion of IL6 by monocytes of MM DCs as detected by both qualitative and quantitative PCR (Fig1e). Next we generated DCs from the stem cells of these two samples using our lab derived two step method. It was found that these DCs were equivalent in terms of cell yield (Fig.2a) antigen uptake (Fig.2b) migration (Fig.2c) and T cell proliferation ability (Fig. 2 d).

In order to test whether CTLs generated from MM -SC-DCs elicited similar killing activity, in vitro CTL assay was performed using U266 multiple myeloma cell line. K562 cell line was used as a negative control. Briefly Cell lines were loaded with Calcein –AM dye and cultured with or without CTLs. The killing was monitored by reading the fluorescence in culture supernatants by fluorimeter. Surprisingly the CTLs from MM-SC-DCs were less efficient in killing as compared to HD –SC-DCs (Fig3a). We

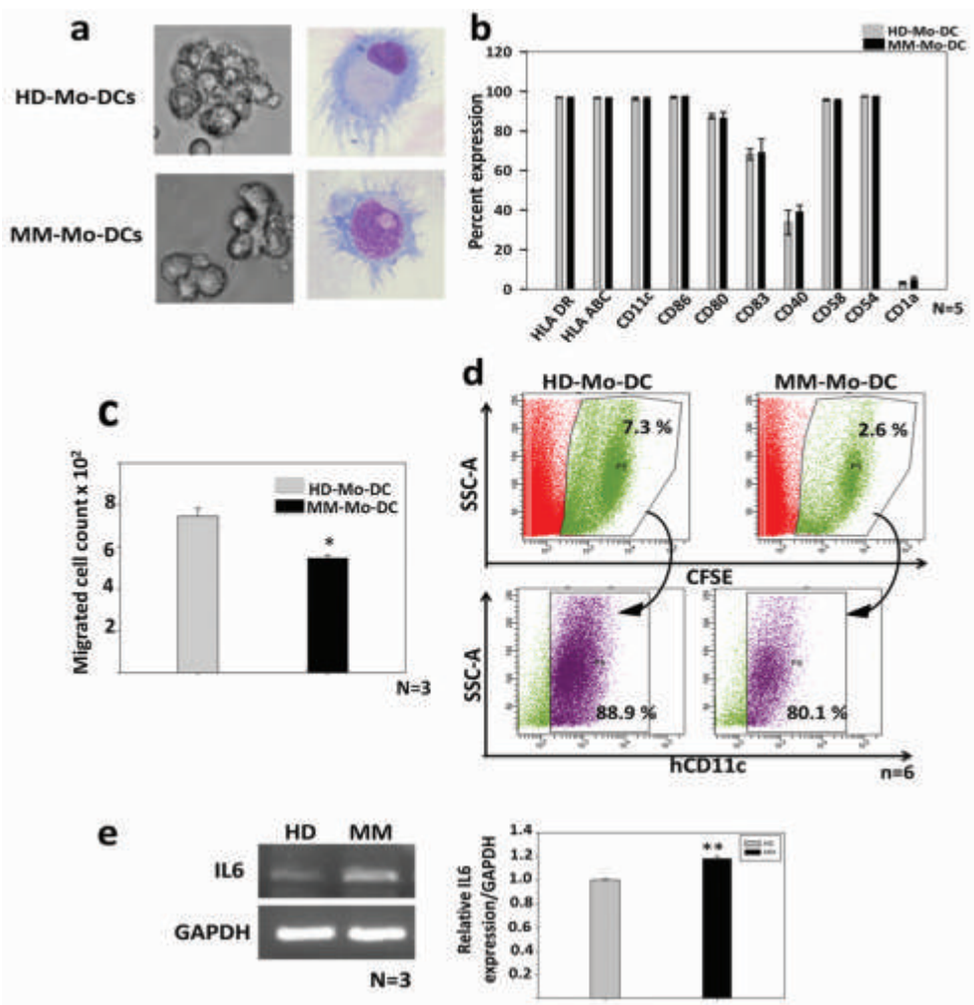

Fig. 1: Monocyte derived DCs from MM samples are compromised in function:
(a) Morphology of HD-Mo-DCs and MM-Mo-DCs (b) DC marker expression was equivalent. in both the types of DCs(c) In vitro and (d) in vivo migration of MM-Mo-DCs was significantly lower as compared to HD-Mo-DCs. (e) Expression of IL-6 on monocytes of MM samples was higher than on HD monocytes.

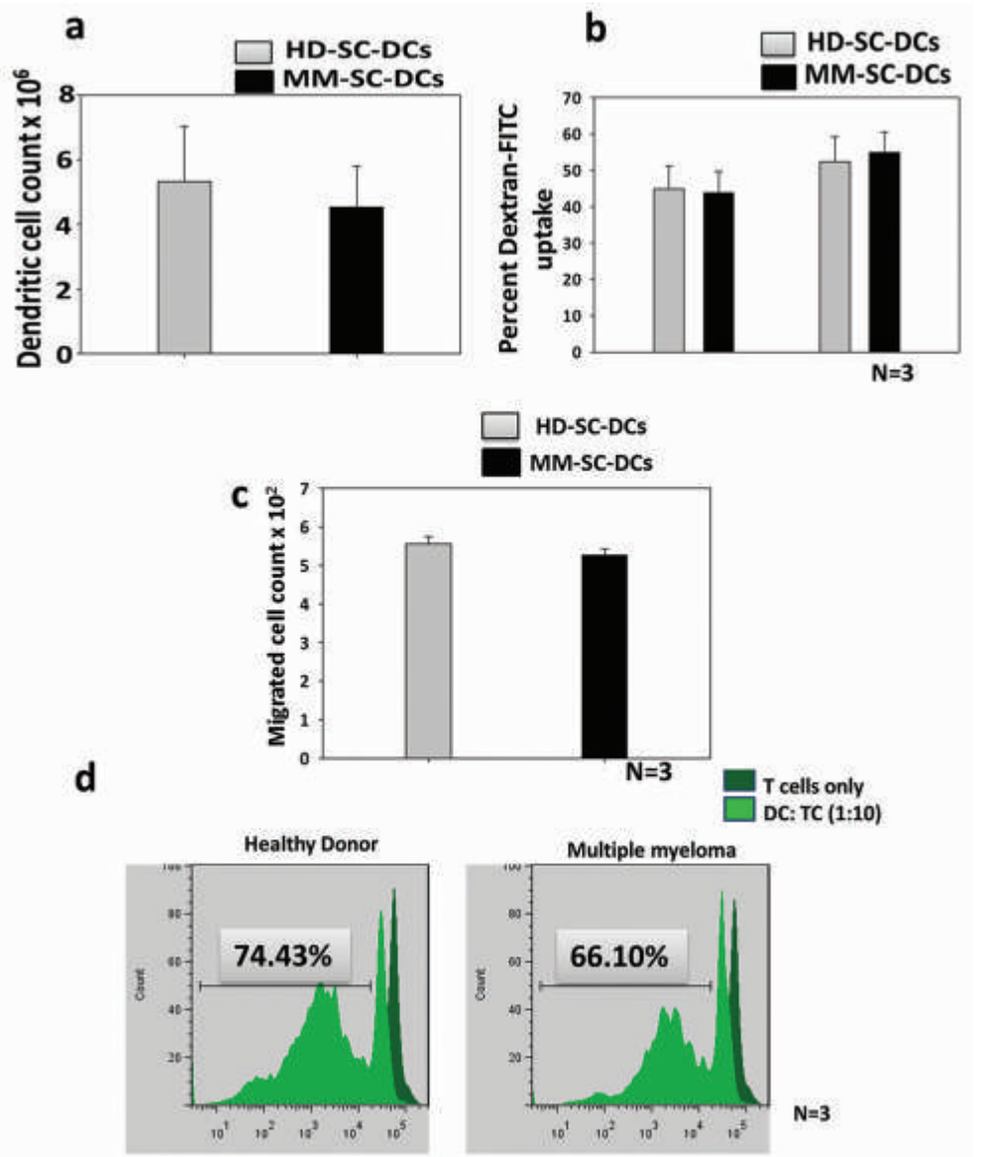

Fig. 2: DCs from MM samples which are generated by using two step method were functionally equivalent to healthy sample DCs: (a) SC-DC yield from HD and MM samples (b) Antigen uptake (c) Migration toward CCL-19 and (d) allo-T cell stimulation of HD-SC-DCs and MM-SC-DCs was similar.

then checked the proliferation of autologous and allogeneic T cells with DCs from MM. As seen in Fig. 3b the autologous T cells showed reduced proliferation by Ki67 staining indicating that perhaps the reduced CTL activity in MM DC set was not due to defective DCs but due to exhaustion of autologous T cells.

Future Research Plans

- We plan to compare in vivo CTL activity of SC-DC s from MM and healthy samples.

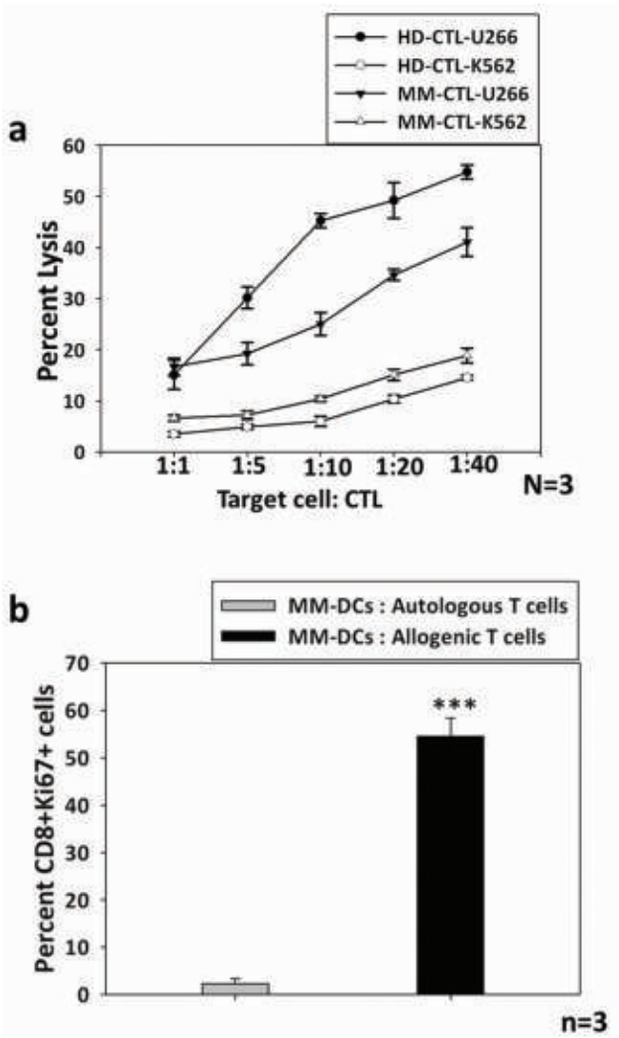

Fig. 3: CTLs generated from MM had low killing activity against multiple myeloma cell line: a) Killing of U266 cell line in vitro by CTLs from HD-SC-DCs and MM-SC-DCs with autologous T cells. (b) Ki-67 expression goes down on autologous naïve T cells as compared to allogeneic naïve T cells co cultured with MM-SC-DCs.

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Towards Deciphering the Mechanism of Huntington's Disease

Background

Huntington's disease (HD) is a severe neurodegenerative disorder caused due to expansion of poly Q repeats in the exon 1 of Huntingtin (Htt) gene. Associated phenotypes range from chorea, problems in movement, cognition and behavior. HD is thought to be a protein misfolding associated disease, as the expansion of polyQ repeats cause the Htt protein to misfold, aggregate and be toxic. The toxicity is associated with Htt aggregates affecting several cellular pathways like transcription, axonal transport, mitochondrial energy generation, autophagy, protein degradation, etc. Till date no treatment for HD is known. Using a Drosophila model for HD we observed cellular translation to be defective in cases of pathogenic Htt expression. We found pathogenic Htt can misregulate a translation factor by physical sequestration and thereby changing its dynamic nature to a non-dynamic one. On overexpressing this translation factor we were able to rescue the toxicity and behavioral defects associated with pathogenic Htt. Our data suggest that the rescue is not due to a decrease in Htt aggregation but through a change in the translatory status of the cell. We are further addressing if by using small molecule regulators of translation, the Htt associated phenotypes can be rescued.

Participants

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Structural and Functional Studies on Mycobacterial Proteins

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Mohd. Yousuf Ansari, *SRF*
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Background

Our laboratory has been interested in structure-function properties of Mycobacterial proteins. We combine different biophysical, biochemical, structural and computational approaches in attempting to understand the functions of these chosen proteins. Our principal interests are in two major families of proteins: heat shock proteins (chaperones), and those involved in electron transfer reactions. In the latter, we have worked on various proteins involved in maintaining intracellular redox balance over the years. Recently our attention has been on an important enzyme- ribonucleotide reductase, which uses free radical chemistry, and undergoes many cycles of oxidation and reduction during catalysis. In the period under review, we have made substantial progress in this work, which is reported below.

Ribonucleotide Reducatases (RNRs) catalyze de novo biosynthesis of deoxyribonucleotides by reduction of corresponding ribonucleotides. The catalytic reaction of all RNRs represents a classic example of biomolecules employing free radical chemistry, where the organic free radicals are maintained in stable form within the enzyme and used as and when required. While the synthesis of ribonucleotides is achieved by simple building blocks such as amino acids, tetrahydrofolates, NH_4^+, CO_2 and Phosphoribosyl-1-pyrophosphate, the synthesis of deoxyribonucleotides can only be carried out by the RNRs through a complex catalytic process. Thus, it has been hypothesized that the ancestral forms of RNRs must have been responsible for the synthesis of DNA precursors during transition from the RNA to the DNA world.

The catalytic mechanism of RNR's starts with the abstraction of a hydrogen from the 3' position of the ribose moiety by a transient thiyl radical, leading to

formation of substrate radical. Radical formation makes the 2' OH group more susceptible to acid attack by one of the cysteine residues of redox-active cysteine pair, facilitating the removal of the protonated OH group in the form of a water molecule and 2' ketyl radical is formed. The two redox-active cysteines get oxidized to a disulfide anion radical upon protonation of the ketyl radical at 2' position. Finally, the abstracted hydrogen atom is returned to the 3' position by the attacking cysteine residue, that becomes thiyl radical. Thus, the entire catalytic process of RNRs can be sub-categorized into two components; viz, radical generation and substrate reduction that occur in different protein sub-units. While the reduction of the substrate is essentially the same for all RNRs, the radical generator component is different in different RNRs and form the basis of their classification. In addition, requirement of oxygen also serves as a basis for RNR classification. Class I RNRs have a stable Tyrosyl radical that oxidizes the substrate to a radical form. The stable tyrosyl radical is formed in a different subunit through the metal cofactor, i.e. dinuclear iron centre. Since, oxygen is one of the components for the tyrosyl radical generation, these enzymes require aerobic conditions. Class I RNRs have been further grouped as Ia and Ib. While class 1a enzymes are prevalent in eukaryotes, prokaryotes, bacteriophages, and viruses, occurrence of class Ib is restricted only to prokaryotes. The subunit composition of both these classes involves non-identical subunits R1 (α) and R2 (β) forming a α2β2 tetramer. The substrate-binding catalytic site is located in the large R1 subunit, while the smaller R2 subunit harbors the metallo-cofactor Fe-O-Fe (or recently reported Mn-O-Mn) per monomer that forms a stable tyrosyl radical within the same subunit.

The structures of R1 from both these sub-classes reveal a highly similar catalytic site as well as the substrate-specificity site. However, class Ib enzyme lacks a separate N-terminal ATP cone, otherwise present in the Ia enzyme where the nucleotide binding (ATP or dATP) decides the overall activity of the enzyme. The regulation of activity of RNRs happens in two ways. The first one involves the overall activity of the enzyme, that regulates the size of the nucleotide pool. The N-terminal region of the larger subunit harbors ATP-binding domain that is responsible for the switch-on/off of the enzyme activity. Thus, when ATP is bound to this site, the enzyme becomes functional, while, an increase in concentration of dATP leads to the inactivity of the enzyme. The second level of regulation happens at the specificity site and maintains the level of different deoxynucleotides in the cell. Thus, binding of a particular effector at the allosteric site allows only a particular nucleotide at the substrate binding site. Hence, dATP leads to reduction of CDP and UDP; TTP (formed from dUDP) leads to reduction of GDP, while, dGTP binding leads to reduction of ADP at the substrate-binding site.

Mycobacterium tuberculosis possesses only the Class 1b Ribonucleotide reductase that has been shown to be essential for its survival. Class 1b RNR are composed of two homodimeric subunits: α subunit encoded by nrdE gene that has the catalytic site for substrate reduction, allosteric site for regulation and redox active thiols/disulphide required for catalysis, and β subunit encoded by *nrdF* gene, which has oxygen-linked diferric iron centre and tyrosyl radical. The oxidized NrdE formed at the end of one catalytic cycle is reduced by a thioredoxin-like protein; NrdH. NrdH itself is maintained in the reduced form by thioredoxin reductase (TrxR). NrdI, a flavodoxin like protein is reported to form oxidants by oxygen reduction for radical generation in NrdF *(Boal et al., 2010)*. Apart from these, the cluster of genes that codes for RNR also has an oxidoreductase Rv3049c, the role of which is yet unknown. Our goal is to understand the protein-protein interaction among different subunits and gain structural insights in the reaction mechanism of RNR's in *Mycobacterial species*. To understand this reduction process, genes associated in mycobacterial species (NrdE, NrdF, NrdI, and Rv3049c) will be cloned, purified and structurally characterized. We are particularly interested in the following questions:

- Given the fact that Class Ib RNRs lack the N-terminal switch-on/off domain, are there alternate pathways that decide their overall activityΔIf so, what are theyΔ
- What is their structural organization of Class Ib RNR as a biologically-active tetramerΔ
- What is the mechanism of long-range Proton-coupled electron transfer that takes place from the smaller subunit to the active-site in the larger subunit, covering >35 Å distanceΔ
- What is the mechanism of allosteric regulation in presence of different allosteric effectors/ substratesΔ

Aims and Objectives

- Cloning, purification, biophysical, structural and functional characterisation of purified NrdE, NrdF and NrdI from *M. tuberculosis* and related species.

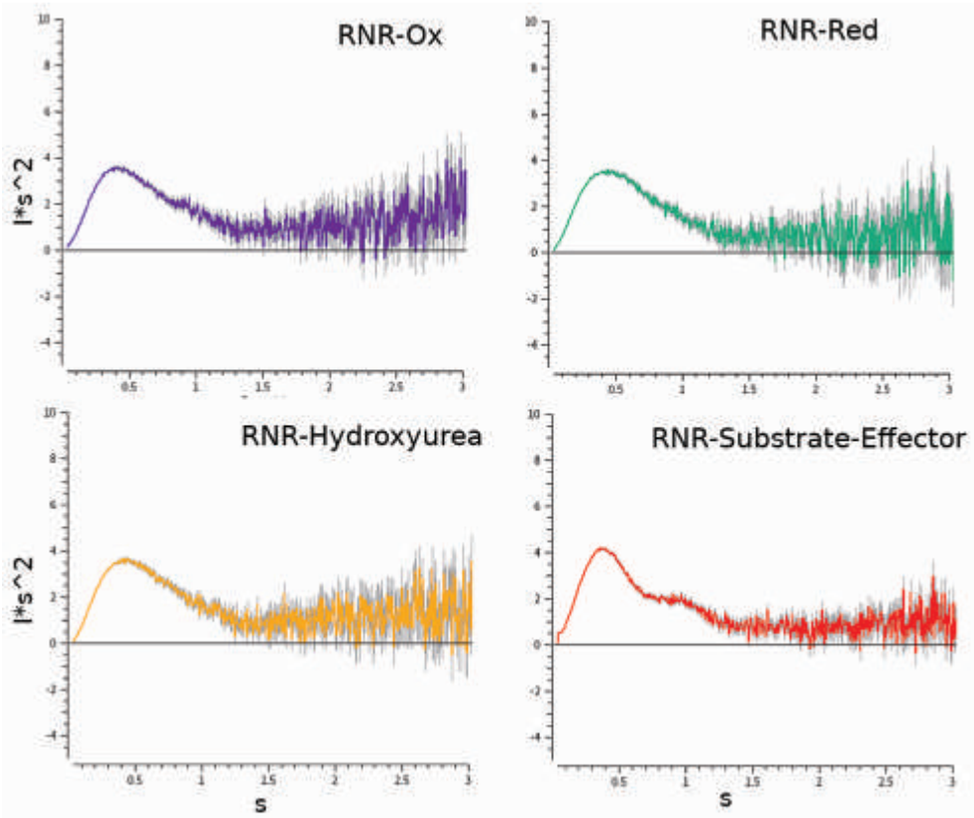

Fig.1: Kratky plots for RNR under various conditions. RNR-Ox and RNR-Red represent the air oxidized and reduced RNR complexes. It can be seen that the RNR complex with substrate effector has a different profile than the other three profiles.

- Obtain high resolution crystal structures of biologically active R1E-R2F complex from *M. tuberculosis* and other related species harboring class Ib enzyme.
- Decipher the regions of structural differences in the oxidized and reduced R1E subunit, that is primarily concerned with the catalytic activity.
- Biochemical and structural investigation of the biological assembly in complex with different effectors in order to understand the overall activity regulation of Class Ib RNR in absence of dATP/ATP binding allosteric site (N-term ATP cone).
- Structural study of NrdF-NrdI and NrdE-NrdF-NrdI complexes in active state to understand the radical transfer mechanism

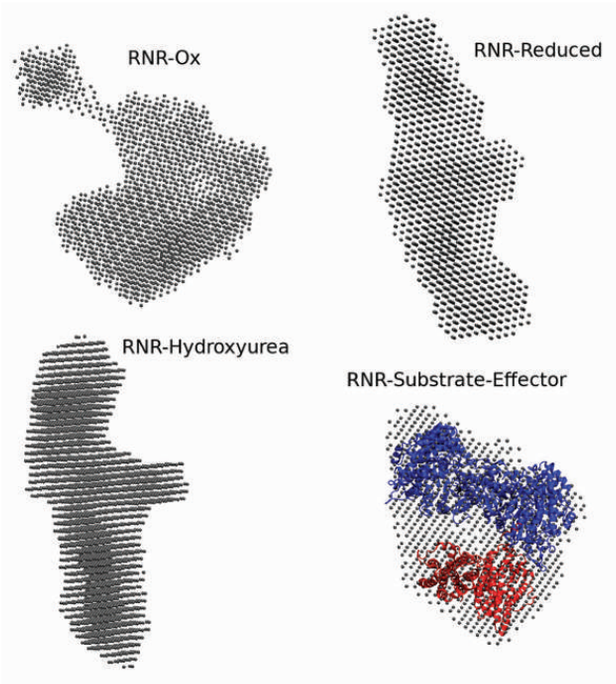

Fig. 2: *Ab initio* reconstructed models of RNR from *M. thermoresistibile* under different conditions. The last panel shows SAXS-based model superposed over low resolution crystal structure of RNR from *S. typhimurium* (PDB ID 2BQ1).

Work Done

The components of RNR holocomplex copurify as a stable complex

During the period under review, we have been able to successfully clone, express and purify both the radical harboring small subunit and catalytic large subunit from *M.*

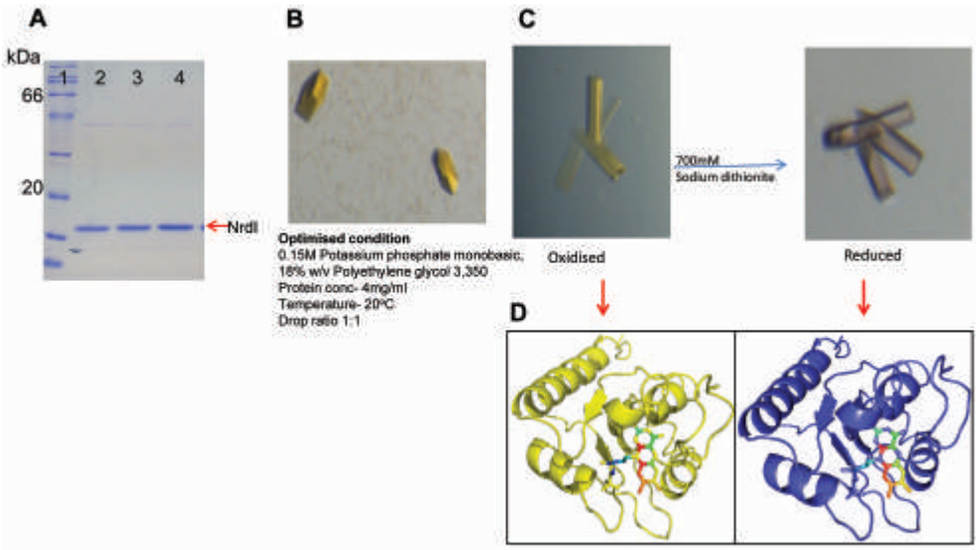

Fig. 3: A. Purified protein fractions (indicated by red arrow) loaded on 15% SDS-gel. B. Crystals of *M. thermoresistible* NrdI. C. Crystals of *M. thermoresistible* NrdI in oxidized and reduced form, D. Structures of *M. thermoresistible* NrdI in oxidized and reduced form.

thermoresistibile as a stable complex. Moreover, we also cloned, expressed and purified NrdI and Rv3049c proteins. Multi-angle Light Scattering coupled to Size Exclusion Chromatography (SEC-MALS) results confirm the size of purified NrdE:NrdF complex to be of ~210-220 kDa, that corresponds to the biologically-active α2β2 tetramer (Table 1).

X-ray solution studies of RNR holocomplex by Small Angle X-ray Scattering (SAXS)

For SAXS analysis, RNR NrdE:NrdF complex was subjected to 4 different conditions, namely, (1) Air-oxidation, (2) reduction with TCEP, (3) Radical scavenged with Hydroxyurea and (4) substrate-effector bound state (ADP-dGTP). The double log plots of Intensity (I) versus scattering vector (s) under different conditions and for different concentrations of protein indicate slight inter-particle interactions. Kratky plots of the same point towards unstructured regions, that may either be due to protein unfolding as a result of high intensity x-rays or mobile regions within the protein structure (Fig. 1). Rg values were found to be significantly different for Oxidized and Substrate-effector bound RNR as compared to reduced and radical scavenged states (Table 2). These observations are encouraging and need to be probed further. Using SAXS data as reference, *ab initio* models were reconstructed and compared with a low resolution crystal structure of RNR from *S. typhimurium* (PDB id 2BQ1). While models from the reduced and hydroxyurea treated states differed drastically from the crystal structure, a partial similarity was observed for the oxidized state. In presence of substrate and effector however, RNR was observed to assume a more compact shape that closely resembles the crystal structure (Fig. 2). Thus, preliminary data suggest large scale shape changes under different conditions observed.

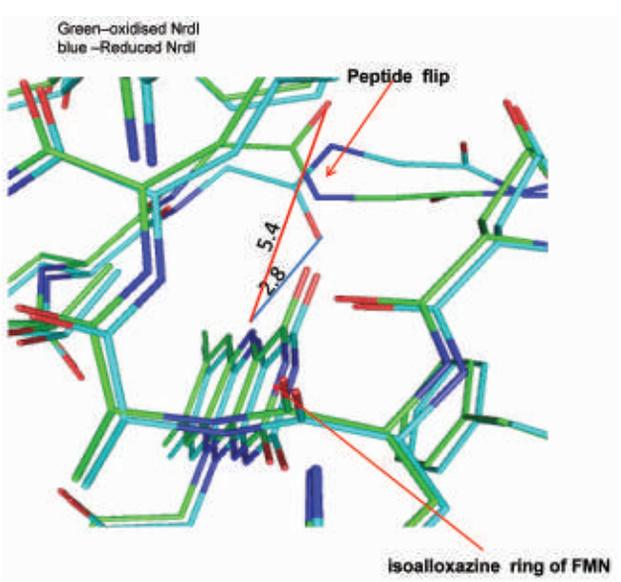

Figure 4: Superposition of structure of *M. thermoresistible* NrdI in oxidised and reduced form. A peptide flip is seen in the reduced structure in order to form a hydrogen bond with the isoalloxazine ring of FMN.

Crystal structures of NrdI in oxidized and reduced forms

We were able to obtain yellow coloured crystals of NrdI protein, the yellow colour being ascribed to the FMN cofactor. Crystals of

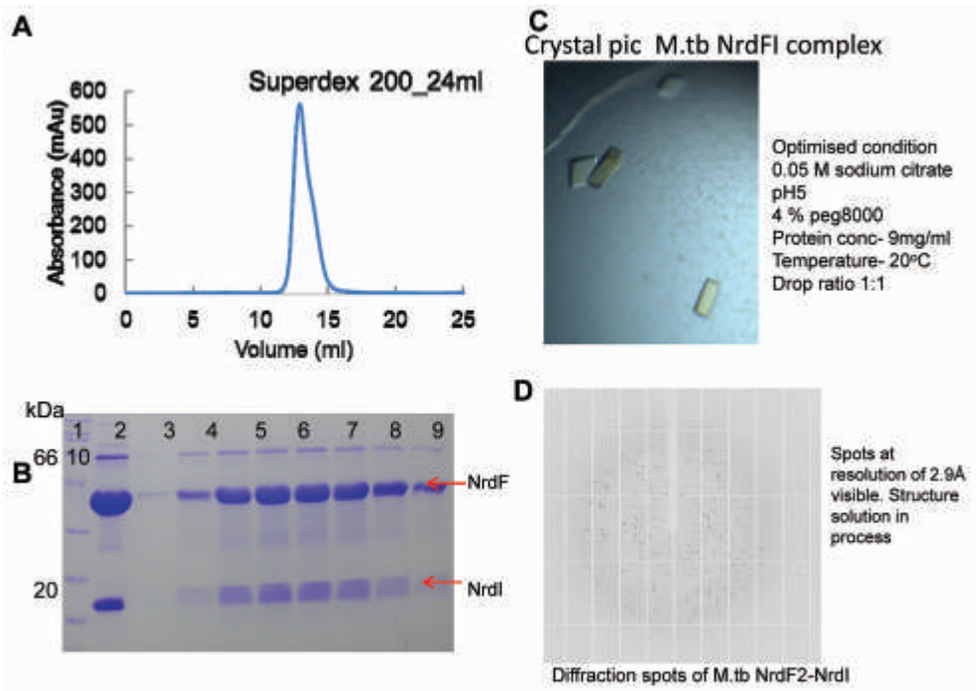

Fig. 5: A. Size exclusion chromatography profile of M. tuberculosis $(His)_6$-NrdF2 and $(His)_6$-MBP NrdI complex. B. Purified protein fractions (indicated by red arrow) loaded on 15% SDS-gel. C. Crystals of *M. tuberculosis* NrdF2-NrdI complex. D. Diffraction image of the complex crystals.

Table 1: SEC-MALS parameters for RNR in absence and presence of cognate effector-substrate pair (dATP-CDP).

	RNR-Reduced	RNR-Red-dATP-CDP
Masses		
Injected mass (µg)	200	200
Cal. Mass (µg)	11.59	5.9
Mass recovery (%)	5.8	2.9
Mass fraction (%)	100	100
Molar Mass moment (g/mol)		
Mn	$2.12x10^5$ (±0.613%)	$2.18x10^5$ (±0.669%)
Mp	$2.122x10^5$ (±0.684%)	$2.18x10^5$ (±0.664%)
Mw	$2.12x10^5$ (±0.613%)	$2.18x10^5$ (±0.669%)
Polydispersity (Mw/Mn)	1.000 (±0.867)	1.000 (±0.946)

Table 2: Various parameters for SAXS data of RNR under different conditions.

	Conc (mg/ml)	Guinier Analysis			P(r)			M.W (kDa) (Bayesian Inference)
		R_g (nm)	I_0	Fidelity	R_g (nm)	Porod volume (V)	D_{max} (nm)	
RNR_Ox	1	5.14±0.08	75.21±0.34	0.97	5.11	302.05	15.54	242.6
RNR_Red	1	5.41±0.13	77.48±0.44	0.98	5.53	317.6	19.53	208
RNR_HU	1	5.38±0.1	79.32±0.31	0.98	5.44	310.07	17.61	208
RNR_Lig	3	4.99±0.08	87.35±0.43	0.98	5.05	354.04	18.36	208

I_0 :Forward scattering intensity

NrdI in oxidized and reduced condition (soaked in sodium dithionite) were diffracted and their structures were solved to a resolution of 1.2Å **(Fig 3)**. The structure of NrdI shows flavodoxin fold with parallel β-sheet sandwiched between α-helical layers. Presence of flavin mononucleotide is also seen. When FMN is reduced, a peptide flip is observed at a loop near N5 atom of the isoalloxazine ring. This peptide flip leads to the formation of hydrogen bond between carbonyl group of glycine and N5 atom of the flavin ring **(Fig.4)**. Similarly, the complex of *M. tuberculosis* NrdF2-NrdI has been successfully purified and crystals hits are obtained. The original conditions were optimized to obtain diffraction quality crystal. The diffraction data for these crystals are obtained at ~ 3.2 Å. Structure solution of this complex is in process **(Fig.5)**.

Future Research Plans

- Optimization of purification conditions for *M. thermoresistible* NrdE-NrdI and *M. thermoresistible* NrdE-NrdF-NrdI ternary complex.
- Optimization of crystallization conditions to obtain diffraction quality crystals for the Rv3049c-NrdF2 complex in which microcrystals are observed.
- Interaction study to demonstrate the thermodynamic parameters of interaction among NrdH, NrdE, nrdF, NrdI and Rv3049c.
- Attempt to crystallize the NrdE protein and its complex with NrdF2.

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Project Title: Role of Long Non-coding RNAs in Intricate Host-virus interaction

Background

All living organisms encounter a wide range of viruses throughout their life span and hence, there is a requirement of an effective antiviral response for controlling these viruses and ensuring survival of the host. Both in lower and high organisms, the immunity to viral infection is caused by a variety of specific and nonspecific mechanisms, which involves many proteins and signaling pathways. Although unlike vertebrates, invertebrates lack adaptive immunity and possess very primitive innate immune responses against viruses. Nevertheless, invertebrates are able to combat viral infection very effectively, suggesting that they rely on alternative mechanisms for antiviral defense. Long non-coding RNAs (lncRNAs) have been recently described as critical regulators in viral infections. Non - coding RNA represents a subset of RNAs, greater than 200 nucleotides in length that does not code any protein, but contains information and remains functionally active by regulating other genes. The main goal of this study is to understand the role of lncRNAs mediated defense and counter-defense in the antiviral immune system of the domesticated silkworm, *Bombyx mori* against its viral pathogen, *Bombyx mori nucleopolyhedrosis virus* (BmNPV).

Aims and Objectives

- Identification and characterization of lncRNAs from *B. mori*
- Functional analysis of differentially expressed lncRNAs upon BmNPV infection.

Work Achieved

RNA extraction and lncRNA library preparation: Newly molted 5 instar larvae of *B. mori* were orally fed with the purified BmNPV suspension containing occlusion bodies. The fat body and midgut tissues from uninfected and BmNPV infected larvae were extracted at different time point of post infection. Total

Participants

Rohit Joshi, *Project Assistant*

Collaborators

Dr Arun Kumar KP, *CDFD, Hyderabad*

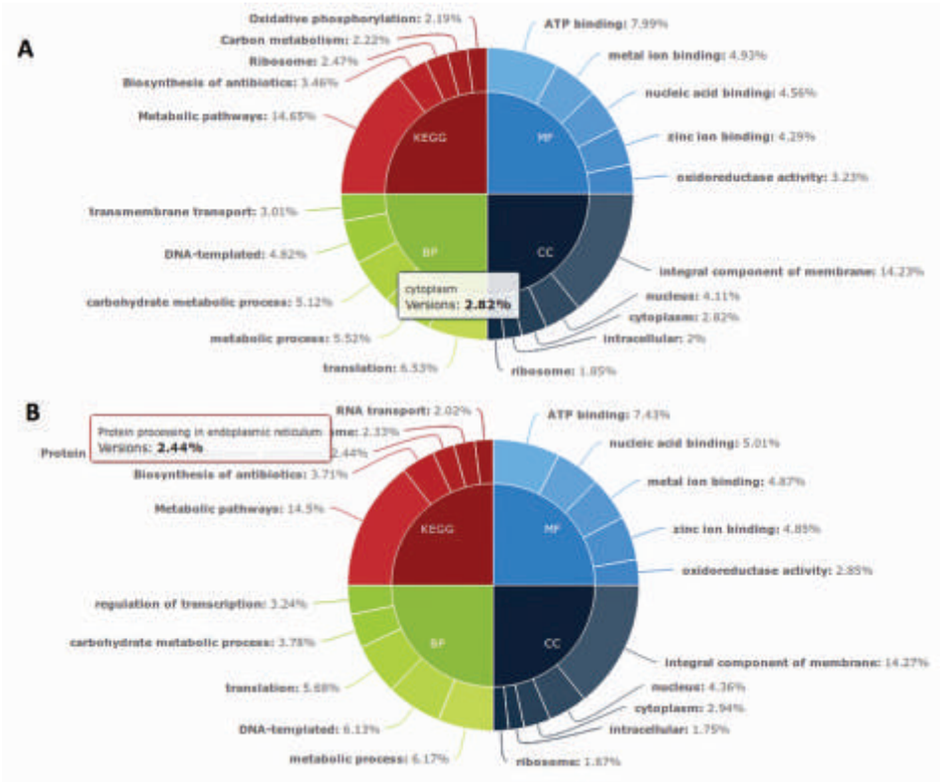

Fig 1: Piedonut presentation of GO summary and KEGG Pathway of differentially expressed lncRNA upon BmNPV infection in (A) Fat body and (B) Midgut tissues.

RNA was isolated from these tissues collected at different time points using standard TRIzol extraction protocol. Infection was then confirmed by RT-qRCR using early and late expressing genes of the virus in all infected samples. Total RNA from different time points was then pooled and three biological replicates from each of the samples were subjected to non-polyA and polyA RNA library construction and sequencing.

RNASeq Data Analysis: Stringent quality control of paired end sequence reads of all the samples was done using NGSQCTool kit. Paired end sequence reads with Phred score >Q20 was taken for further analysis. TopHat pipeline was used for alignment and Cufflink and Cuffdiff pipeline was used for identification of transcript coding regions followed by quantitation and annotation using default parameters. Control replicates and treatment replicates have been pooled for differential expression analysis using CuffDiff. Transcripts with log2ratio > =2 were considered as differentially expressed.

LncRNA prediction and characterization: Based on the assembly results, transcripts with FPKM = 0 or shorter than 200 nt were removed. The open reading frame (ORF) was predicted using TransDecoder and transcripts with an ORF that was longer than 100aa were excluded. Coding-Potential Assessment Tool (CPAT) was used to distinguish mRNA from lncRNA and transcripts with predicted protein-coding potential were removed. Pfam Scan was used to identify occurrence of any of the known protein family domains documented in the Pfam database. Transcripts with similarity to known protein sequences in the Swiss-Prot database and known protein-coding domains in the Pfam (AB) database were discarded.

Biological Pathways and Gene Ontology Enrichment Analysis: GO ontologies and KEGG pathways that harbour expressed transcripts were identified using DAVID Functional Tool.

Future Research Plans

- Functional annotation of differentially expressed lncRNAs in the BmNPV resistant and susceptible strains of *B. mori*.

CONFERENCES / WORKSHOPS

- Presented poster in the 3rd India International Science Festival (IISF-2017), Anna University, Chennai, 15-16 October 2017.
- Invited talk for 9th RNA Group Meet on "Role of Virus-Encoded miRNAs in Safeguarding Antiviral Host Response" BHU, Varanasi, 26-28th October 2017.

EXTRAMURAL FUNDING

- *DST-INSPIRE Faculty Project : Ended on 25th Dec 2017.

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

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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 complexity 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

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

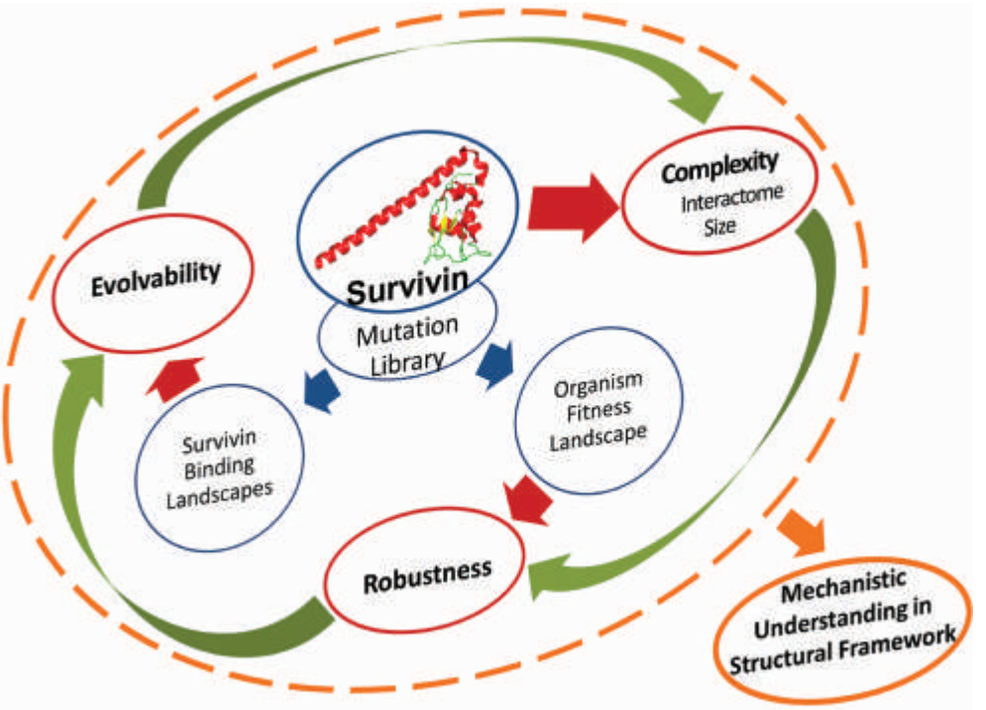

Fig. 1: The scheme depicts the primary goals and approaches of the work. The green arrows indicate the accepted relationship between complexity, robustness and evolvability of biological systems.

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.

Using ß-lactamase based protein fragment complementation assay (PCA), survivin interactions with peptide from binding partners is established.

Future Research Plans

- A 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)

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Host Cell Factors in HIV Pathogenesis and Identification of New Anti-viral Lead Molecules

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 significantly but is not the ultimate answer to AIDS patients as a therapy 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 Done

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

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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. An earlier 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 NF-κB sites on the c-Rel promoter. 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. We have 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. Our initial results suggest that selected isoforms of HSP40 play positive or negative role in HIV-1 replication.

In recent times, Hsp90 has also emerged as an important cellular factor for viral pathogenesis. The ability of Hsp90 to perform diverse range of functions arises from its differently compartmentalized five isoforms, thus each of them taking part in unique as well as similar cellular processes. In cytoplasm, Hsp90 is expressed as two inducible isoforms i.e. Hsp90AA1 and Hsp90AA2 (together known as Hsp90α) and one constitutive isoform Hsp90AB1 (Hsp90β). Other than the cytosolic isoforms, Hsp90 also has one endoplasmic isoform Hsp90B1 and one mitochondrial isoform TRAP1 (Tumor Necrosis Factor Receptor-Associated Protein-1). We have now initiated studies to look at the role of HSP90 isoforms in viral replication and infectivity. Our preliminary results suggest that HSP90 isoforms might 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. We have reported that HspBP1 inhibits HIV-1 gene expression and replication by restricting p65 from binding to NF-κB enhancer sequence on the viral promoter. We have also observed that HIV-1 down-modulates the expression of nucleotide exchange factor, HSPBP1. As we were curious to understand if this regulation occurs through the promoter of HSPBP1, which has not been characterized till date, we have created different constructs of upstream sequences of HSPBP1 UTR region. Our initial results indicate that a small segment of DNA (-70 to +30) around the TSS shows promoter activity. This could potentially be termed as the core promoter of HSPBP1. Our preliminary results also suggest that HIV-1 down regulates the activity of the above-mentioned promoter construct.

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. In this context, we are currently trying to functionally characterize the role of Death associated protein Kinase (DAPK/ZIPK) that has been shown to interact with Nef protein both *in vitro* and *in vivo*. Furthermore, HIV-1 infection is known to be associated with the hijacking of a number of cellular factors including non-coding RNAs. Mammalian miRNAs are small non-coding RNAs that are potent regulators of gene expression. In their mature form, they bind primarily to the 3'UTR of target mRNAs through seed complementarity, leading to silencing or reduced levels of protein expression, either through translational repression or mRNA degradation. Although there is significant literature suggesting that host miRNAs play a fairly important role in HIV-1 infection, however, a comprehensive elucidation of the role of micro RNAs in HIV-1 replication and infectivity remains to be done. Thus, we have initiated identification of deregulated miRNAs through bioinformatic analysis of existing GEO database which has led to identification of 16 differentially expreseed miRNAs during HIV-1 infection. These miRNA

expression changes have ben further validated in HIV-1 infected PBMCs. Selected miRNAs from this list is currently being functionally characterized including looking at the role of their targets.

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 of repurposing and 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. Out of these molecules, we have studied DMRC-05 in detail to understand its mechanism of action governing its anti-HIV activity. Further studies suggest that the anti-HIV activity of DMRC-05 is not a cell type, virus isolate or viral load dependent phenomena. We have also observed that this molecule does not inhibit viral enzymes Reverse Transcriptase, Integrase or Protease and also does not block virus entry to the cell. Our results indicate that DMRC-05 induces proteasomal degradation of HSP90 that ultimately increases levels of HSP70, leading to the inhibition of NF-κB pathway. This results in inhibiton of HIV-1 transcription and thus viral replication. Finally, combination of DMRC-05 with Indinavir (IND) and Tenofovir (TDF) showed significant increase in anti-HIV activity. Hence, using DMRC-05 along with the currently available drug regimen may help us to fight the problems of cytotoxicity and emergence of drug resistant viral strains to a significant extent.

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 death 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.

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Diversity Dynamics of the Fermentation of *Idli* Batter

Background

Pulses and cereals are major dietary constituents of individuals in developing countries. Presence of complex proteins, insoluble fibres and anti-nutritional factors make cereal diet less salutary. These difficult-to-digest cereals are converted to nutrition rich food by simple house-hold fermentations and this practice is an important part of provincial legacy. During the process, fermentative microorganisms play important role in preparation and preservation of food and also attribute to the taste and flavour of the final product. A small acid leavened steamed cake, *idli*, is a naturally fermented Indian food, popular for its excellent organoleptic properties, soft and spongy texture and subtle aroma. *idli* is prepared from dehulled cotyledons of black gram [*lentil; Vigna mungo L.*] and parboiled rice [*Oryza sativa L.*]. Constituents are soaked separately, pulverized and mixed to obtain a coarse batter. The batter is allowed to ferment overnight at ambient temperature, without any starter culture. After fermentation, the batter is steamed to obtain final product in a cake form. Lowering of batter pH due to acid production and leavening leading to dough rising are two major changes that occur during *idli* batter fermentation. The only fundamental work describing the role of microorganisms in the fermentation of *idli* was reported using laboratory fermented batter. The report highlighted that the acid and gas required for batter leavening are generated solely by the heterofermentative *Leuconostoc mesenteroides*, *Enterococcus faecalis* and *Pediococcus cerevisiae*. The recently developed high-throughput DNA sequencing methods have been employed to find out different microorganisms participating in complex food fermentations. However, detailed analysis of the microbial community structure and its role in the fermentation of *idli* batter using modern methods has received little attention.

Participants

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Aims and Objectives

- To study the microbial biodiversity of *idli* batter using culture-dependent and culture-independent approaches.
- To study the gene expression profile of the organisms active during the process of *idli* batter fermentation.
- To profile the volatile organic compounds and metabolites generated during the fermentation of *idli* batter.

Work Done

The microbial composition of *idli* batter was studied by both culture-dependent and culture-independent approaches. Using spread plate technique and based on colony characters, a total of 354 bacterial isolates were obtained from three fully fermented *idli* batter samples. The identification of bacteria from these three samples revealed that microbial community of all samples was essentially similar with most isolates belonging to orders Lactobacillales and Bacillales (Phylum Firmicutes) and Enterobacteriales (Phylum Proteobacteria). The Lactic Acid Bacteria (LAB) constitute nearly 50-60% indicating LAB may play important role in the fermentation of *idli* batter. From all three samples, different Weissella species were isolated which had the capacity to ferment glucose and maltose producing acid and gas indicating their hetero-fermentative metabolism. The similarity in the bacterial flora responsible for *idli* fermentation from different samples was confirmed by DGGE. Furthermore, exploration of the complete bacterial community by amplicon sequencing of the 16S rRNA V3 region using Illumina Miseq revealed that phylum Firmicutes was the most dominant, contributing on an average 64%, followed by Proteobacteria (30%) and followed by Actinobacteria (2%). At the genus level, *Weissella* (phylum Firmicutes) was the most abundant genus. The major LAB enabling this process were found to be *Weissella*, *Enterococcus* and *Streptococcus*, among others such as *Lactobacillus*, *Pediococcus*, *Desemzia*, *Pilibacter*, *Lactococcus*, *Vagococcus* and *Lactovum*, contributing more than 94% of the total population. Employing genus specific primers, the predominant microbiota was quantified in the *idli* batter by real time quantitative PCR, further substantiating the results obtained from amplicon data.

Idli batter fermentation is a result of natural cereal fermentation and is independent of back-slopping process. It normally takes 12 -15 hours to obtain the fermented batter. In laboratory, it was observed that with the progression of fermentation, there was an increase in the total acid content leading to sour conditions and the pH decreased from 6.3 at 0 hr to 4.5 after 24 hrs. Simultaneously, gas production had taken place with increase in batter volume and reduction in bulk density of the batter. The serial dilutions of samples collected at different stages of fermentation were plated on different bacteriological media and incubated for 48 hours at 37°C which highlighted a concomitant increase in bacterial count as the fermentation continued. The kinetics of bacterial growth was near logarithmic up to the first 12 hours and the trend of gas production paralleled the bacterial growth.

In addition, a peculiar succession pattern in the bacterial diversity during the fermentation of *idli* batter was revealed from the DGGE images highlighting presence of few bands in the early time points of fermentation which later disappeared and some new bands appeared in the later stages of fermentation. This was also confirmed by partial 16S rRNA gene sequencing. To explore the changes in the microbiota structure across different time points of the fermentation process, we computed the weighted Unifrac distances based on the OTU data. The wide Unifrac distance distributions indicated the difference in the microbial structure at different times of the process, thereby confirming a microbial succession during the process.

For enhanced understanding of the role played by the microbiota present in the *idli* batter during the fermentation process, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) program was used. It predicts the functional composition of the microbiota using 16S rRNA amplicon profile. It was found that the major metabolic processes such as carbohydrate and amino acid metabolism, energy metabolism, lipid and nucleotide metabolism, glycan biosynthesis and metabolism of co-factors and vitamins were slow during the first 9 hours of the fermentation, which then progressed rapidly and remained vigorous till the end of the process. *Weissella*, *Enterococcus*, *Streptococcus* and *Bacillus* (phylum Firmicutes) and Klebsiella, Erwinia and *Citrobacter* (phylum Proteobacteria) are the major players in all the diverse metabolisms. The overall reconstruction showed increased starch and sugar metabolism, glycolysis and pentose phosphate pathway and higher pyruvate metabolism supporting the fermentation process. An enhanced amino acid and nucleotide metabolism indicated rapid proliferation of the microflora enabling the fermentation of *idli* batter. These organisms also contributed to the synthesis of short chain fatty acids and B group vitamins. The important enzymes active in lactic acid fermentations such as lactate dehydrogenase and alcohol

dehydrogenase were found to be contributed by these organisms. *Weissella* appears to be contributing most to these important enzymes, thereby highlighting its abundance during the process.

Future Research Plans

- To perform HS-SPME GC-MS and GC-MS based identification of volatiles and metabolites respectively generated during the fermentation of *idli* batter.
- To isolate organisms by culturomics and assess their probiotic potential by in vitro assays.

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Hemozoin Induced Phenotypic Changes in Human Monocytes

Background

Malaria is an infectious disease affecting populations in tropical and subtropical regions. Of the five *Plasmodium* species, *P. falciparum* causes the most severe form of human malaria with several complications. It is well established that only the erythrocytic stage of parasites is responsible for malaria symptoms. During schizont rupture, the parasite releases several pyrogens, toxins and the malarial pigment, hemozoin (Hz), into the bloodstream, along with merozoites. These factors activate immune cells, which augment several proinflammatory cytokines, chemokines and other soluble factors. These factors influence the hypothalamus in the brain, which results in fever like conditions, and probably contributes to the severe pathophysiology associated with malaria.

During the intraerythrocytic cycle, the parasites digest hemoglobin in the food vacuole, resulting in the production of potentially-toxic heme metabolites, which the parasite detoxifies by converting it to an insoluble crystal called malarial pigment or Hz. Hz is released into the blood circulation after completion of the erythrocytic cycle (48h), and is avidly phagocytized by human phagocytic cells such as monocytes, neutrophils and dendritic cells. Hz-containing monocytes are frequently encountered in patients and also related to disease severity. Hz has been reported to alter several functions of monocytes, such as repeated phagocytosis, bactericidal abilities, oxidative burst, MHC Class II expression, and antigen presentation and maturation of dendritic cells. The phagocytosis of Hz by monocytes results in the stimulation and secretion of pro- and anti-inflammatory cytokines, thereby influencing the immune response.

Monocytes and macrophages are heterogeneous in nature and can mature into the pro-inflammatory (M1) or anti-inflammatory M2 (M2a, M2b, M2c)

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type, depending on the nature of microenvironmental signals. The response is reflected by an alteration in receptor expression, cytokine production, effector function and chemokine repertoires. IL-10, a well-characterized anti-inflammatory cytokine produced by monocytes, plays a crucial role in M2 polarization, and is produced through IL10R, STAT3 and NF-κB-mediated pathways. While p38- MAPK, PI3K/AKT and NF-κB signaling have been implicated in IL-10 synthesis and M2 polarization separately, their relevance in M2 polarization as a function of IL-10 production is not clearly understood. Monocyte phenotypes have been reported to play an important role in many diseases. The M2 polarization of monocytes has been shown to aggravate the clinical symptoms in parasitic and bacterial diseases. Immunomodulators are emerging as a new class of agents to control different infections. Artemisinins have been reported to decrease the levels of IL-10, IL-1β, IL-6, and TNF-α in disease, through the p38-MAPK, NF-κB and PI3K/AKT pathways. Chloroquine (CHQ) has also been demonstrated to act through the MAPK and NF-κB pathways, and decrease IL-1β, IL-6 and TNF-α levels in mononuclear phagocytes, in different diseases.

Recent studies have enhanced our understanding of the importance of phenotypic changes in monocytes in modulating immune responses in several infectious diseases and have highlighted their potential for development of new therapeutics. While the role of Hz is well established in immunosuppression, its involvement in polarization of monocytes has not been studied. The phenotypic switching of monocytes to M1/M2 has an indirect role in the activation of TH1 and TH2 response, thereby making it an important topic in immunomodulation and pathology in malaria. Along these lines, a study was designed to investigate the role of natural hemozoin (nHz) on monocyte phenotype modulation, with the following objectives.

Aims and Objectives

- To investigate the role of Hz in the modulation of adherent monocyte phenotype.
- Differential gene expression profiling of Hz and sHz- fed monocytes.

Work Achieved

Primary human monocytes isolated from peripheral blood were used for all the experiments. Adherent monocytes exposed to Hz showed elevated levels of transcripts, secreted cytokines and chemokines IL-10, CCL17, CCL1, and showed expression of the mannose-binding lectin receptor (CD206) and arginase activity, which are signatures of M2 phenotype. Hz attenuated HLA-DR expression, nitric oxide (NO) and reactive oxygen species (ROS) production, which are the features of M1 phenotype. This suggested that Hz induces M2-like phenotype and functional properties in human monocytes. Our data also implicated the involvement of p38-MAPK, PI3K/AKT and NF-κB signaling pathways in skewing of Hz-fed monocytes towards M2-like type, and suppression of mitogen-stimulated lymphocyte proliferation. Importantly, the antimalarial drugs, chloroquine (CHQ) and artemisinin (ART) partially reversed the activation of Hz-induced monocytes towards the M2-like phenotype. Considering the limitations of the current therapeutic options for malaria, we propose that these drugs may be re-examined for their potential as immunomodulators and candidates for adjunctive treatment in malaria. Further, it would be interesting to find immunomodulators which can reverse the M2 phenotype and help in eliciting an efficient immune response against the parasite, or aid in enhancing the current therapy.

β-hematin (sHz) is widely used as a structural analog of Hz. However, there are differences in the immune responses induced by natural Hz and sHz. Hence, to understand the similarities and differences between the responses in monocytes, we determined the gene profiles of Hz and sHz-fed monocytes using microarray. Monocytes fed with inert latex beads served as phagocytosis control and showed the least number of differentially regulated genes. This suggests that mere phagocytosis does not alter gene expression in monocytes. sHz did not result in the expression of M2-specific IL-10 and the mannose receptor CD206. The data also revealed that Hz, but not sHz, induced M2-like characters in adherent human monocytes. The differences in sHz and Hz-induced responses were seen in terms of differential gene expression, functional processes and pathways in human monocytes. Thus, we conclude that use of Hz for immune-related assays might give more relevant information than sHz in monocytes. Considering the fact that most antimalarials act by inhibiting hematin formation, the use of sHz for anti-malarial screening is still useful.

Future Research Plans

- Validation of differential gene expression profiling in Hz and sHz-fed human monocytes.

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Identification of Potential Targets and Biomarkers for Breast Cancer Using Quantitative Proteomics and Metabolomics Approaches

Background

Globally, the most prevalent and highly incident oncological disease among women, breast cancer tends to be one of the leading causes of cancer related deaths in Asia. An estimated incidence of 14.9 million breast cancer related cases and 8.2 million breast cancer mortalities were reported globally for the year 2013 and Asian countries exhibit 37.5% of the global breast cancer incidence. Even though decades of research have been devoted towards the discovery of novel and most effective treatment strategies for breast cancer cure, the high number of new cancer cases persists due to lack of early diagnosis and clinical screening setup. The latter especially holds true for developing countries where regular clinical check-ups are not the part of society. The research on biomarker discovery has evolved through various hurdles and new upcoming biomarkers give hope towards early disease detection which is the key towards successful treatment of breast cancer. There are four major subtypes in practice for clinical perspective of breast cancer diagnosis and treatment namely, Luminal A (LA), Luminal B (LB), HER-2 enriched (HE) and Triple Negative (TN). The presence of these surface markers have been exploited to develop drugs that can block these receptors, and prevent tumor development/ progression. This discovery had enabled breast cancer to be curable up to a certain extent, but identification of the breast cancer pertaining to a specific subtype at early stage still remains a challenge.

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,

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assessment of prognosis, prediction of therapeutic effect and treatment monitoring. In this work, we plan to identify potential biomarkers for breast cancer using high throughput mass spectrometry based proteomic and metabolomic approaches in Indian scenario.

Aims and Objectives

- Investigation of tissue proteome and phospho proteome alterations in breast cancer intrinsic subtypes using label-free quantitative proteomics.
- Identification of urinary metabolomic alterations in breast cancer using targeted and untargeted metabolomic approaches.
- Elucidation of urinary volatome alterations in IDC type of breast cancer towards non-invasive potential biosignatures.

Work Done

Investigation of tissue proteome and phospho proteome alterations in breast cancer intrinsic subtypes using label-free 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 label-free quantitative proteomic approach and identified 307 differentially regulated tissue proteins pertaining to different subtypes where in LA subtype 270 proteins showed up-regulation while 27 showed down-regulation. In LB subtype 291 proteins showed up-regulation while 28 showed down-regulation, while 81 proteins showed up-regulation and 130 proteins showed down-regulation in HE subtype. In TN subtype, 154 showed up-regulation whereas 97 showed down-regulation (Fig. 1A). Two proteins were specific for Luminal A, seven for Luminal B, three for HER2 enriched and three proteins were specific for TN subtype (Fig. 1B). The differential expression of 110 proteins was observed in all subtypes suggesting them to be breast cancer specific rather than any subtype. The most common pathways observed in all the subtypes involving most of the proteins were ribosomal, carbon metabolism, protein processing in endoplasmic reticulum, glycolysis and gluconeogenesis, regulation of actin cytoskeleton, focal adhesion and ECM-receptor interaction.

To study the phosphoproteomic alterations in the different subtypes of breast cancer, quantitative label-free proteomics was applied which revealed 141 differentially expressed phosphoproteins. Total of 2363 phosphopeptides were

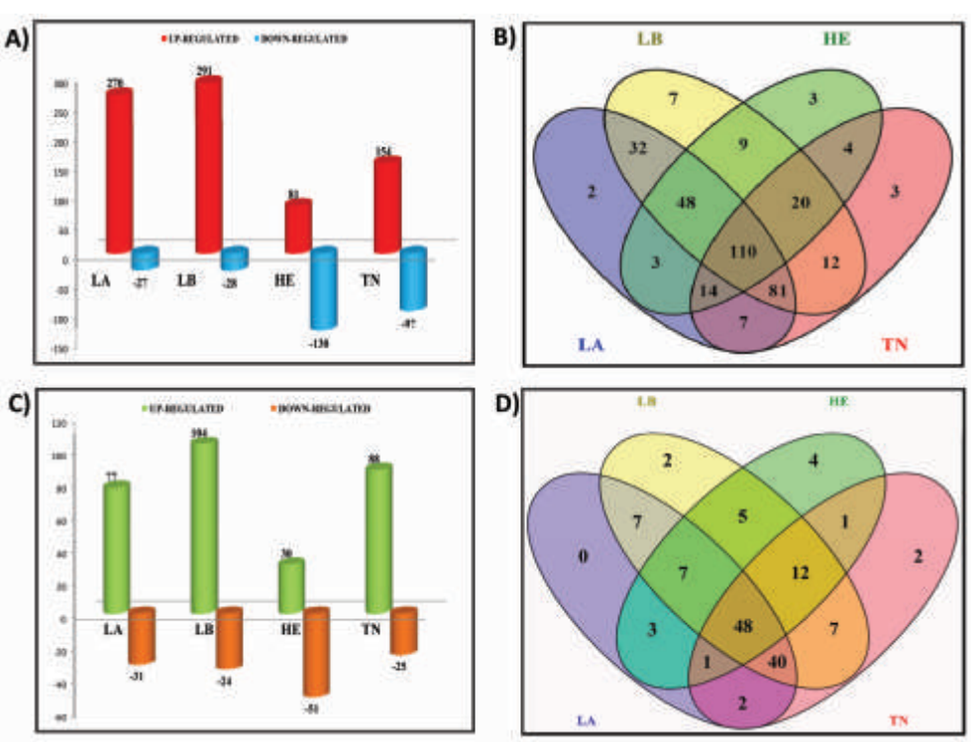

Fig. 1: A) Differential expression pattern of 355 proteins in different subtypes of breast cancer, B) Differential expression distribution of 355 proteins in different subtypes of breast cancer, C) Differential expression pattern of 141 phosphoproteins in different subtypes of breast cancer, D) Differential expression distribution of 141 phosphoproteins in different subtypes of breast cancer.

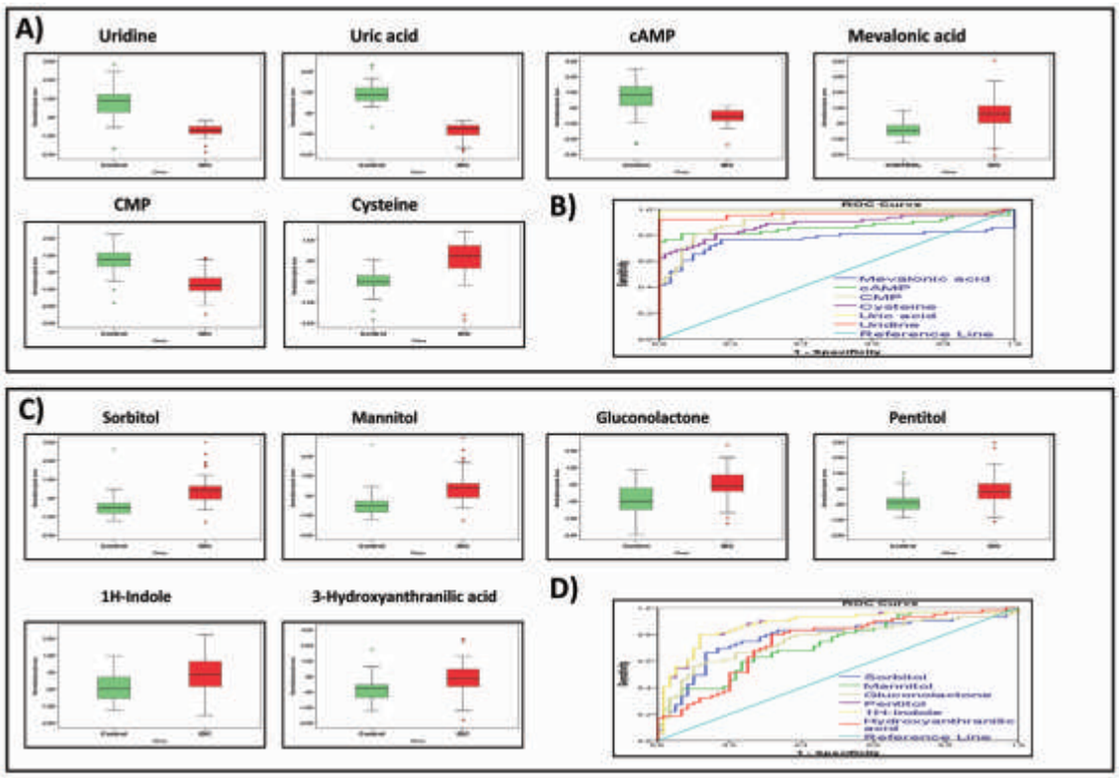

Fig. 2: Marker metabolites identified in LC-MRM/MS and GC-MS analysis: A) Box-and-whisker plots illustrating concentration differences between BC (Red) and healthy control (Green) for the top six marker metabolites from LC-MRM/MS analysis based on VIP score, B) The ROC curve analysis of the six marker metabolites uridine (AUC-0.96), uric acid (AUC-0.99), cAMP (AUC-0.87), mevalonic acid (AUC-0.77), CMP (AUC-0.91) and cysteine (AUC-0.88) from LC-MRM/MS analysis. The plot depicts discriminative ability of these metabolites for BC subjects from healthy controls, C) Box-and-whisker plots illustrating concentration differences between BC (Red) and healthy control (Green) for the top six marker metabolites from GC-MS analysis based on VIP score, D) The ROC curve analysis of the six marker metabolites sorbitol (AUC-0.87), mannitol (AUC-0.87), gluconolactone (AUC-0.74), pentitol (AUC-0.78), 1H-Indole (AUC-0.73) and 3-hydroxyanthranilic acid (AUC-0.76) from GC-MS analysis.

identified, which were manually curated to generate the subtype level information. The phosphopeptides that were identified in all the subtypes were considered to calculate the fold change. This meticulous analysis revealed 311 differentially expressed phosphopeptides belonging to 141 phosphoproteins in which 108 proteins showed differential expression in LA subtype and 128 proteins in the LB subtype. In case of HE subtype, 81 proteins showed differential expression while 113 showed differential expression in TN subtype (Fig. 1C). These 141 proteins were shown to consist of 197 phosphosites, where 183 sites contained phosphorylation at the serine (S) residue, 13 sites with threonine (T) and 1 site with the tyrosine (Y) residue. 48 phosphoproteins were shown to be differentially expressed in all subtypes. Among the Luminal subtypes, Luminal B showed 2 phosphoproteins to be specifically differentially expressed where both proteins showed up-regulation while none was observed in LA subtype. Four proteins were found to be specifically associated with the HE subtype where interestingly all showed down-regulation while two proteins showed specific differential expression in TN subtype where, one protein showed up-regulation while one showed down-regulation (Fig. 1D).

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

Breast cancer is usually detected in advanced stages due to its asymptomatic nature and lack of specific and sensitive screening markers, which ultimately leads to low survival rate. A non-invasive early diagnostic approach which will reduce economic burden and minimize apprehension among high risk population would be the ideal strategy to develop efficient screening methodology. This study aims towards investigating the urinary metabolic alterations of breast cancer by targeted (LC-MRM/MS) and untargeted (GC-MS) approaches for the development of non-invasive screening methods. Urinary metabolic alterations of breast cancer subjects (63) and control subjects (63) were explored by targeted (LC-MRM/MS) and

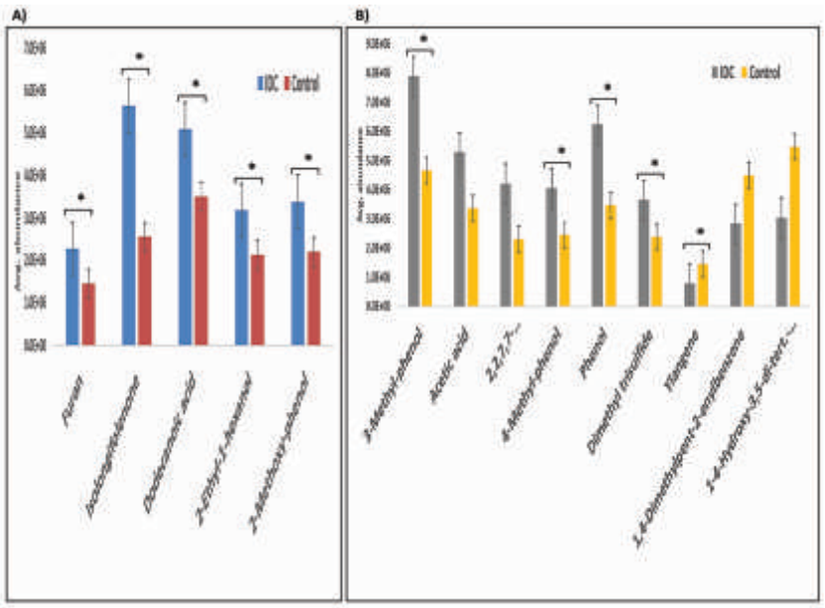

Fig. 3. The bar graph representing the expression profile of significantly altered VOCs as seen in the external cohort of subjects. A) The five verified VOCs with SIM mode GC-MS analysis and B) The semi -quantitative chromatographic areas graph for the nine VOCs. The bars are mean abundance values of the VOCs in samples acquired in duplicates with error bars as SEM. The star marks represents the p-value < 0.05. In panel B, the 3rd compound from left is 2,2,7,7-Tetramethyltricyclo6.2.1.01,6undec-4-en-3-one and the last compound is 1-4-Hydroxy-3,5-di-tert.-butylphenyl-2-methyl-3-morpholinopropan-1-one.

untargeted (GC-MS) approaches. Breast cancer specific urinary metabolomics signature was extracted by applying both univariate and multivariate statistical tools. Statistical analysis identified 39 urinary metabolites with the highest contribution to metabolomic alterations specific to breast cancer. Out of which, 19 metabolites were identified from targeted LC-MRM/MS analysis, while 20 were identified from the untargeted GC-MS analysis. Receiver operator characteristic (ROC) curve analysis revealed six most discriminatory metabolites from each type of approach that could differentiate between breast cancer subjects and controls with higher sensitivity and specificity (Fig. 2). Furthermore, metabolic pathway analysis depicted several dysregulated pathways in breast cancer including sugar, amino acid, nucleotide metabolism, TCA cycle etc. This study provides valuable inputs regarding discriminatory urinary metabolites with potential as non-invasive screening tool for breast cancer.

Elucidation of urinary volatome alterations in IDC type of breast cancer towards non-invasive early diagnosis biosignatures

Worldwide, breast cancer is the most prevalent and highly incident oncological disease among women. Breast cancer effective management, as for any form of cancer, would greatly benefit from an early diagnosis. This, however, due to various socio-economic reasons, is very far for the reality of developing countries like India, where cancer diagnosis is often carried out at late stages when the disease management is troublesome. In this work, we aimed to evaluate a simple analytical methodology to identify set of volatile organic compounds (VOCs) in urine samples, as biosignature for invasive ductal carcinoma (IDC) type of breast cancer. Using solid-phase microextraction followed by gas chromatography/mass spectrometry, a panel of 14 urinary VOCs was identified to discriminate IDC from healthy group through multivariate statistical treatments (Fig. 3). Furthermore, metabolic pathway analysis revealed various dysregulated pathways involved in IDC type of breast cancer patients hinting that their detailed investigations could lead to novel mechanistic insights into the disease pathophysiology. In addition, we verified the expression pattern of these 14 VOCs in another external cohort of 59 urinary samples and found their expression pattern to be consistent with the primary sample set. To our knowledge, this is the first study exploring the IDC type of breast cancer volatomic alterations in Indian patients.

Future Research Plans

- Investigation of chemo-resistance protein markers in breast cancer using quantitative proteomics.
- Identification of glycoproteome alterations in breast cancer using quantitative glycoprororoteomics approach.
- Identification of total lipid alterations in breast cancer towards early diagnosis.

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Reciprocity of CD40 Receptor is Attributed to Site-specific Occupancy by the Different Amino Acid Residues of CD40L

Background

CD40 receptor is expressed in all APC (Macrophages, Dendritic cells, Bcells) and helps in the bridging of innate to the adaptive immune response by interacting with the CD154 expressed on the surface of Tcells. During Tcell-APC interaction TCR-MHC provides the initial signal for the Tcells to proliferate, while doing so the CD40L-CD40 acts as a secondary signal to differentiate the Tcells into different subsets by release of the different cytokines by APC. These different subsets can be important in redefining the progression or regressing or just maintaining the immune status depending of the circumstances under which this Tcells-APC interact, like infection, autoimmune response etc. While doing so the Tcells also initiates a set of signals which are required for the antibody isotype switching from IgM to IgG in Bcells and also for affinity maturation. Defect in the antibody isotype switching can lead to hyper-IgM (xIgM) syndrome. These patients have mutations in their CD40L gene which further suggest that different residues of CD154 have different function ascribed along with the cumulative function as a receptor.

Considering these facts, we propose that the differential ability of the CD40 receptor to initiated different sets of signals are function of the different sites occupied by the ligand in the given receptor ligand pair.

Aims and Objectives

- Identification of CD154 mutants & characterization them as agonist, super agonist or antagonist.
- Validation of the cytokine production and different signaling intermediates using Leishmania as a model.

Work Done

Human and mouse CD40 ligand were aligned using sequence homology tool (Fig. 1) to identify the amino acids which are reported in Hyper XigM syndrome in humans and the corresponding amino acids in mouse were chosen. Published reports on bioinformatics studies showed that the region from S128 to N157 of CD154 was important in the binding of CD154 with CD40. Moreover, those amino acids were selected in this region which was either reported to be important in hydrogen bonding, hydrophobic interactions or electrostatic interactions. Peptides were generated using these selected amino acid mutations (Tab. 1) from the region D116 to N157 of the mouse CD154 and were commercially synthesized with 98% purity for performing the biological functional assays.

Peritoneal derived mice primary macrophages were stimulated with different peptides for 15 minutes to check the phosphorylation status of known downstream signaling intermediates which are counteractive & activated upon CD40 engagement (Fig. 2a). It was observed that different peptides had differential ability to initiate the downstream signal upon engaging with receptor. CD40 NA/LE was used as a positive control for the experiment. Densitometry analysis was performed using Quantity one from Bio-Rad the values of which are shown in the (Fig. 2b). It was observed that Peptide 5, 7 & 9 had a counteractive response considering the phosphorylation of greater p38, a pro-inflammatory signaling pathway intermediate to ERK1/2, an anti-inflammatory pathway intermediate. Further we will be checking the cytokine inducing ability of these selected peptides in mice macrophage.

The mechanism of ligand-receptor complex triggering a response remained unknown however; this is the first effort that characterizes how ligand residues can trigger differential receptor responses. We have characterized those ligands that evoke opposite responses. Now those select ligands will be sued for receptor clustering studies.

Future Research Plans

- In vivo implication of the selected mutants after invitro screening.
- Understanding the mechanism of this differential signaling using raft non raft fractionation.
- Role of different TRAF domains of CD40 receptor in differential signaling.

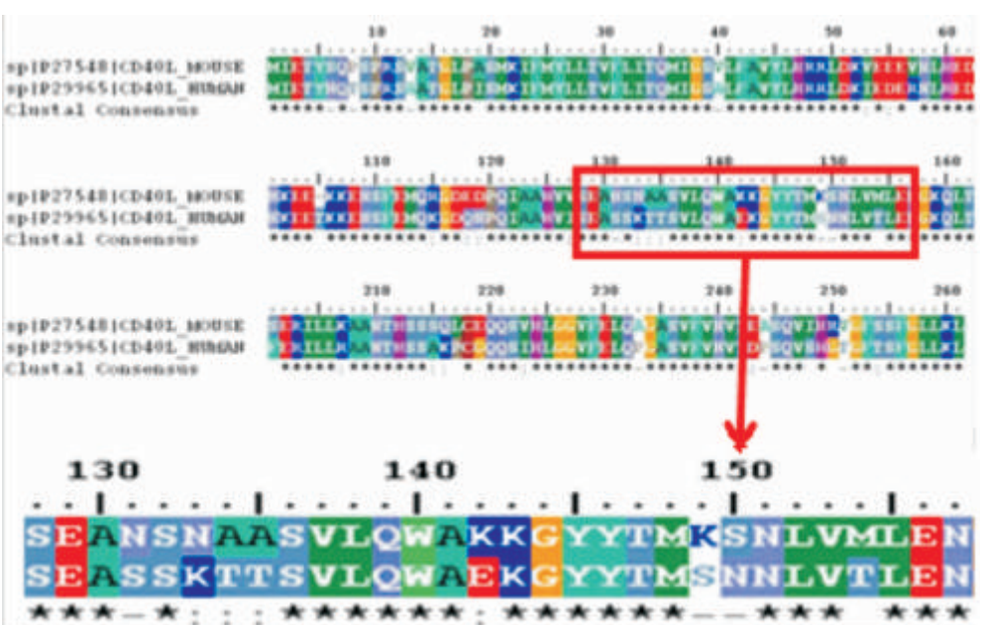

Fig. 1: Clustal sequence alignment of full length human and mouse CD40L Parameters used: Matrix:Blosum62, GOP: 10, GEP: 0.1

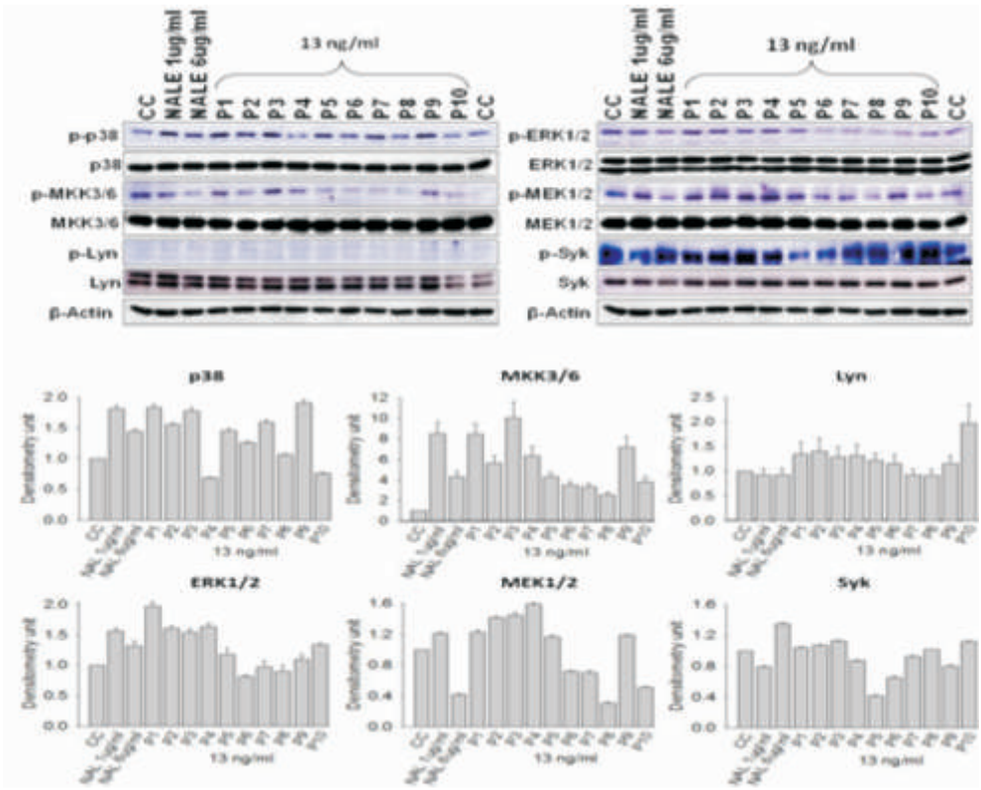

Fig. 2: Differential activation of different signaling intermediates 2a) Phosphorylation of CD40 signaling intermediates under stimulation of different peptides at a concentration of 13 ng/ml for 15mins. 2b) Densitometry of the different signaling intermediates using Quantity one Bio-Rad.

No	Amino acid sequence	Position	Amino acid	Change
1	DEDPQIAAHVVSEANSNAASVLQWAKKGYYTMKSNLVMLEN	Wild type		
2	DEDPQIAAHVV**V**EANSNAASVLQWAKKGYYTMKSNLVMLEN	127	S	V
3	DEDPQIAAHVVS**V**ANSNAASVLQWAKKGYYTMKSNLVMLEN	128	E	V
4	DEDPQIAAHVV**VV**ANSNAASVLQWAKKGYYTMKSNLVMLEN	127-128	SE	VV
5	DEDPQIAAHVVSEA**V**SNAASVLQWAKKGYYTMKSNLVMLEN	130	N	V
6	DEDPQIAAHVVSEAN**V**NAASVLQWAKKGYYTMKSNLVMLEN	131	S	V
7	DEDPQIAAHVVSEANSNAASVLQWA**V**KGYYTMKSNLVMLEN	141	K	V
8	DEDPQIAAHVVSEANSNAASVLQWAK**V**GYYTMKSNLVMLEN	142	K	V
9	DEDPQIAAHVVSEANSNAASVLQWAKK**V**YYTMKSNLVMLEN	143	G	V
10	DEDPQIAAHVVSEANSNAASVLQWAKKGYYTM**V**SNLVMLEN	148	K	V

Table 1: Amino acid sequence of 41 mer peptides along with amino acid substitution position in mice.

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Complement Evasion by *Aspergillus fumigatus*

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Background

Aspergillus fumigatus, an opportunistic airborne human pathogen, is known to cause a spectrum of diseases depending on the immune status of an individual. Hosts with immune hypersensitivity are predisposed to allergic aspergillosis and aspergilloma whereas those with compromised immune status are susceptible to invasive aspergillosis. Immunocompetent individuals, on the other hand, are capable of efficiently eliminating the inhaled conidia by innate immune mechanisms. The last two decades have seen a significant increase in the invasive aspergillosis cases owing to the widespread use of immune suppressant drugs, particularly in hemato-oncology patients and transplant recipients. Thus, our laboratory in collaboration with Institut Pasteur, Paris and NIRRH, Mumbai, initiated a study to understand the virulence factors employed by the fungus to evade the innate immune responses, particularly the complement system.

Aims and Objectives

- To identify proteases that confer protection to *A. fumigatus* conidia from the host complement system.
- To define the role of conidia-associated proteases in *A. fumigatus* virulence.

Work Done

The successful establishment of an *Aspergillus* infection would require efficient evasion of the complement system since this system is an important arm of the innate immunity, which is triggered within minutes after the entry of foreign bodies. Earlier studies have shown that one of the *A. fumigatus* alkaline proteases, Alp1p, secreted by the hyphal morphotype, is known to degrade the complement components. In particular, it was shown to degrade C3, C4 and C5. Alp1p, however, is secreted only by the hyphal morphotype and therefore it

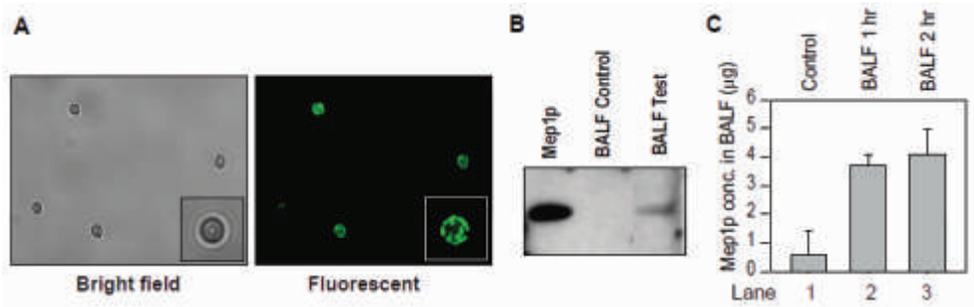

Fig. 1: Secretion of proteases from *A. fumigatus* conidia in collagen-containing medium. (A) Detection of Mep1p in the permeabilized conidia by immunofluorescence. (B) Mep1p in BALF of mice after exposure to WT conidia for 2 h. Released Mep1p was detected by Western blot analysis using polyclonal anti-Mep1p antibody. (C) Mep1p in BALF quantitated by ELISA (mean ± SD of three experiments; values represent the Mep1p concentration in 5 ml of BALF collected from each mouse).

was not clear how conidia exposed to the complement system immediately after inhalation defend the complement attack. In the present study, we therefore, sought to determine how conidia are protected from complement.

Conidia-derived Mep1p degrades complement components

The *A. fumigatus* morphotype that mainly enters the human lungs and gets exposed to the complement system is conidia. We thus hypothesized that conidia strores a protease(s) that is released upon its exposure to the lung environment and cleaves the complement components. To test this posit, we cultured wild-type (WT) conidia in the medium containing collagen (to mimic the lung environment) for less than 2 h and assessed the activity of the culture supernatant (CS) against complement proteins C3b and C4b, which are expected to be generated as a result of complement activation induced by conidia. The CS showed limited cleavage of C3b, but efficiently cleaved C4b. Interestingly, the proteolytic activity was specifically directed against the α'-chain of C4b. This indicated that a protease (or proteases) stored in the conidia and released early on, is/are responsible for cleaving C4b. Next, to determine the class of conidial protease responsible for cleaving C4b, we inhibited the protease activity by adding various inhibitors. Intriguingly, the activity was inhibited only by EDTA apart from heat inactivation suggesting that C4b is cleaved by metalloprotease(s).

Mep1p and Alp1p are the two major endoproteases secreted at neutral pH by *A. fumigatus* when grown in the presence of protein as the nitrogen source. We thus next examined if conidia release Mep1p after culture in the liquid medium containing collagen. Western blot analysis revealed that Mep1p, but not Alp1p, was released by conidia into the collagen medium early on. Mep1p was also released into the medium containing albumin, but not into other classical Aspergillus culture medium (Aspergillus minimal medium, BRIAN medium or Sabouraud medium; data not shown), suggesting that the release of Mep1p is medium specific. Moreover, permeabilization of the conidial cell wall followed by probing with anti-Mep1p antibody revealed the presence of Mep1p, suggesting that Mep1p is indeed stored in the conidial cell wall (Fig.1A). To ascertain whether Mep1p is also released in the lung during infection, we challenged mice with *A. fumigatus* conidia for 2 h and collected the bronchoalveolar lavage fluid (BALF). As shown in Fig. 1B, there was a band corresponding to Mep1p on Western blot upon probing SDS-PAGE separated BALF using polyclonal anti-Mep1p antibody, suggesting Mep1p is also released into the lung environment. These results were also confirmed by ELISA (Fig. 1C).

Next, to ascertain that Mep1p does have the complement degrading activity, we expressed it using the *Pichia* expression system. We also expressed Alp1p for comparison purpose, and three other major proteases (Pep1p, Pep2p and DppVp). Examination of the proteolytic activity of these recombinant proteases against the complement proteins C3b and C4b showed that unlike Alp1p, Mep1p possesses proteolytic activity only towards C4b; the other three proteases did not show any proteolytic activity towards C3b or C4b at the reaction conditions tested (Fig. 2A). Further, as expected, the activity of Mep1p could be inhibited by EDTA (Fig. 2B), but not by other protease inhibitors. To examine whether the proteolytic activity of Mep1p is specific towards C4b, or is also directed against other complement components as well, Mep1p was incubated with different complement components – C1q, C2, factor B, factor D, properdin, MBL, ficolins (-1, -2 and -3), complement regulators like C4BP and factor H (FH) as well as IgG. Mep1p

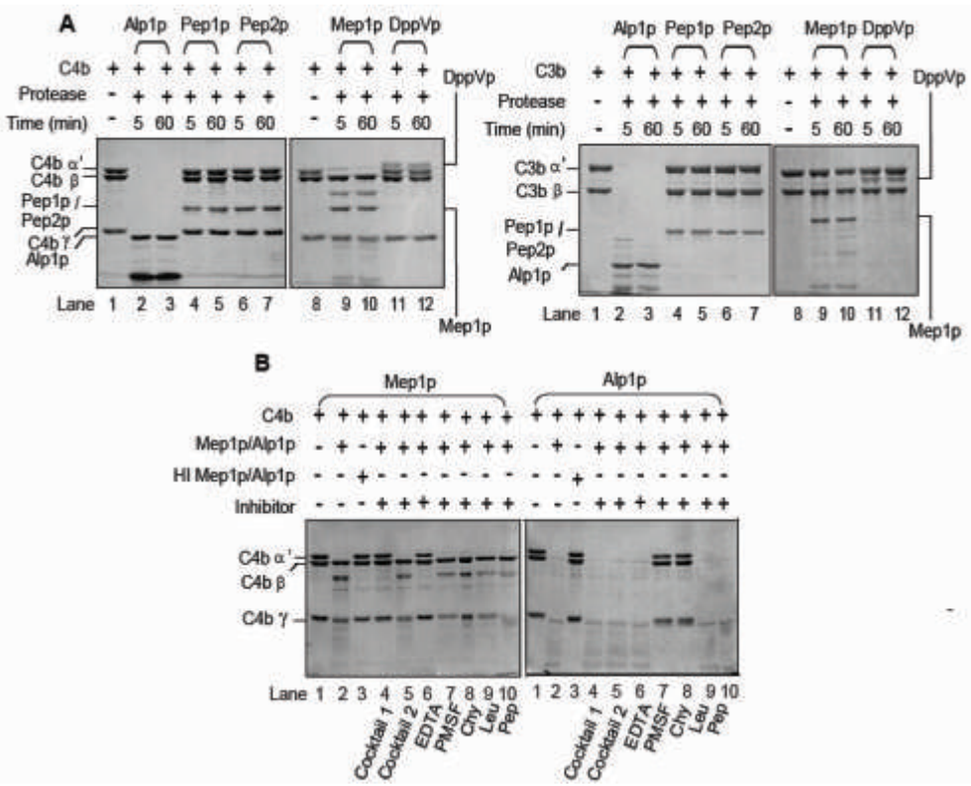

Fig. 2: Proteolytic activity of *A. fumigatus* proteases towards the human complement proteins C4b and C3b. (A) Proteolytic activity of recombinant proteases (Alp1p, Pep1p, Pep2p, Mep1p or DPPVp) for C4b (left two panels) and C3b (right two panels). (B) Inhibition of proteolytic activity of Mep1p and Alp1p by various classes of protease inhibitors. Inhibitors: Cocktail 1, Roche complete mini cocktail; Cocktail 2, Roche complete mini, EDTA-free; PMSF, Phenylmethylsulfonyl fluoride; Chy, Chymostatin; Leu, Leupeptin; Pep, Pepstatin.

efficiently cleaved properdin, MBL, ficolin-1 and C4BP, and showed limited activity towards ficolin-2, -3, IgG and FH suggesting Mep1p targets multiple complement components.

Mep1p inactivates C3, C4 and C5

The complement components C3, C4 and C5 are structural homologs and are key molecules involved in activation of the complement pathways. We thus next pursued to determine whether Mep1p has the ability to cleave these molecules. Mep1p converted the α-chains of these molecules into α'-like chains leading to generation of C3b-, C4b- and C5b-like fragments. Sequencing of the N-terminals of Mep1p generated α'-chains of the cleaved fragments revealed that the Mep1p cleavage sites on these proteins are 1-3 residues away from the physiological convertase cleaving sites. Amongst these molecules, C4b- and C5b-like fragments were completely degraded upon prolong incubation with Mep1p, while C3b-like fragment remained stable. Nevertheless, C3b-like fragment is cleaved by the physiological complement regulators FH and factor I (FI) suggesting Mep1p cleavage inactivates all the three major complement components.

Physiological activation of C3, C4 and C5 into C3b, C4b and C5b result in generation of anaphylatoxins C3a, C4a and C5a. We therefore also examined if Mep1p inactivates C3a, C4a and C5a. Incubation of C3a, C4a and C5a with Mep1p resulted in the cleavage of these anaphylatoxins into smaller fragments. Following N-terminal sequencing and high-resolution Orbitrap mass spectrometry analysis of the Mep1p cleaved C3a, C4a and C5a, it was observed that all the three anaphylatoxins were cleaved at the C-terminus which removed the C-terminal arginine, suggesting that Mep1p inactivates the anaphylatoxins.

Mep1p inhibits all the three major pathways of the complement system

The above results suggested that Mep1p targets the early complement components like properdin, MBL, ficolins, C3 and C4 as well as one of the terminal components C5. It is therefore expected that such Mep1p-mediated inactivation of complement would result in inhibition of all the three major pathways of complement activation. Hence, we next determined whether Mep1p is capable of inhibiting complement pathways when human serum is used as a source of complement. For this, we employed the Wieslab complement

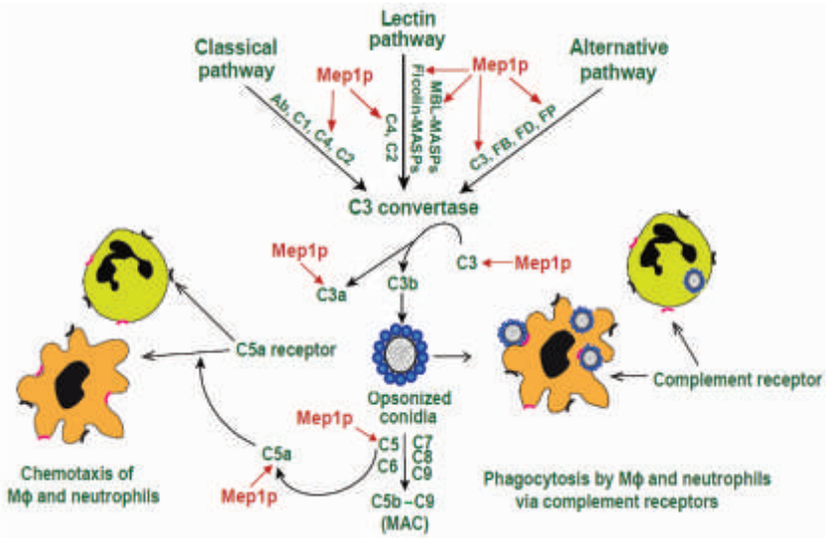

Fig. 3: Model for Mep1p-mediated immune evasion by *A. fumigatus*. Mep1p released from WT conidia is efficient in cleaving pattern recognition molecules such as properdin, MBL and ficolin-1, and the major complement proteins C3, C4 and C5, resulting in reduced opsonization of conidia as well as the MAC formation on their surface. Inhibition of opsonization by Mep1p results in decreased phagocytosis of conidia by macrophages (Mφ) and is also expected to result in decreased phagocytosis by neutrophils. Mep1p also inactivates C5a, which is a potent chemoattractant for Mφ and neutrophils and thus is expected to reduce chemotaxis of these cells. Together our data suggest that Mep1p-mediated complement subversion would result in decreased clearance of conidia, which would facilitate their colonization and invasion.

screen ELISA. The results showed the Mep1p inhibits the classical, alternative and lectin pathways with the IC_{50} of about 0.9 µM, 0.7 µM and 0.7 µM, respectively.

Phagocytosis is an essential step in clearing inhaled *A. fumigatus* conidia and is suggested to be dependent on the conidial opsonization by complement components which are recognized by the complement receptors on the phagocytes. As Mep1p showed inactivation of different complement pathways, we also investigated whether it inhibits C3b deposition on the conidial surface. Pre-treatment of human serum with Mep1p resulted in significant reduction in C3b deposition on conidia, suggesting that Mep1p indeed depletes C3 from the serum. And as expected, reduction in C3b deposition inhibited the phagocytosis of conidia by human monocyte-derived macrophages.

Virulence of Mep1p-deficient conidia in a murine model

Next, we sought to determine the virulence of Mep1p-deficient conidia using a murine model of aspergillosis. Herein, we employed the cyclophosphamide-immunosuppressed mouse model. There was no significant difference between the body weight and survival curves of the mice intranasally challenged with wild-type and Mep1p-deficient conidia. To determine whether deletion of Mep1p alters the Aspergillus secretome, we examined the protein profile on SDS-PAGE. The profile of Mep1p-deficient conidial collagen culture supernatant was different from that of the wild-type collagen culture supernatant. Moreover, unlike the wild-type conidial collagen culture supernatant that could not degrade C3 efficiently, the Mep1p-deficient conidial collagen culture supernatant completely degraded C3. Thus, it appears that the stress induced due to Mep1p deletion alters the released protease composition. Disabling Mep1p by mAb during wild-type condia infection is expected to reveal the role of Mep1p in virulence. Development of such a mAb is underway.

In summary, our data reveal that Mep1p is efficient in inhibiting all the three major complement activation pathways, and as a result, complement-mediated opsonization and phagocytosis of conidia and generation of C5a, a potent chemoattractant for neutrophils and macrophages. We thus propose that the release of Mep1p from conidia in the lung environment helps it to subvert the complement system and consequently inhibit the conidial clearance by neutrophils and macrophages (Fig. 3).

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.

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Degradation of FBXO31 by APC/C is Regulated by AKT- and ATM-mediated Pshosphorylation

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Background

Cancer is one of the leading causes of death worldwide and it happens due to the uncontrolled proliferation of cells. Function of two classes of genes, namely oncogenes (cancer promoting genes) and tumor suppressors (cancer inhibiting genes), is altered in cancer. Usually oncogenes are activated and tumor suppressor genes become non-functional during progression of cancer. Therefore, understanding the expression pattern as well as cellular function of these genes is important for developing an effective cancer therapy. While significant progress has been made in understanding the pathology of cancer for further improvement of therapy, it is important to identify new therapeutic targets for relief from undesired additional effects. Consequently, understanding the molecular players involved in the regulation of cancer progression and metastasis is the key to developing improved treatment strategies.

The ubiquitous ubiquitin-proteasome machinery is essential for normal cellular function and is also involved in many diseases including cancer. Inhibitors and activators of E3 ubiquitin ligases are therefore promising targets for therapy, as they dictate the proteins to be ubiquitylated and the manner of their ubiquitylation. Thus, an in-depth understanding of this class of genes is important.

FBXO31 is a member of the F-box protein family and component of 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, Par6c and promotes their polyubiquitylation mediated proteasomal degradation.

Recently, it has been shown that FBXO31 functions as a dedicated DNA damage checkpoint protein by arresting cells at G1 phase of the cell cycle through facilitating the proteasomal degradation of cyclin D1 and MDM2.

Cellular proteins are maintained at the basal level either through transcriptional, post-transcriptional or post translational regulation. Post-transcriptional and post-translational regulations have emerged as the major players in malignancy. Small non-coding RNAs and microRNAs (miRNAs) have emerged as crucial gene regulators at the post-transcriptional level and their expression levels are frequently altered in cancer and other diseases. Correspondingly, E3 uiquitin ligases are closely associated with the post-translational regulation of tumor suppressors and oncogenes.

Given the predominant role of FBXO31 in growth arrest and DNA damage checkpoint activation, it becomes important to understand its comprehensive regulation. Last year, we demonstrated that miR-93 and miR-106a regulate FBXO31 at the post-transcriptional level. In addition to the post-transcriptional level, we have found that FBXO31 is also regulated at the post-translational level by APC/C complex.

Aims and Objectives

- To understand the post-translational regulation of tumor suppressor FBXO31.
- To decipher the molecular mechanism of regulation of FBXO31 at the post-translational level.

Work Done

Aim 1: To understand the post-translational regulation of tumor suppressor FBXO31

To begin studying the mechanism by which FBXO31 is maintained at low levels in unstressed cells, we first monitored endogenous FBXO31 expression throughout the cell cycle. Briefly, immortalized human embryonic kidney (HEK293T) cells were synchronized by treatment with the DNA replication inhibitor hydroxyurea (HU), which arrests cells at the G1/S boundary, and following HU release FBXO31 levels were detected by immunoblotting. As controls, we also monitored the cell cycle-regulated proteins cyclin A and cyclin B. Consistent with previous reports, we found that FBXO31 levels oscillated during the cell cycle: FBXO31 levels were low in late G1 to S, high in early G2 to G2/M, and then low from late M to G1 (Figure 1A). *FBXO31* mRNA levels did not change during the cell cycle, confirming that cell cycle-regulation of FBXO31 occurred at the post-transcriptional level.

Notably, the cell cycle-regulated pattern of FBXO31 protein levels showed similarity to those of cyclin B, which is degraded during mitotic exit and G1 by APC/C coactivators CDC20 and CDH1, respectively, suggesting that FBXO31 levels may be regulated by a similar mechanism. Consistent with this possibility, FBXO31 contains multiple D-box motifs, which, as stated above, reside in APC/C substrates targeted by CDH1 and CDC20. We therefore investigated the involvement of APC/C in regulating FBXO31. As a first test of this idea, we generated HEK293T cells stably expressing a control non-silencing (NS) shRNA or an shRNA targeting APC2 or APC11, two catalytic subunits of APC/C, and monitored FBXO31 protein levels by immunoblotting. Knockdown of APC2 (Figure 1B) resulted in increased FBXO31 protein levels compared to that obtained with a NS shRNA.

Next, we performed a series of experiments to determine whether FBXO31 levels were regulated by CDH1 and/or CDC20. First, we ectopically expressed CDH1 or CDC20 in HEK293T cells and monitored the levels of FBXO31, or as a control cyclin B, by immunoblotting. Ectopic expression of either CDH1 (Figure 1C) or CDC20 (Figure 1D) decreased levels of cyclin B, as expected, and also resulted in decreased levels of FBXO31. By contrast, ectopic expression of CDH1 or CDC20 failed to reduce FBXO31 levels in APC2-depleted cells (Figure 1E). Addition of a proteasome inhibitor, MG132, blocked the ability of ectopically expressed CDH1 or CDC20 to decrease FBXO31 levels (Figure 1C and 1D), indicating that the CDH1- or CDC20-mediated decrease in FBXO31 resulted from proteasomal degradation. Consistent with this conclusion, *FBXO31* mRNA levels were unaffected by ectopic expression of CDH1 or CDC20. Finally, we performed a cycloheximide chase/immunoblot assay, and found that the half-life of endogenous FBXO31 was markedly reduced following ectopic expression of CDH1 or CDC20 (Figure 1F and 1G).

We next performed a reciprocal set of experiments, in which we knocked down CDH1 or CDC20 and monitored FBXO31 levels. The immunoblots of Figure 1H and Figure 1I show that shRNA-mediated knockdown of CDH1 or CDC20 in HEK293T cells resulted in increased FBXO31 protein levels. Consistent with these results, the half-life of endogenous FBXO31 was substantially longer in CDH1- or CDC20-depleted HEK293T cells than in NS shRNA-expressing cells (Figure 1J and 1K).

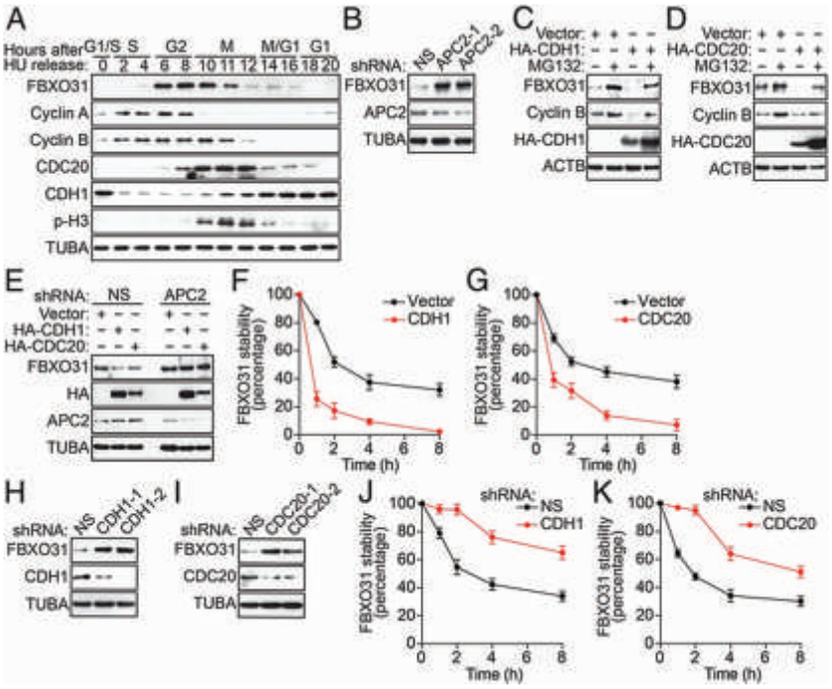

Fig. 1: FBXO31 is regulated through proteasomal degradation promoted by the APC/C coactivators CDH1 and CDC20. (A) Immunoblot monitoring FBXO31, cyclin A, cyclin B, CDH1, CDC20, and etaboli-histone H3 (p-H3) in HEK293T cells following HU synchronization and release. p-H3 was used as a marker for mitotic cells. α-Tubulin (TUBA) was monitored as loading control. (B) Immunoblot analysis monitoring the levels of FBXO31 in HEK293T cells expressing a NS shRNA or one of two unrelated APC2 shRNAs. (C and D) Immunoblot monitoring FBXO31 and cyclin B levels in HEK293T cells expressing vector or HA-CDH1 (C) or HA-CDC20 (D) in the presence or absence of MG132. β-Actin (ACTB) was monitored as a loading control. I Immunoblot monitoring FBXO31 levels in HEK293T cells expressing vector, HA-CDH1, or HA-CDC20 and either a NS or a APC2 shRNA. (F and G) Quantification of cycloheximide chase/immunoblot assay monitoring FBXO31 stability in HEK293T cells expressing vector, HA-CDH1 (F), or HA-CDC20 (G). (H and I) Immunoblot monitoring FBXO31 levels in HEK293T cells expressing a NS shRNA or one of two unrelated CDH1 (H) or CDC20 (I) shRNAs. (J and K) Quantification of cycloheximide chase/immunoblot assay monitoring FBXO31 stability in HEK293T cells expressing a NS, CDH1 (J), or CDC20 (K) shRNA. Data are represented as mean ± SD.

Collectively, these results demonstrate that APC/C regulates FBXO31 levels through CDH1 and CDC20.

Aim 2: Decipher the molecular mechanism of regulation of FBXO31 at the post-translational level

CDH1 and CDC20 interact with FBXO31 through its first D-Box motif, which is required for proteasomal degradation

To test whether CDH1 and CDC20 interact with FBXO31, we performed a series of co-immunoprecipitation experiments. In all cases, HEK293T cells were pretreated with MG132 prior to preparation of protein extracts. Our immunoprecipitation results revealed that FBXO31 could be detected in CDH1 or CDC20 immunoprecipitates and, conversely, CDH1 or CDC20 could be detected in the FBXO31 immunoprecipitates. Similar results were obtained in reciprocal co-immunoprecipitation experiments using ectopically expressed proteins.

To determine whether the interaction between FBXO31 and CDH1 or CDC20 occurred in a cell cycle-dependent manner, HEK293T cells were synchronized using HU and, following HU release, extracts from cells in G1/S, G2/M or G1 phase were immunoprecipitated using an anti-CDH1 or anti-CDC20 antibody. Our immunoprecipitation results revealed that FBXO31 interacted with CDH1 predominantly in the G1/S and G1 phases, whereas CDC20 interacted with FBXO31 in G2/M.

Both CDH1 and CDC20 interact with their substrates through a recognition motif known as a D-box, which consists of a simple core sequence of an arginine followed two residues later by a leucine (RxxL). Analysis of the FBXO31 protein sequence revealed eight conserved putative D-box motifs. Previous studies with cyclin B have demonstrated that it contains two D-box motifs, the first of which is required to mediate protein degradation. Therefore, as a first step toward delineating the functional D-box motif(s) in FBXO31, we generated an FBXO31 mutant in which residues 1-67, including the first D-box motif, were deleted (FBXO31(ΔD1)). We ectopically co-expressed wild-type Flag-FBXO31 (FBXO31(WT)) or Flag-FBXO31(ΔD1) in

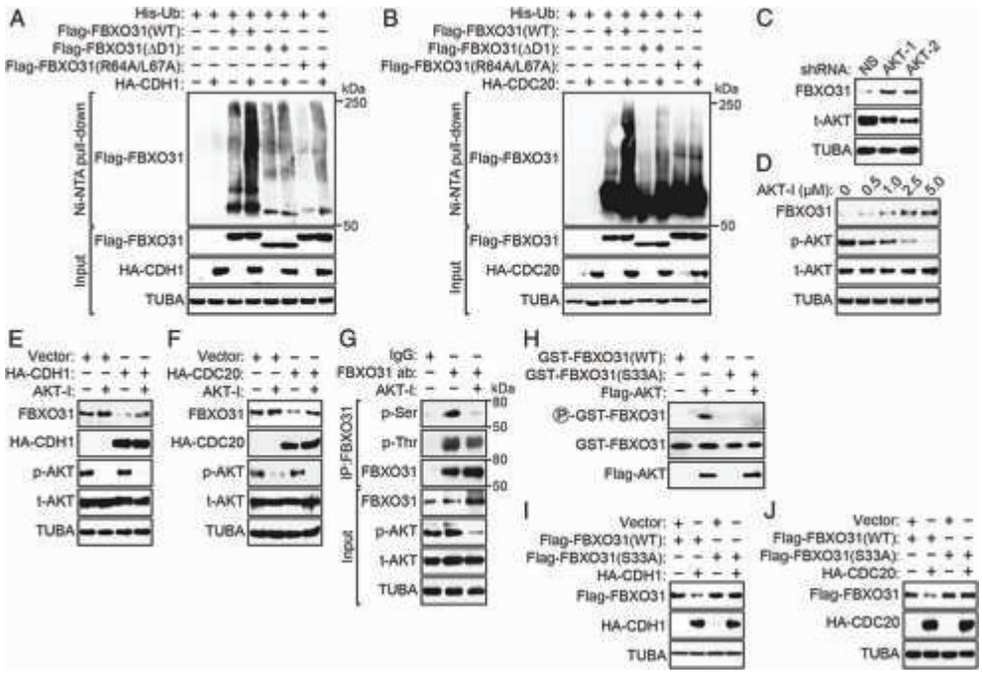

Fig. 2: Polyubiquitination of FBXO31 by CDH1 or CDC20 is dependent on phosphorylation by AKT kinase. (A and B) In vivo ubiquitination assay. HEK293T cells were cotransfected with plasmids expressing His-ubiquitin, Flag-FBXO31(WT), Flag-FBXO31(ΔD1), or Flag-FBXO31(R64A/L67A) and HA-CDH1 (A) or HA-CDC20 (B). Proteins bound to His-ubiquitin were purified by Ni-NTA pull-down, washed, and eluted in imidazole. Ubiquitinated Flag-FBXO31 was detected using an anti-Flag antibody. (C) Immunoblot monitoring the levels of FBXO31 and total AKT (t-AKT) in HEK293T cells expressing a NS shRNA or one of two unrelated AKT shRNAs. (D) Immunoblot monitoring FBXO31, phosphorylated AKT (p-AKT), and t-AKT in HEK293T cells treated with various concentrations of AKT inhibitor (AKT-I). (E and F) Immunoblot monitoring endogenous FBXO31 in HEK293T cells expressing HA-CDH1 I or HA-CDC20 (F) in the presence or the absence of AKT-I. (G) Coimmunoprecipitation monitoring the presence of phosphorylated Ser (p-Ser) or phosphorylated Thr (p-Thr) in the FBXO31 immunoprecipitate from HEK293T cells treated in the presence or the absence of AKT-I. (H) In vitro kinase assay monitoring the ability of purified AKT to phosphorylate GST-FBXO31(WT) or GST-FBXO31(S33A). (I and J) Immunoblot monitoring levels of Flag-FBXO31(WT) or Flag-FBXO31(S33A) in HEK293T cells expressing vector, HA-CDH1 (I), or HA-CDC20 (J).

the presence or absence of ectopically expressed CDH1 or CDC20 and monitored FBXO31 levels using an anti-Flag antibody. Our immunoblotting results demonstrate that the FBXO31(ΔD1) mutant was not degraded following ectopic expression of CDH1 or CDC20. The co-immunoprecipitation experiments confirmed that unlike FBXO31(WT), FBXO31(D1) did not detectably interact with CDH1 or CDC20.

Polyubiquitination of FBXO31 by CDH1 or CDC20 is Dependent on Phosphorylation by AKT Kinase.

Previous studies demonstrated that CDH1 and CDC20 interact with and promote polyubiquitylation of their substrates. We therefore assessed whether CDH1 and CDC20 could promote polyubiquitination of FBXO31. In the first experiment, we performed an in vivo ubiquitination assay in which HEK293T cells were co-transfected with plasmids expressing His-ubiquitin, HA-CDH1 or HA-CDC20, and either Flag-FBXO31(WT), Flag-FBXO31(ΔD1), or Flag-FBXO31(R64A/L67A). His-ubiquitin-conjugated proteins were purified using nickel beads under stringent, denaturing conditions, followed by immunoblotting with an anti-Flag antibody. The results confirmed that ectopic expression of CDH1 (Figure 2A) or CDC20 (Figure 2B) promoted polyubiquitination of FBXO31(WT), but not the FBXO31(ΔD1) or FBXO31(R64A/L67A) mutant.

Generally, recognition by E3 ubiquitin ligases requires phosphorylation of the substrate, which serves as a signal for ubiquitin-dependent destruction. Previous studies have shown that AKT has a prominent role in promoting progression through the cell cycle by acting on diverse downstream factors involved in controlling the G1/S and G2/M transitions. We therefore hypothesized that AKT could mediate the phosphorylation of FBXO31 required for its degradation by APC/C. To determine whether AKT was involved in regulating FBXO31 stability, we expressed an shRNA targeting AKT in HEK293T cells and monitored FBXO31 levels by immunoblot analysis. Figure 2C shows that shRNA-mediated knockdown of

AKT led to increased FBXO31 levels. Consistent with this result, treatment of HEK293T cells with a specific AKT chemical inhibitor resulted in increased FBXO31 protein levels in a dose-dependent manner (Figure 2D). We also observed that following ectopic expression of CDH1 or CDC20, degradation of endogenous FBXO31 was decreased by the AKT inhibitor (Figure 2E and 2F). Likewise, polyubiquitination of endogenous FBXO31 was also significantly reduced by the AKT inhibitor.

Although we could not find a consensus AKT phosphorylation site in FBXO31, previous reports have demonstrated that AKT can phosphorylate substrates independent of the consensus sequence. As a first step to determine which residue of FBXO31 was phosphorylated by AKT, we immunoprecipitated FBXO31 in the presence or absence of AKT inhibitor followed by immunoblotting with an anti-phosphorylated-Ser (p-Ser) or anti-phosphorylated-Thr (p-Thr) antibody to detect phosphorylated residues. The results of figure 2G show that the p-Ser signal in the FBXO31 immunoprecipitate was abrogated by treatment with the AKT inhibitor, whereas the p-Thr signal was only modestly affected. To predict which specific serine residue(s) of FBXO31 are likely to be phosphorylated by AKT, we queried the PhosphoSite database, which revealed Ser-33 as the most highly reported phosphorylated residue in FBXO31. To determine whether AKT phosphorylates FBXO31 at Ser-33, we constructed an FBXO31 derivative in which Ser-33 was mutated to Ala (FBXO31(S33A)). The in vitro kinase assay of figure 2H shows that AKT could phosphorylate FBXO31(WT) but not FBXO31(S33A), demonstrating that AKT directly phosphorylates FBXO31 at Ser-33.

Next, we tested the ability of CDH1 and CDC20 to degrade the phosphorylation-defective FBXO31(S33A) mutant. Immunoblot analysis showed that FBXO31(S33A) was not degraded following ectopic expression of CDH1 (Figure 2I) or CDC20 (Figure 2J).

Next, we performed an in vitro ubiquitination assay. In brief, we assembled reaction mixtures containing FBXO31 and cofactors (E1, E2, ubiquitin and ATP) in the presence or absence of AKT and either CDH1 or CDC20 immunopurified from transfected HEK293T cells (i.e., associated with APC/C). Data revealed that immunopurified CDH1 or CDC20 promoted polyubiquitination of FBXO31 in vitro in the presence but not absence of AKT. Collectively, these results show that polyubiquitination of FBXO31 directed by CDH1 or CDC20 and subsequent degradation is dependent upon phosphorylation of FBXO31 by AKT.

Interaction between FBXO31 and CDH1 or CDC20 is Perturbed Following DNA Damage.

Previous we showed that diverse DNA damaging agents increase FBXO31 levels, indicating that stabilization of FBXO31 is a general response to genotoxic stress. We therefore sought to determine whether the stabilization of FBXO31 following DNA damage is due to the loss of interaction with CDH1 and CDC20. To test this idea, we performed co-immunoprecipitation in HEK293T cells that were either untreated or treated with a DNA damaging agent. Following treatment with ionizing radiation (IR), the interaction between endogenous FBXO31 and CDH1 or CDC20 was lost (Figure 3A).

Our previous study showed that DNA damage-induced stabilization of FBXO31 is mediated post-translationally by ATM, which phosphorylates FBXO31 at Ser-278/Gln-279. To determine whether the loss of the FBXO31–CDH1 or

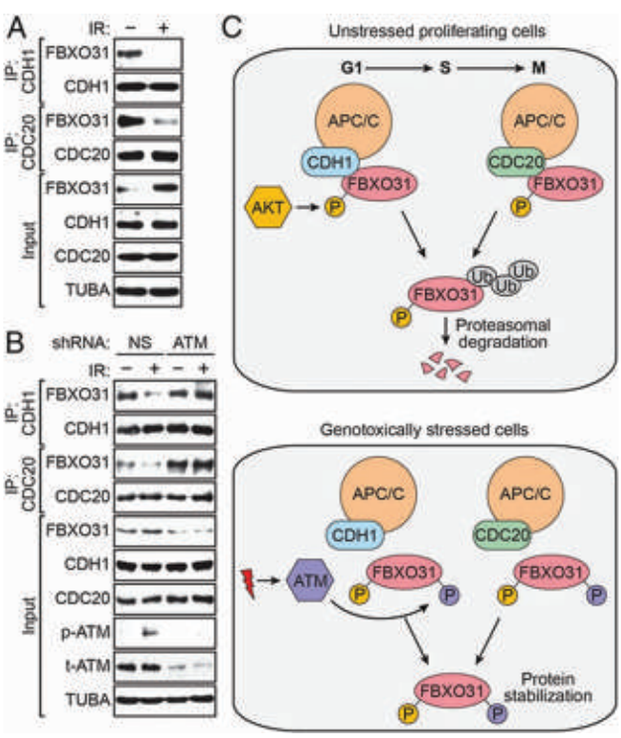

Fig. 3: Interaction between FBXO31 and CDH1 or CDC20 is perturbed following DNA damage. (A) Coimmunoprecipitation monitoring the interaction between endogenous FBXO31 and CDH1 or CDC20 in asynchronous HEK293T cells treated with or without IR. (B) Coimmunoprecipitation monitoring the interaction between endogenous FBXO31 and CDH1 or CDC20 in HEK293T cells expressing a NS or ATM shRNA, with or without IR. (C) Model for regulation of FBXO31 by APC/C.

FBXO31–CDC20 interaction following DNA damage was due to phosphorylation of FBXO31 by ATM, we performed co-immunoprecipitation experiments in irradiated HEK293T cells following shRNA-mediated depletion of ATM. As shown in Figure 3B, CDH1 and CDC20 failed to interact with FBXO31 in irradiated cells expressing a control NS shRNA. By contrast, in ATM-depleted cells, the interaction of FBXO31 with CDH1 or CDC20 still occurred following irradiation (Figure 3B). These results suggest that ATM-mediated phosphorylation of FBXO31 on Ser-278 prevents interaction between FBXO31 and CDH1 or CDC20, which leads to stabilization of FBXO31 following DNA damage.

Summary

The tumor suppressor protein FBXO31 is maintained at low levels in unstressed (normal cycling) cells, but rapidly increases following genotoxic stress (DNA damage), leading to induction of growth arrest or senescence. The mechanism that underlies physiological regulation of FBXO31 is unknown. Here we show that in unstressed cells FBXO31 is maintained at low levels by anaphase-promoting complex/cyclosome (APC/C), a ubiquitin ligase that targets cell cycle-regulatory substrates for proteasomal degradation. APC/C coactivators CDH1 and CDC20 interact with FBXO31 and coordinate its degradation, which requires phosphorylation by the pro-survival kinase AKT (Figure 3C upper panel). Following genotoxic stress, FBXO31 is phosphorylated by another kinase, ATM, disrupting its interaction with CDH1 and CDC20 and preventing its degradation (Figure 3C lower panel). Thus, a kinase switchaltered phosphorylation underlies physiological regulation of FBXO31 levels.

Future Research Plans

- In the coming year, we would like to investigate whether FBXO31 has any role in other phases of cell cycle as well as in signaling pathway associated with cancer.
- Determination of how FBXO31 is regulated at different phases of cell cycle.

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Role of RNA-protein Interactions in *Plasmodium falciparum* Infection

Background

Plasmodium falciparum is a causative agent for malaria and has a complex life cycle in human and mosquito hosts. Translation repression of specific set of mRNA has been reported in gametocyte stages of this parasite. A conserved element present in the 3'UTR of some of these transcripts was identified. Biochemical studies have identified components of the RNA storage and/or translation inhibitor complex but it is not yet clear how the complex is specifically recruited on the RNA targeted for translation regulation. We used the 3'UTR region of translationally regulated transcripts to identify Phosphatidyl-inositol 5-phosphate 4-kinase (PIP4K2A) as the protein that associates with these RNAs. We further show that recombinant PIP4K2A has the RNA binding activity and can associate specifically with *Plasmodium* 3'UTR RNAs. Immunostainings show that hPIP4K2A is imported into the Plasmodium parasite from RBC. These results identify a novel RNA binding role for PIP4K2A that may play a role in *Plasmodium* propagation.

Aims and Objectives

- To identify specific proteins that interact with Parasite RNA sequences.
- To characterize the role of this interaction in *Plasmodium* propagation.

Work Done

Translationally repressed transcripts have been identified in both *P. berghei* and *P. falciparum*. We believed that specific proteins may associate with these transcripts and play a role in translation regulation. We performed RNA-EMSA experiments with protein extracts prepared from mixed stage *P. falciparum* and Pf1 or 1059 RNA (the 3'UTR of P. falciparum Pfs28, and *P. berghei* Rad51

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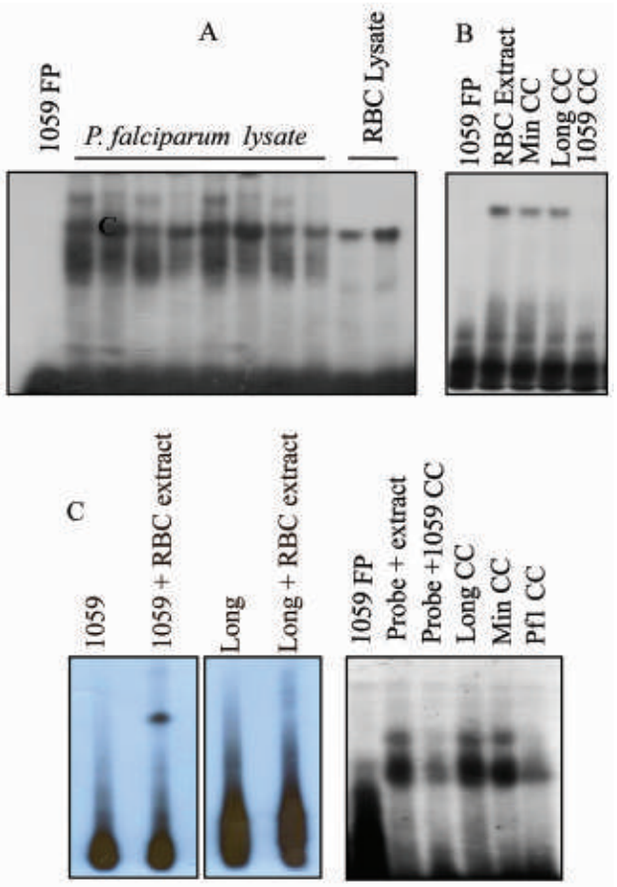

Fig. 1: *Plasmodium* UTR RNAs interacts with host erythrocytic factors (A) RNA EMSA analysis with α-P32UTP labeled 1059 UTR and extract from *P.falciparum* or erythrocytes. The labeled RNA and extract were incubated and the complex formed were resolved on 6% PAGE. The sample in each lane is indicated. (B) Competitive RNA EMSA with α-P32UTP 1059 UTR RNA, RBC extract and hundred fold excess of unlabeled competitor RNA. The competitor RNA used is indicated, 1059 is self competitor while Long and Min are unrelated non specific RNAs that do not compete for the factors. (C) RNA EMSA analysis with α-P32UTP labeled 1059 UTR RNA (left panel) or Long RNA (middle panel) and extract from erythrocytes. The complex were resolved on 6% PAGE. The sample in each lane is indicated. Competitive RNA EMSA with α-P32UTP 1059 UTR, RBC extract and unlabeled etabolizi RNA (right panel). The competitor RNA used is indicated.

homolog respectively), transcripts that are known to be translationally repressed in gametocyte stages. We find that RNA-protein complex is formed with parasite extract, which could be competed with specific (1059 or Pf1) competitor but not by nonspecific RNA (Fig. 1A). Uninfected human erythrocytic lysate was also used to serve as negative control. To our surprise we observed a specific complex formation with lysates from uninfected erythrocytes. We further confirmed the specificity of complex formation by using molar excess of specific (1059) or non- specific (Long and Min) RNA competitors. Addition of 1059 specific cold competitor abolishes the RNA protein complex while non-specific competitor RNA's Min and Long did not inhibit the complex formation, confirming the specificity of complex (Fig. 1B). Pf1 UTR (RNA corresponding to the 3'UTR of Pfs28 one of the transcripts from *P. falciparum* that is translationally regulated during gametocyte stage) compete the complex formed by 1059 probe and the proteins, suggesting that Pf1 and 1059 UTR recruits common factors from host RBC (Fig 1C).

Plasmodium UTR recruits a multi-protein complex from human erythrocytes and *Plasmodium* lysate

To identify the molecular weight of proteins present in the complex that is recruited/assembled on 1059 UTR from *Plasmodium* parasite and host erythrocyte lysate, U.V cross linking experiment was performed. Radiolabeled 1059 probe was cross-linked to proteins from *P. falciparum* or erythrocytes. Four distinct protein bands having molecular weight between 20-80kDa were observed in erythrocytic lysate as well as in *P. falciparum* mix stage lysate (Fig.2A). Interestingly, as we have hypothesized a conserved mechanism of UTR mediated translation regulation across genus *Plasmodium*, UV cross-linking experiment suggests recruitment of similar proteins on both the UTR's Pf1 and 1059 (Fig. 2B).

Human erythrocytic protein PIP4K2A (Phosphatidyl inositol 5 phosphate 4 kinase) interacts with *Plasmodium* UTR

In order to identify the proteins that interact with the 3'UTR RNA we biotinylated the 1059 RNA and used it to purify specific RNA binding proteins from RBC lysate. A non-specific biotinylated RNA (Min RNA) was used for control pull down experiments. A specific band corresponding to 50 kDa was observed only in 1059 UTR lane in 1X TE+ 1M NaCl elution (Fig. 2C). This specific band was cut out and the proteins present were analyzed by MS/MS after trypsin digestion and Phosphatidyl inositol 5 phosphate 4 kinase (PIP4K2A) was identified as one of the protein that was present in the band. Phosphoinositide signaling has been studied extensively across genus *Plasmodium*, many of the kinase have well defined role in *Plasmodium* parasite propagation. Interestingly PIP4K2A family of enzymes appeared relatively late in the evolution of eukaryotes as it is absent in all the unicellular eukaryotes including yeast and bioinformatic analysis of *Plasmodium* genome and *Plasmodium* database indicate the absence of PIP4K2A in *Plasmodium*.

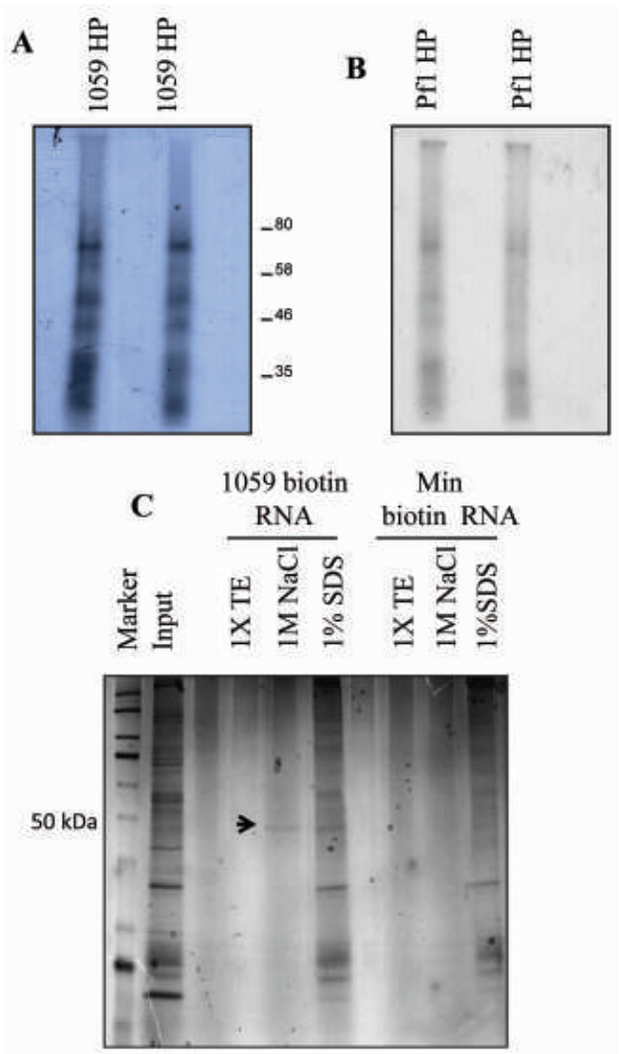

Fig. 2: Multiple proteins from erythrocyte and *P. falciparum* interact with 1059 and or Pf1 UTR. *Plasmodium falciparum* extract was incubated with 1059 (A) or Pf1 (B) P^{32} labeled RNA EMSA reaction was exposed to UV followed by RNAse digestion and the labeled proteins were resolved on 10% PAGE. (C) Biotinylated 1059 RNA was used to pull down specific interacting proteins from RBC lysate. The bound proteins were eluted sequentially with TE, 1M NaCl and lastly with 1% SDS. The eluted proteins were resolved and silver stained. The specific 46 kDa band observed in 1059 RNA column but not in nonspecific control Min RNA was identified as PIP4K2A by mass spectrometry.

Recombinant PIP4K2A has RNA binding activity and interacts with *Plasmodium* UTR element

Results from the U.V cross linking and biotin RNA pull down experiments suggest formation of a multi-protein complex with *Plasmodium* UTR 1059 or Pf1, having host erythrocyte protein PIP4K2A as one of the factor. Sequence analysis of PIP4K2A did not reveal any specific RNA binding domains thus we wanted to assay if it has any RNA binding activity or it interacts with RNA through other proteins. We cloned and expressed human PIP4K2A in bacteria and purified it as a His-tagged protein (Fig. 3A) and assayed for its RNA binding activity by RNA mobility shift assay. Recombinant His-PIP4K2A binds specifically to 1059 or Pf1 UTR, suggesting direct binding of PIP4K2A to the *Plasmodium* UTRs (Fig. 3B).

hPIP4K2A is imported into *Plasmodium falciparum*

Plasmodium parasite during its erythrocytic life cycle exports and imports various proteins to and from host erythrocyte. We assessed if hPIP4K2A is also imported from host erythrocyte into *Plasmodium* parasite by immunostaining. PIP4K2A specific staining was observed in erythrocytes, and increased staining was observed in side the DAPI positive *P. falciparum* parasite, indicating that PIP4K2A is abundantly present in human erythrocyte and is enriched inside the DAPI positive *P. falciparum* parasite. PIP4K2A distribution in *Plasmodium* parasite is homogeneous and is present throughout the parasite cytoplasm (Fig. 3C). This suggest that *Plasmodium* parasites import host erythrocytic protein PIP4K2A.

It is believed that the main role of PIP4K2A is in regulating the levels of PI5P in mammalian cells. PIP4K2A is predominantly a cytoplasmic protein, however its substrate is membrane bound. Phospho inositides and their kinases also play an important role in the growth of *P. falciparum*. Parasite export several proteins to the erythrocytes that require the interaction of these proteins to lipid associated Phosphatidyl Inositol 3 Phosphate in the parasite endoplasmic reticulum. Proteins regulating these have been identified as specific target for drugs against malaria. We have shown that one member of this pathway, PIP4K2A is imported into the parasite from the host where it associates with specific parasite RNA. The exact mechanism of how PIP4K2A is imported into the parasite is still unclear. Although we show that PIP4K2A interact with few specific RNA it is possible that it may bind to other targets in both host and parasite. In case of PIP4K2a, the relationship between the RNA binding activity and the kinase activity is not known, however it is possible that the RNA binding may regulate the localization and kinase activity of PIP4K2a and thereby affecting its interacting partners. RNA binding activity of PIP4K2A may be an important function of PIP4K2A apart from its role in inositides metabolism.

Future Research Plans

- We have identified human PIP4K2A as an RNA binding protein that associate specifically with few Plasmodium transcripts. We believe that PIP4K2A may interact with many other transcripts in plasmodium and also it is likely that it

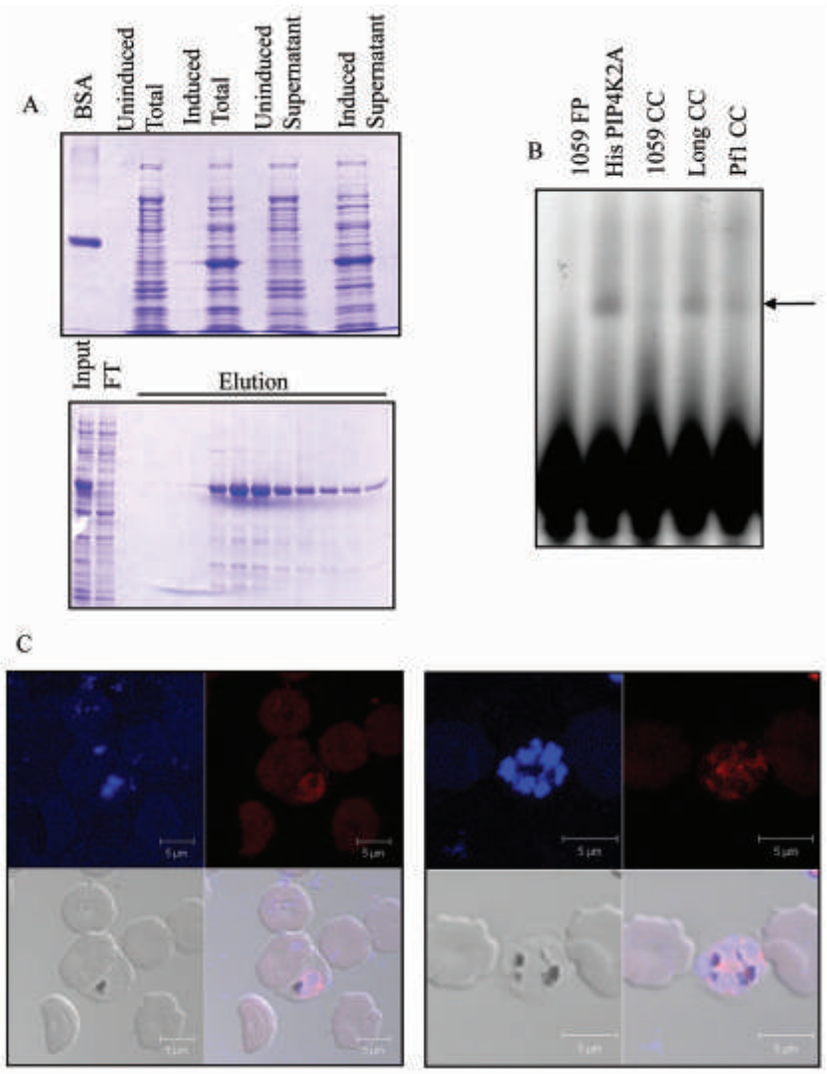

Fig. 3: hPIP4K can bind to *Plasmodium* RNA directly and is imported in to *Plasmodium* parasite (A) Human PIP4K2A was cloned and expressed in BL21 strain. The recombinant protein production was induced by IPTG. The protein extracts were prepared and analyzed on SDS-PAGE (Top panel). The recombinant His-tagged protein was purified using Ni-NTA column (lower panel). The sample in each lane is indicated. (B) Purified recombinant His-tagged PIP4K2A was used in EMSA reaction with unlabeled 1059 RNA or Pf1 (specific competitor) or Long-RNA (non-specific) used as competitors. The RNA-Protein complex is shown by an arrow and the competitor used is indicated in each lane. (C) Immunostaining of *P. falciparum* infected erythrocytes (DAPI; blue) with PIP4K2A rabbit monoclonal Ab (red). The dark particulate stain is due to parasite hemozoin.

may associate with specific mammalian transcripts as well. We will be characterizing the transcripts from *Plasmodium* and human cells that specifically associate with PIP4K2A.

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Understanding the Mechanism of Transformation Elicited by a novel Long Non-coding RNA - Ginir

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Background

It is getting increasingly apparent that the genomes of many species are pervasively transcribed, resulting in the production of numerous long noncoding RNAs (lncRNAs). At the same time, it is now appreciated that many types of DNA regulatory elements, such as enhancers and promoters, regularly initiate bi-directional transcription. Thus, discerning functional noncoding transcripts from a vast transcriptome plays critical role in mediating gene regulation in higher eukaryotes. We have identified a pair of long noncoding RNA termed as Ginir/Giniras from mouse cells and have performed extensive studies to elaborate its role in cell growth and transformation. Using a series of several *in vitro* and *in vivo* experiments we have shown that excess amounts of one of the transcript Ginir is responsible for causing unregulated cell growth in mouse cells and ultimately leading to malignant transformation.

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

Aims and Objectives

- To study cell cycle dependent expression of long noncoding RNA Ginir.
- To investigate whether biological effects of Ginir are mediated through interaction with specific protein partners like Kif20b.
- To evaluate the network of Kif20b interacting proteins and determine role of Ginir in the Kif20b interactome.
- To perform differential gene expression and pathway analyses of Ginir over-

expressing cells by whole transcriptome sequencing and validate the specific targets by gene over-expression and knock-down studies in mouse cells.

Work Done

Our earlier studies have extensively elaborated on identification and functional characterization of a novel linc RNA pair Ginir/Giniras in mouse cells and shown its role in maintenance of genomic stability and cellular homeostasis. Our previous studies showed that Ginir was able to exert regulatory role during cell cycle division and thereby ensure genomic stability. We found that in mouse cells like NIH/3T3 fibroblasts where Ginir expression was ectopically induced, it led to cells showing malignant transformation. Also, transformed cells showed higher expression of Ginir as compared to normal untransformed cells. 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 over-expressed cells to decipher mechanistic clues for Ginir action and function. Since, interaction of non-coding RNA pair –Ginir/Giniras with proteins could be one of the ways through which Ginir would 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 interaction of Cep112 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. We additionally in the Mass-spec analyses found that another microtubule associated protein Kif20b was an interacting protein partner for Ginir. Next, we validated the interaction of Ginir RNA with Kif20b protein. Kif20b is a member of kinesin 6 superfamily of proteins, also known as M-phase

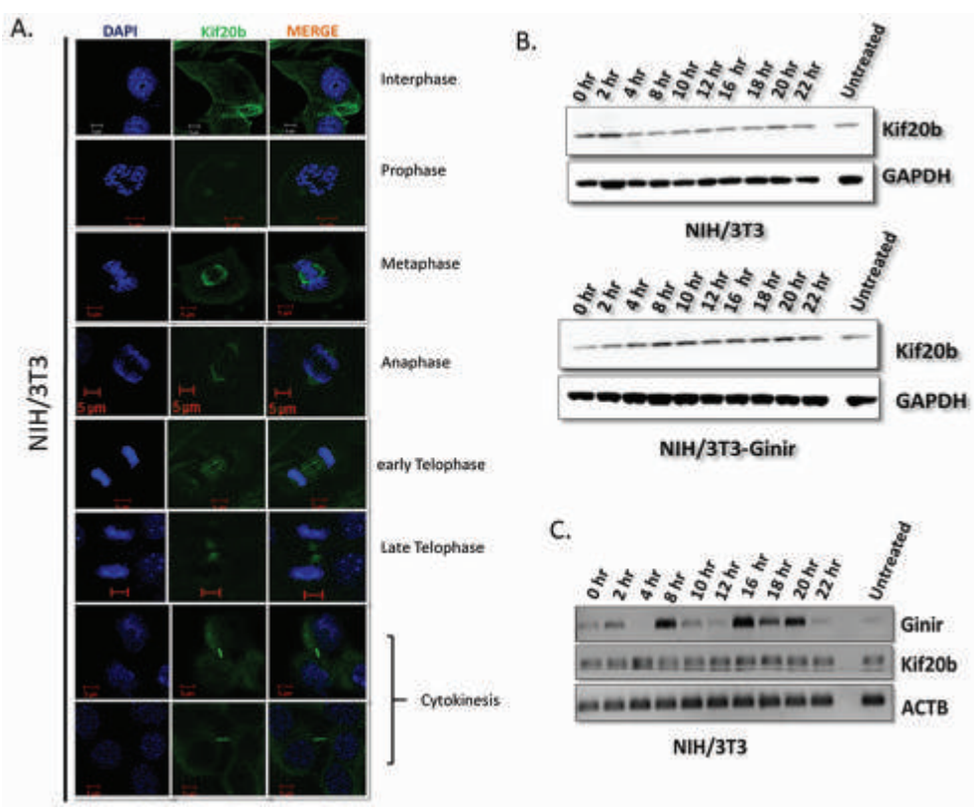

Fig. 1: Cell cycle dependent expression of Ginir and Kif20b

A) Western blotting for Kif20b after release from Aphidicolin at indicated time points.
B) Immunostaining for Kif20b in NIH/3T3 cells at different stages of cell cycle.
C) SemiQ RT PCR showing expression of Kif20b and Ginir after release from Aphidicolin at indicated time points

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.

Cell cycle dependent expression of Ginir and Kif20b

Next, to understand role of Ginir and its interacting protein Kif20b in cell cycle regulation, we analyzed for expression and localization of Ginir and Kif20b. We were interested to know the variation of its expression and localization in different phases of cell cycle. To achieve this, we used Aphidicolin to synchronize cells at G1/S phase. Aphidicolin is a cell cycle inhibitor which blocks the cells by acting on DNA polymerase subunits. We obtained effective synchronization of NIH/3T3 cells at a concentration of 5 µg/ml incubated for 14 h., wherein more than 80% of the cells showed arrest in G1/S phase analyzed through cell cycle analysis with PI staining. The cells were then released and analyzed for expression of Kif20b at both protein and RNA level at every 2 h interval (Fig. 1A, B). We also analyzed Ginir expression at these indicated time points through strand-specific semi quantitative RT-PCR (Fig.1C). Ginir showed significant increment at the 8 and 16 hr (proceeding towards the M phase). Kif20b, on the other hand, did not show much variation in the expression levels both at RNA and protein levels in both NIH/3T3 and NIH/3T3-Ginir cells. However, the immunofluorescence experiment (Fig.1B) showed variation in intra-cellular localization of Kif20b. It showed prominent localization at midbody in the late telophase and cytokinesis step. Kif20b was earlier reported to be important for completion of proper cytokinesis. Its localization at midbody might be important for its function during cytokinesis.

Aurora kinase A interaction with kif20b disrupts in Ginir overexpressed cells

To study the effect of interaction of Ginir with Kif20b, we determined further downstream protein interactors of Kif20b to analyze 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. In attempt to validate the interaction of these proteins with Kif20b, we performed Co-immunoprecipitation experiments and found that AurkA interacted with Kif20b. However, the cells in which Ginir wasover expressed, this interaction of AurKA with Kif20b wasdisrupted (Fig.2A). Furthermore, 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

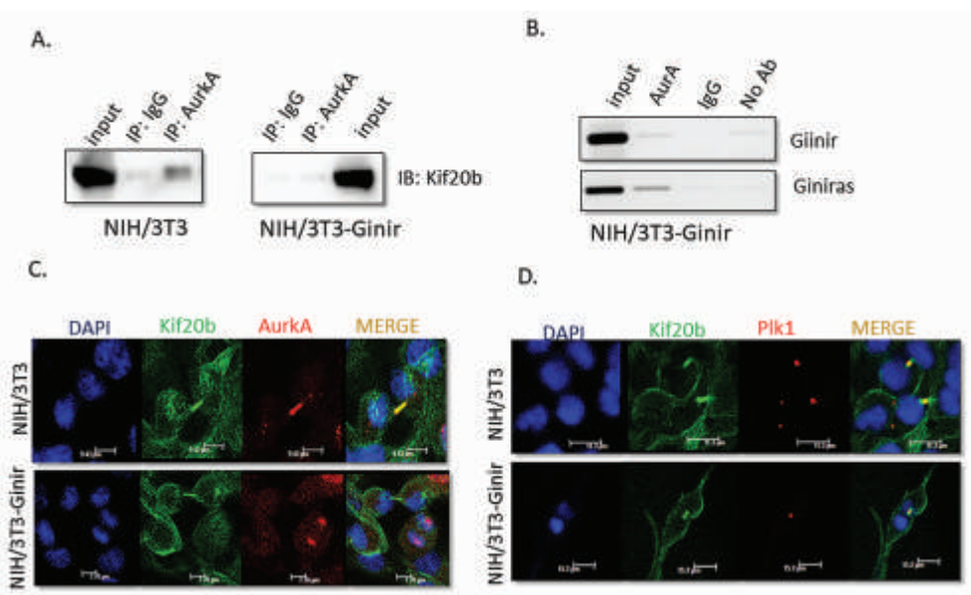

Fig. 2: Aurora kinase A interacts with Ginir and Giniras along with Kif20b

A) Co-immunoprecipitation with Aurora kinase A followed by western blotting for Kif20b in NIH/3T3 and NIH/3T3-Ginir cells.
B) UV crosslinking RNA-IP with Aurora kinase A followed by SemiQ RT PCR(strand specific) for enrichment of Ginir and Giniras.
C) Immunofluorescence for Kif20b and Aurora kinase A at telophase stage of the cell cycle in NIH/3T3 and NIH/3T3-Ginir cells.
D) Immunofluorescence for Kif20b and Plk1 at telophase stage of the cell cycle in NIH/3T3 and NIH/3T3-Ginir cells

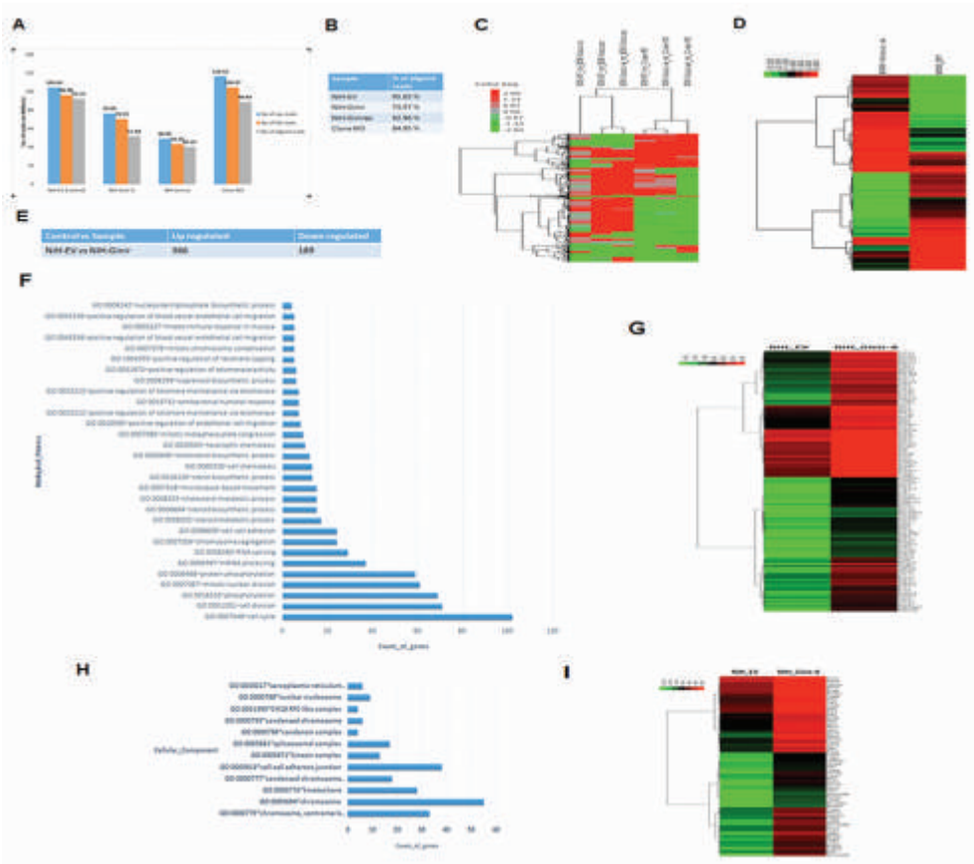

Fig.3: RNA-Seq analyses of Ginir expressing cells

A) Graph showing number of high quality reads and aligned reads out of total number of raw reads for NIH-EV, NIH-Ginir-A, NIH-Giniras and Clone-M3 cells.
B) Table showing percentage of aligned reads for NIH-EV, NIH-Ginir-A, NIH-Giniras and Clone-M3
C) Heat map representing differentially expressed genes through whole transcriptome sequencing in NIH-Ginir vs NIH-EV; NIH-EV vs NIH-Giniras; NIHGiniras vs NIH-Ginir; NIH-EV vs Clone M3; NIH-Ginir vs Clone M3; NIH-Giniras vs Clone M3
D) Heat map representing differentially expressed genes through whole transcriptome sequencing in NIH-Ginir as compared to NIH-EV
E) Total number of up and down regulated genes in NIH-Ginir cells in comparison to NIH-EV control cells using p-value less than 0.05 using Cuffdiff analysis
F) Enrichment in GO terms for the genes upregulated in NIH-Ginir compared to NIH-EV for 'Biological Process'
G) Heat map representing the relative expression of the genes showing enrichment of these GO terms.
H) Enrichment in GO terms for the genes upregulated in NIH-Ginir compared to NIH-EV for 'Cellular Component'
I) Heat map representing the relative expression of these genes showing enrichment of these GO terms.

cytokinetic furrow (Fig.2C) and not at any other cellular location or phase of cell cycle. The co-localization of AurkA at midbody was only observed in NIH/3T3 cells but not in Ginir over expressed cells which is also supported by our Co-IP data. Absence of AurkA at midbody was found to be specific as another important midbody marker Plk1 was observed at midbody in both NIH/3T3 and NIH/3T3-Ginir cells (Fig.2D). Our aim is to study the significance of these interactions in regulation of cytokinesis in Ginir over expressingcell system (endogenous and exogenous).

Aurora kinase A interacts with both Ginir and Giniras along with Kif20b

To study the association of Aurka with Ginir RNA, we performed UV crosslinking RNA-IP and found that Ginir was enriched in elutes after immunoprecipitation with Aurka. Semi quantitative PCR with strand specific cDNAs for sense and antisense strands showed that AurkA interacted with both Ginir and its antisense counterpart Giniras (Fig.2B).

Whole transcriptome analysis of cells over expressing Ginir

Further, to investigate the differential gene expression upon

Ginir over-expression, we performed whole transcriptome sequencing of NIH/3T3-EV, NIH/3T3-Ginir (A), NIH/3T3-Giniras and Clone M3 cell-lines. The high quality reads obtained with the above-mentioned cell-lines is shown in Fig.3A, B. Differential gene expression from these cells lines is represented in heat map in Fig.3C. Heat map in Fig.3D represents differential gene expression in NIH/3T3-Ginir cells as compared to control NIH/3T3 (NIH/3T3-EV) showing a total of 366 upregulated and 189 downregulated genes in NIH/3T3-Ginir cells compared to NIH/3T3-EV (Fig.3E) ($P \leq 0.05$) To further identify the differential gene enrichment of the functional terms to biological pathways, we performed Gene Ontology (GO) analysis of the gene data sets. The results of this analysis and heat-map showed that the enrichment terms were mainly concentrated to pathways important in cell-cycle, cell division, mitosis, RNA splicing, RNA processing and cell-cell adhesion (Fig.3F, G). Analysis of the cellular components involved in these processes, indicated that most of these genes resided in the centromeric regions of the chromosomes, the kinetochores and to the cell-cell adherens junctions (Fig.3H, I).

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.

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Human Microbiome: Indian Perspective

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Background

Humans are colonized for life with more than trillions of bacteria, creating a vast diverse ecosystem whose contributions to human health remain poorly understood. In recent years, there has been a revolution in Biology due to advances in sequencing technology that has helped understand how and why humans harbor multitudes of microbes. Advances in research in recent years have now made it possible to understand this unexplored reservoir of microbes. Increasing evidence suggests that the human 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. Ultimately, understanding the characteristics of microbiota may lead to natural therapeutics for human diseases and understanding of life evolution.

Aims and Objectives

- Determinants shaping the microbiome composition in Indian population.
- Eco-physiology of gut microbial members.
- Role of gut microbial community in healthy individuals.
- Microbial diversity of extreme environments.
- Role of microbiome in partially understood gastrointestinal and respiratory diseases and metabolic disorders.

Work Done

Microbiome of healthy individuals

Joint family structure, a common practice in rural India provides unique

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opportunity for in depth analysis of any factor affecting human microbiome by keeping other microbiome confounding factors constant. We have studied gut, oral, and skin microbiome of 54 healthy individuals belonging to six patrilineal joint families with three generations staying in the same household from the endogamous Indian sub-population. Analysis revealed presence of many core OTUs shared between subjects at different body sites and the genus *Prevotella* being dominant in gut as well as in oral cavity.

Beta diversity analysis using differentially abundant OTUs in gut, oral and skin microbiome showed grouping across the age groups . A higher abundance of phylum *Proteobacteria* was observed with age in the gut microbiome whereas, in the oral microbiome higher abundance of phylum *Fusobacteria* was observed with the age. However, in the skin microbiome no statistically significant correlations were observed in the relative abundance of bacterial genera relating to age suggesting transient nature of skin microbiome. In conclusion, age has minimal effect on human microbiome if other known microbiome confounding factors kept constant.

Core Indian Microbiome

Here we explore the possibility of understanding the core gut microbiome of a healthy Indian population. While it may not always represent the most dominant phyla but can be thought to be important for certain critical metabolic functions. QIIME analysis for core microbiota revealed the presence of genus *Prevotella* in 80% of the population while 55% of the population harbored genus *Megasphaera, Roseburia* and *Bacteroides* along with *Prevotella* in their gut. Although the sequences from core I showed taxonomic identity as *Prevotella copri* using GreenGenes database, phylogenetic analysis reveals distinct clade which we believe is indicative of a different species of *Prevotella* present in Indian population. The microbiome community as well as the core OTUs showed higher abundance of genes responsible for carbohydrate metabolism. These observations can be supported with the average higher consumption of carbohydrate-rich and plant-derived foods in the diet of Indian population. Collectively, these findings suggest association of carbohydrate rich diet in enrichment of gut bacteria with diverse CAZymes in the Indian population.

Understanding the changes and influence of maternal gut microbes during pregnancy on the infants early microbial gut flora

The gut microbiome profiles from 20 mother-infant pairs at

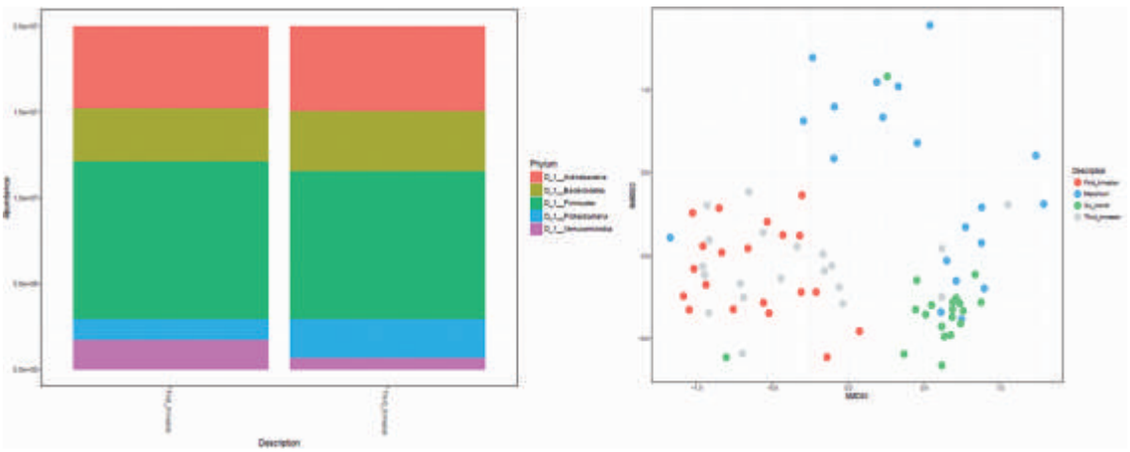

Fig.1a. Gut microbial diversity during pregnancy. A. Phylum level distribution of the 5 most dominant bacterial taxa during pregnancy. B. NMDS plot illustrating the differences in gut bacterial diversity during pregnancy and early infancy

different time point's viz. first trimester and third trimester from mothers and meconium and six months fecal samples from infants were studied. Results show that gut microbiome profile changes during the length of pregnancy (Fig 1a and. The meconium samples show a huge diversity which suggesting that there is a diverse gut bacterial community structure in new born infants which stabilizes as the age increases. The further analysis showed that *Escherichia- Shigella*, *Bifidobacterium* and *Streptococcus* were more abundant in six month samples, while *Enterococcus*, *Enterobacter* and *Klebsiella* were abundant in meconium samples. The presence of facultative aerobes during early life in infant gut can be attributed to the aerobic environment in gut in early life which becomes anaerobic over a period of days. The shift to more anaerobic environment explains the colonization of gut by anaerobes like *Bifidobacteria*, *Streptoccous* and *Escherichia*.

Human Milk Microbiota

The main and continuous source of microbial inoculum for the gut of infants is mother milk. It's is quite interesting to know the various factors affecting the milk microbiota of lactating mothers and its effect on infants gut microbial flora.The study aims to characterize the diversity of the microbiota in human milk samples collected from healthy mothers in India and compare it with the microbiota from other populations.Our analysis shows that the most abundant bacterial phyla in Indian healthy mothers is *Firmicutes* followed by *Proteobacteria* and *Actinobacteria*. While at the genus level *Megasphaera* is the most abundant genus contributing 40.28% of the total bacterial community in the milk. Further, the core milk microbiota analysis shows that *Megasphaera*, *Streptococcus*, *Staphylococcus*, *Bacillus*, and *Williamsia* are the core bacterial genera at a detection level of 0.01. The NMDS profile of microbial diversity

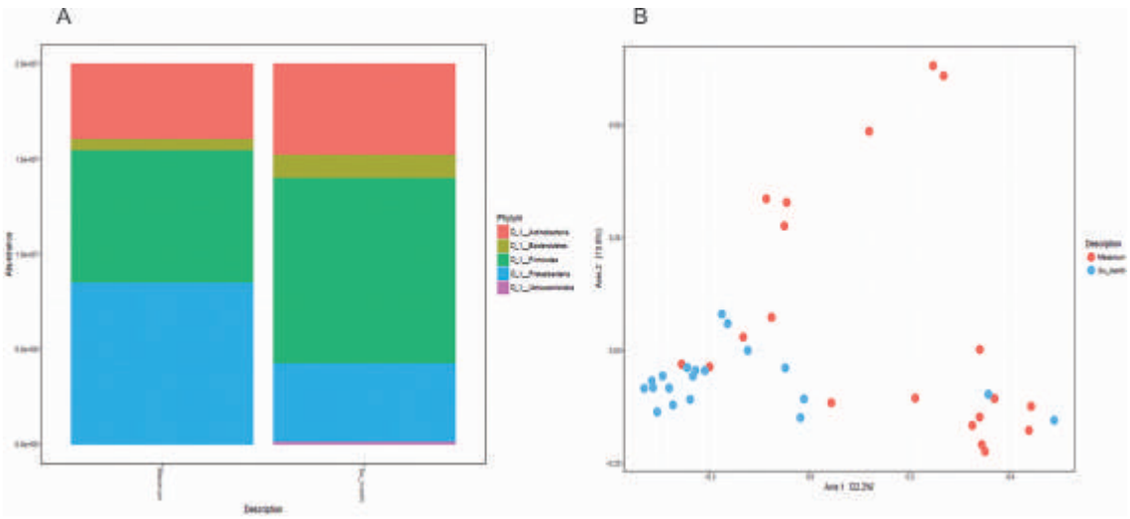

Fig.1b. Gut microbial diversity during early infancy. A. Phylum level distribution of the 5 most dominant bacterial taxa during infancy. B. NMDS plot illustrating the differences in gut bacterial diversity from early infancy to six months.

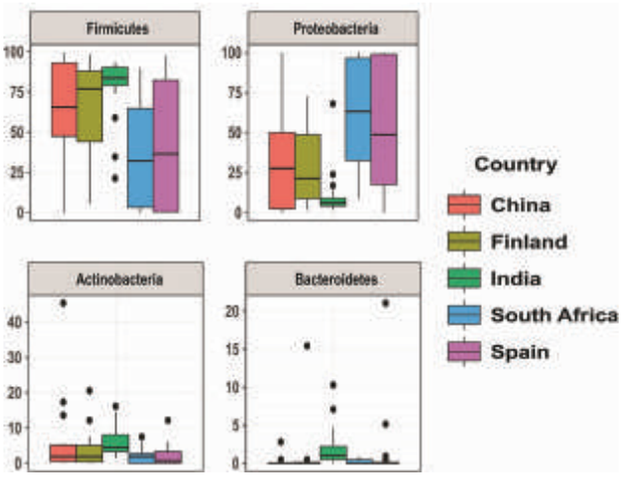

Fig: 2 Boxplots showing significant difference (p>0.05) at phylum level across all population in the breast milk.

shows that milk samples of healthy Indian mothers are distinctly clustered in NMDS plot (Fig 2) .

Type 2 Diabetes and microbiome

Type 2 diabetes (T2D) though considered as a metabolic non-communicable disease has reached to epidemic proportions worldwide. Shedding light on Indian population, we sought to address variation in gut microbiota of newly diagnosed diabetic (New-DMs, n=13), diabetic subjects (Known-DMs, n=50), and diabetic control subjects (NGTs, n=50) and their association with pathophysiology of T2D.

Significant differences in major bacterial phyla were observed in the gut microbiota of diabetic subjects. Notably, phylum *Firmicutes*, *Proteobacteria* and *Actinobacteria* were elevated, while Bacteroidetes was reduced in both New-DMs and Known-DMs subjects ($p<0.05$) Two dominant members of Bacteroidetes phylum viz. *Prevotella* and *Bacteroides* were found to follow inverse relationship with respect to their abundance in study subjects (Fig 3a). Considering high abundance of *Prevotella* in healthy Indian subjects we found that abundance of *Prevotella* decreased and concomitantly, the abundance of *Bacteroides* increased in diabetic subjects. Taxa that were highly discriminant among three study groups and found that *Akkermansia* were observed to be highly depleted in New-DM subjects, *Lactobacillus* found to be increased in both New-DMs and Known-DMs. Lachnospiraceae family, which includes *Lachnospira* significantly increased in New-DMs and *Blautia* was found to be discriminative for Known-DM subjects (Fig 3 b).Based on or analysis, we speculate that certain microbes can be a potential target for gut microbiota-based diabetes therapies.

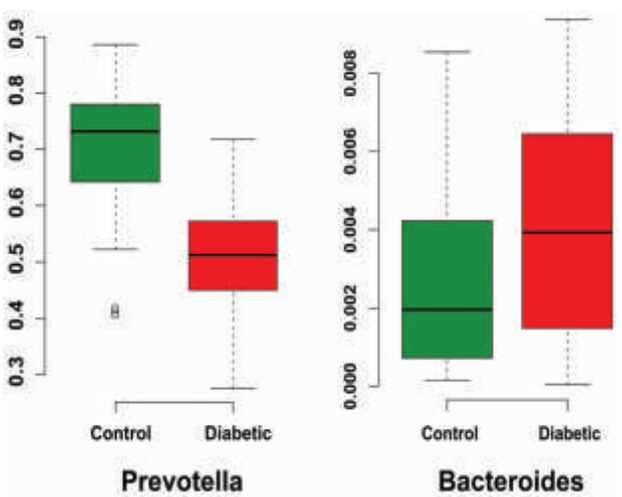

Fig.3a: Abundance of Prevotella and Bacteroides in the study groups.

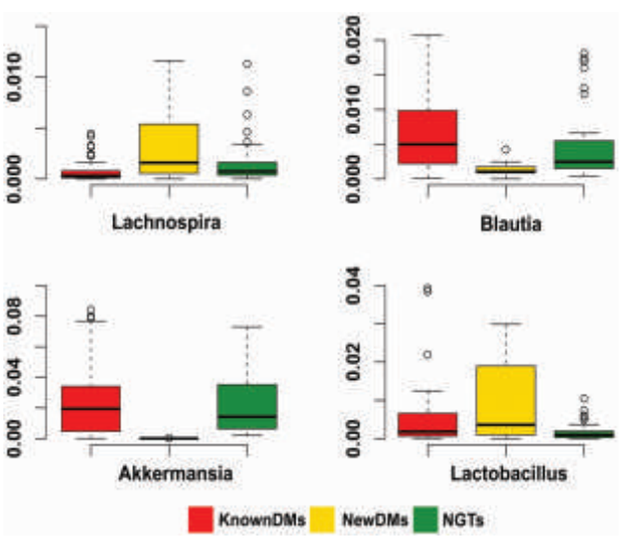

Fig.3b: Abundance of discriminating OTUs (based on Random Forest analysis) among the three groups.

Future Research Plans

- A proposal for pan India study of human microbiome across different endogamous group and geographical regions is submitted for funding.
- Studies on variation of microbiome in some GI disorders like Non Celial Gluten Sensitivity.

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

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Background

Interleukin 6 (IL6) is a pleiotropic cytokine that plays a crucial role in many biological processes including activation of immune system, metabolism, bone homeostasis, pathological pain and in regenerative processes of the body. Together with IL1beta and TNFalpha, IL6 have been elevated in many of the auto immunity and inflammatory disease like rheumatoid arthritis, bowel disease and castle man disease, therefore, it is well recognized target for therapeutic intervention in many inflammatory and auto immune diseases.

IL6 signaling occurs through the complex of IL6-IL6Receptor which leads to the homodimerization of gp130 glycoprotein which is ubiquitously present on every cell membrane. IL6 signals through two ways: IL6 bind to membrane bounded IL6R (mbIL6R) and perform homodimerization of gp130 which leads to signal transduction. This is called IL6 *classical signaling*, whereas IL6 binds to soluble form of IL6R (sIL6R) and mediate IL6 signal transduction in cell which don't express mbIL6R, this is called IL6 *Trans signaling*. Classical signaling is correlated with anti-inflammatory or regenerative response whereas trans signaling is meant for proinflammatory response.

Leishmaniasis is the second most neglected tropical disease after malaria in terms of parasitic disease mortality. It is caused by intracellular protozoan parasite from the genus Leishmania and transmitted through the bite of phlebotomine sandfly which acts as an intermediate host. The disease is associated with the socio-economic status of people. Malnutrition, population displacement, poor housing, environment fluctuation, a weak immune system and lack of resources are the major reasons that causes high disease endemicity in many part of the world including countries of Asia, Africa, South Europe, Central and South America. Every year approximately 0.2 to 0.4 million of

visceral leishmaniasis cases and 0.7 to 1.3 million of cutaneous leishmaniasis cases have been reported which causes annual death of 20,000-30,000 worldwide.

With reference to leishmaniasis, IL6 has been detected in the serum level of VL and CL patient and found to be reduced in VL patient after treatment. This indicates the role of IL6 in pathogenesis of *Leishmania*. Literature review suggested that LPG (a component of Leishmania cell membrane) activates NFkB through interaction of Toll like receptors namely TLR2 and TLR1/6 complex and bring out the release of IL6 and many other proinflammatory cytokines from macrophages. Cytokine IL6 has both pro inflammatory and anti-inflammatory action. Therefore, its role in Leishmaniasis is critical to determine. Some studies suggested that IL6 knockout mice showed enhanced control over infection with rapid parasite killing and stimulation of anti leishmanial Th1 response: increased levels of circulating gamma interferon, accelerated granuloma assembly, and heightened responsiveness to chemotherapy .Further, studies on IL6 deficient C57BL/6 against *L. major* infection showed increased IFNγ concentration in lymph node and decrease foot pad diameter with respect to *L. major* infected IL6 positive C57BL/6.This shows that there is efficient immunity against Leishmania infection in absence of IL6. On contradictory to this, some reports are there which shows IL6 functions in disease protection against *Leishmania donovani*. Adoptive immunotherapy is use as tool to study immunoregulation *in vivo*. Adoptive dendritic cell immunotherapy is being used to enhance host resistance in many leishmanial experimental models. LPS activated bone marrow derived DC (BMDC) are adoptively transfer to mice models and found to enhance resistance whereas IL6 deficient BMDC shows no protective effect which indicate the importance of IL6 production for efficacy of BMDC. Thus IL6 function in host protective against *L. donovani* in dendritic cell.

Turning towards other cytokine which function in leishmaniasis, IL-17 has been recently reported cytokine which has host protective function against *L. donovani* infection. IL 17 is a hallmark cytokine secreted by only Th-17 cells. The differentiation of T naïve cell to Th17 cells is triggered by the combination of IL6 and TGFβ. Here again, host protective role of IL6 come into play. L. braziliensis promastigotes were found to induce a specific pattern of cytokine include IL6, TFG β, IL10 and IL1β in peripheral blood mono nuclear cells from non-endemic healthy resident in in vitro condition. This cytokine pattern has IL6 and TGFβ in it which may contribute to generate Th 17 cells in vivo

Activity of IL6 has also been studied with respect to macrophage activation. IL6 alone has no effect on macrophage activation but 24 hrs or 48 hrs pretreatment of IL6 has dose and time dependent suppression of IFN gamma and TNF alpha signaling in *L. amazonensis* infected macrophage. Macrophages itself express mbIL6R, therefore, it may be hypothesized that during leishmanial infection, IL6 released by macrophage through interaction of TLRs and LPG would do autocrine signaling on macrophages and bring out anti-inflammatory response, i.e. M2 phenotype resulting in parasite survival and disease progression.

SOCS (Suppressor of cytokine signaling) is a negative regulator of JAK-STAT pathway. It occurs in eight form CIS and SOCS1-SOCS7. SOCS1 and SOCS3 are found to function in macrophage polarization (conversion of macrophage into M1 or M2 phenotype). IL6 signaling induced JAK STAT-1 pathway is meant for M1 polarization whereas M2 Polarization is governed by IL6 induced JAK-STAT3 pathway. Further studies reveal that, higher expression of SOCS1 and lower expression of SOCS3 would result in the expression of Arginase I that eventually contribute to M2 phenotype. From the above discussion, it may be hypothesized that the ratio of phosphorylated SOCS1/SOCS3 may determine the macrophage polarization in *Leishmania* infected macrophage.

Aims and Objectives

- To quantitatively perform analyses of IL6 induced signaling through mathematical model.
- To study negative regulation of IL6 signal transduction.

Work Done

3.1. Phylogenetic analysis

Evolutionary conservedness of TLRs among various species forms the basis to predict possible target of leishmania parasite for specific signaling. From the analysis, we found that TLR2, TLR6, TLR9 and TLR10 are found to be conserved during the entire course of evolution.

As per the literature, TLR2 interacts with LPG, is found to be conserved, therefore, leishmania parasite must be targeting conserved receptors so as to infect the whole class of mammals. Furthermore, TLR2 heterodimerize only with TLR1 or TLR6, therefore, from the analysis we concluded that the interaction of

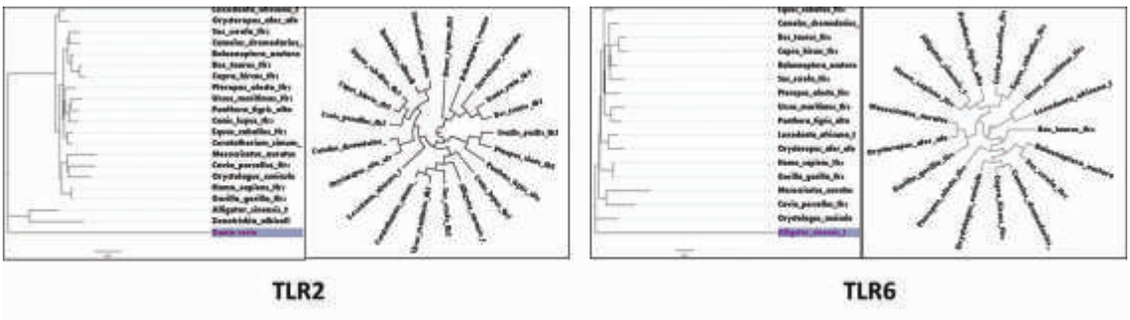

Fig. 1 Phylogenetic tree and molecular clock of TLR 2 and TLR6. Highlighted are the outgroups of TLR.

parasite to TLR through LPG may cause the heterodimerization of TLR2-TLR6 for specific signaling in leishmaniasis (Fig. 1)

3.2. Model reconstruction and simulation

3.2.1. Healthy state model

The network created has 3 compartments viz., membrane, cytoplasm and nucleus, 42 components (signaling proteins), 37 reactions, 54 parameters and 37 kinetic laws. The model was simulated for 80 units (time) using 15 s ODE solver to obtain a desired behavior, in graphic format. The network shows the formation of four components as end product i.e., SOCS3 & AIF from JAK-STAT3 pathway and SOCS1 & iNOS from JAK-STAT1pathway. The JAK-STAT3 pathway will be shut down due to the insertion of negative feedback loop of SOCS3 acting over JAK1/2, therefore AIF and SOCS3 production will cease whereas JAK STAT1 pathway will remain the same, ultimately, resulting in the formation of iNOS. The graph shows the production of iNOS (Fig. 2 and Table1) indicating the healthy state of macrophage.

3.2.2. Diseased state model

Network has 3 compartments viz., membrane, cytoplasm and nucleus, 42 components (signaling proteins), 38 reactions, 71 parametersand 38 kinetic laws were simulated using ODE solver. The diseased state model shows the high production of AIF and very low production of SOCS3. The production of iNOS is nil due to shut down of JAK STAT1 pathway by negative feedback loop insertion of SOCS1 (Fig. 3 and Table 1).

3.3. Analysis of mathematical model

3.3.1. Crosstalk points

There are two crosstalk points identified in both the networks.

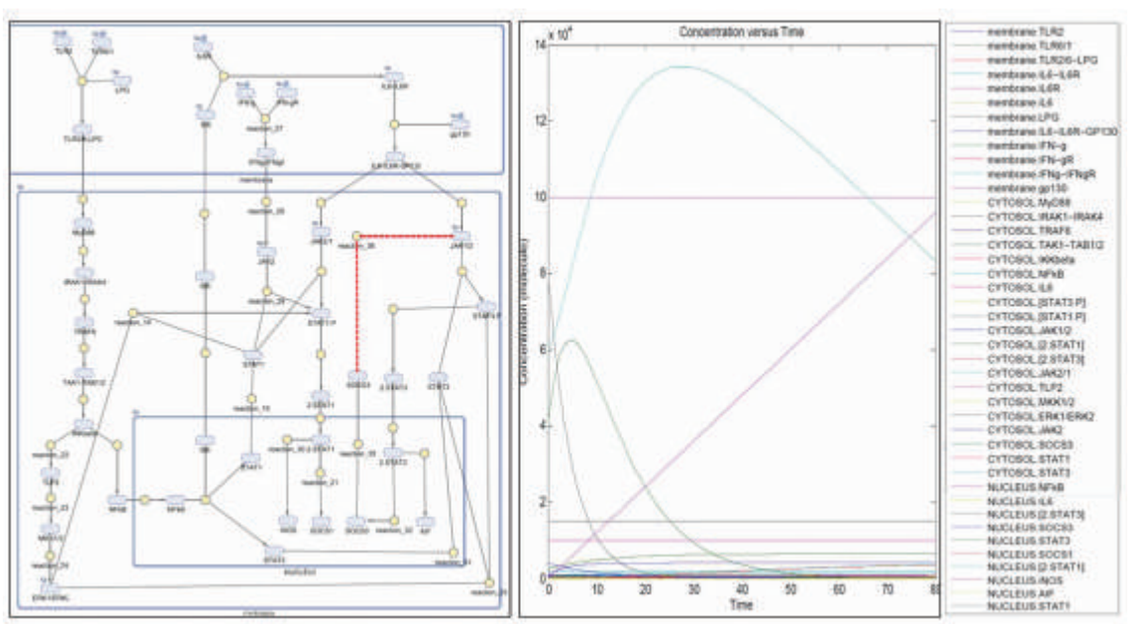

Fig.2: Reconstructed signaling network of TLR2-TLR-6/1-IL6 pathway: With SOCS3 inhibiting IL6 mediated JAK-STAT3 signaling. This will result in no production of AIF; instead iNOS production will be there which signifies parasite killing inside the macrophage. (b) The graph depicts high production of iNOS as well low production of SOCS1 which signifies the healthy state of macrophage. The reconstruction of network is done using MATLAB Simbiology toolbox (7.11.1.866).

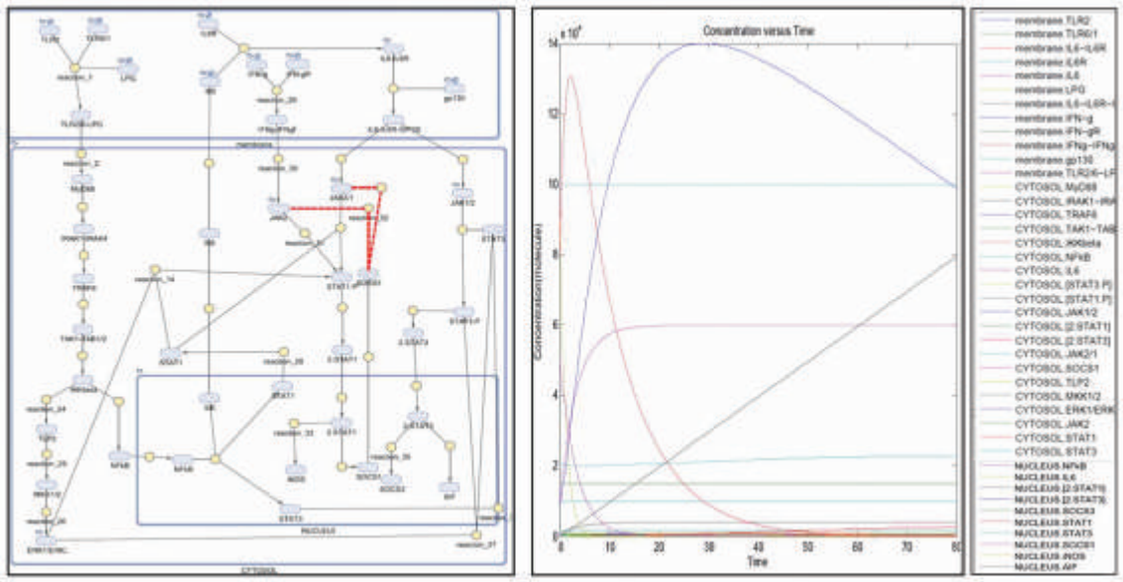

Fig. 3: Reconstructed signaling network of TLR2-TLR-6/1-IL6 pathway: With SOCS1 inhibiting IFN γ and IL6 mediated JAK-STAT1 signaling. This eventually results in no production of iNOS i.e. parasite will survive in macrophage (b) The graph shows high production of AIF as well low production of SOCS3 which signifies the diseased state of macrophage. The reconstruction of network is done using MATLAB SimBiology toolbox (7.11.1.866).

Table 1: Concentrations of SOCS1 and SOCS3 found through mathematical model:

Model	SOCS1	SOCS3	SOCS1/ SOCS3
Healthy state	800	2500	0.32
Diseased state	5000	800	6.2

These are cytoplasmic phosphorylated STAT3 and STAT1 (Fig. 4). STAT3 shows overall 4 degree in the reconstructed network where 2 degree found in TLR2-TLR6/1 pathway which shows that 2 degree must be from IL6 signaling pathway. As compared to STAT3, STAT1 shows overall 5 degree in the network, in which 2 degree belong to TLR2- TLR6/1 pathway, 1 degree belong to IFN gamma pathway therefore 2 degree is from IL6 signaling pathway.

3.3.2. Principal component analysis (PCA)

PCA extracts those components that may have an important effect on the output of the network. In healthy state model, component having highest PCA scores are cytoplasmic phosphorylated STAT1, nuclear homodimerized STAT1, Il6R receptor, membrane IL6-IL6R-gp130 complex, nuclear SOCS1, and cytoplasmic SOCS3 whereas, in diseased state model, cytoplasmic homodimerized STAT3, cytoplasmic SOCS1, and AIF are components which shows high PCA score. PCA scores of the sensitivities show that any change in the parameters associated with these signaling components may affect the whole network in terms of its output, and may be considered as future intervention points to allow modulation from a M2 phenotype to a M1 phenotype (Fig. 4).

3.3.3. Flux analysis

To mimic the negative feedback loop condition in cell, parameters for some reaction were set at "0", therefore their calculated flux come out to be '0'. These reactions are SOCS1 inhibiting IL6 and IFNg mediated JAK2/1-STAT1 activation in diseased state and SOCS3 inhibiting IL6 mediated JAK1/2-STAT3 activation in healthy state. These reactions have been ignored in flux analysis. Reactions that show change in the flux are represented in Fig. 4. Comparison between flux of both networks shows that the production of iNOS is increased after negative feedback loop of SOCS3 inhibiting JAK1/2-STAT3 pathway in healthy state whereas production of AIF is increased after inhibition of JAK1/2-STAT1 pathway by SOCS1 and high AIF production indicate, M2 phenotype i.e. diseased state.

Table 2: Reaction filtered out through model reduction.

REACTION	HEALTHY STATE	DISEASED STATE
TLR2 + TLR6/1 + LPG -> TLR2/6-LPG	✓	✓
IL6-IL6R + gp130 -> IL6-IL6R-GP130	✓	✓
STAT3.P -> 2.STAT3{CYTOSOL}	×	✓
2.STAT3{CYTOSOL} -> 2.STAT3{NUCLEUS}	×	✓
SOCS1{NUCLEUS} -> SOCS1{CYTOSOL}	×	✓
STAT1.P -> 2.STAT1{CYTOSOL}	✓	×
2.STAT1{CYTOSOL} -> 2.STAT1{NUCLEUS}	✓	×

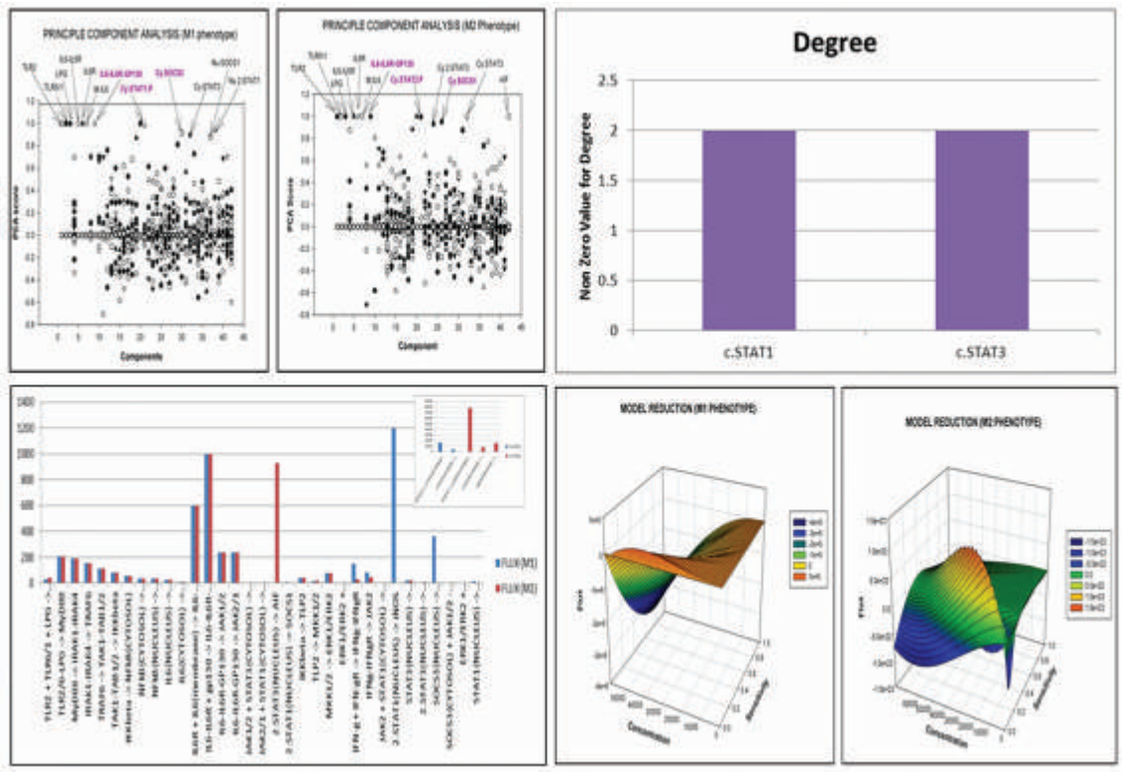

Fig.4 Analysis of TLR2-TLR6/1- IL6 signaling mathematical model : (A) Principle component analysis (B) Cross talk analysis (C) Flux analysis (D) Model Reduction

3.3.4. Model reduction

By performing sensitivity, principle component and flux analysis, both healthy and diseased state models are reduced and following are the important reaction filtered out resulting in 89% reduction in healthy model and 86% reduction in diseased state model (see Table 2). Model reduction may also be present in terms of flux, concentration and sensitivity analysis. A 3D mesh has been created for both healthy and diseased state of the model which shows that in healthy state, the 3D mesh is uniform but due to high flux of three reactions that have been upregulated in the diseased state, the 3D mesh become uneven (Fig. 4).

Future Research Plans

- The crucial components and reactions fished out through the analysis of mathematical model will further be used to design synthetic circuit that will convert M2 phenotype of macrophage to M1 phenotype for the killing of parasite inside macrophages.

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Role of Chromosome 21 Genes in Impaired Neurogenesis in Down Syndrome

Background

In our current work, we have established stem cell model of neurogenesis in Down syndrome (DS). Studies of neural differentiation of iPSCs derived from the Ts65Dn mouse model of DS, and from a human with DS, have made it possible to robustly model the decreased neurogenesis characteristic of this condition. Correlated with reduced neurogenesis, and consistent with it, were changes in the cell cycle, gene expression and PAX6 binding. Shared phenotypes between the mouse DS model and the human DS iPSCs observed provide a platform that enables one to gain further insights into the underlying genetic and mechanistic basis of reduced neurogenesis in cells trisomic for Chr 21, and their in vivo translation.

Among several genes on Chr 21, genes like *Olig1/Olig2*, *Dyrk1a*, *Dscam*, *Usp16* and *App* have currently been shown to play a role in reduced neurogenesis using various model systems. However, studies comparing the role of individual genes side-by-side as well as combinatorial studies have not been performed, leading to an inconsistent understanding of reduced neurogenesis. It is plausible that various genes act at different levels of neural differentiation. We intend to utilize DS stem cell modeling to unravel the role of Chr 21 genes during neural differentiation.

Aims and objectives of the proposed future research

For the studies to be carried out over the next 4-5 years, siRNA and shRNA-based knock down, as well as CRISPR-mediated gene dosage normalization approaches will be used. The objective of these studies will be following:

- To elucidate the role of previously-implicated Chr 21 genes during early

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stages of neurogenesis, i.e. from iPSCs (pluripotent) to neural progenitor cells (NPCs) (multipotent).

- To elucidate the role of previously implicated Chr 21 genes during late stage of neural differentiation, i.e. from NPCs to post mitotic neural differentiation.
- To identify additional Chr 21 genes having a role in reduced neurogenesis.
- To explore downstream signaling pathways leading to reduced neurogenesis using the global genomics, proteomics and epigenomics approach.

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Role of NOX4 in the Regulation of Breast Cancer Cell Migration and Invasion

Background

Despite significant advances in basic research and clinical development, therapies that specifically target metastatic breast cancer remain inadequate, reflecting a need for both insights into the metastasis process and the development of new therapies. Since, metastasis, a multi-step process involving migration and invasion of primary tumour cells to the surrounding tissues and to the distant organs followed by colonization, and cell migration being an initial and crucial event during metastasis, it is essential to target cell migration. In addition to migration, cytoskeleton remodelling also plays important role in movements of proteins, cargo containing vesicles and organelles inside the cell.

An emerging evidence suggest the role of reactive oxygen species (ROS) in the regulation of cytoskeleton remodelling/dynamics, since many proteins participating in these processes are particularly sensitive to redox regulation. The effect of ROS is largely attributed to the ability of ROS to directly modify the proteins that participate in the migration process.The major sources of cellular ROS includes, NADPH oxidases (NOX), mitochondrial electron transport chain, xanthine oxidase, nitric oxide synthase and monoamine oxidase (MAO). Recently the role of NOX proteins in the regulation of cytoskeleton dynamics during cell migration and invasion has been extensively reviewed. It is found that, of the seven identified family members in the NADPH family (five NADPH Oxidases (NOX), NOX1–5, and two NOX homologues, DUOX1 and DUOX2), NOX1, NOX2 and NOX4 have emerged as important regulators of actin cytoskeleton and cytoskeleton supported cell functions such as cell migration and adhesion. In particular NOX4 is deregulated in various types of cancers and involved in cancer proliferation and metastasis. While NOX1–3 and NOX5

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appear to release superoxide (O2-), NOX4 predominantly produces hydrogen peroxide (H_2O_2). This preferential production of H_2O_2 by NOX4 is attributed to a highly conserved histidine in the third extracytosolic loop (E-loop) of NOX4 that accelerates spontaneous dismutation of superoxide to form H_2O_2 before it leaves the enzyme. Also, NOX4 directly interacts with p22phox, which is a prerequisite for H_2O_2 generation. Interestingly, NOX4 is overexpressed in malignant breast cell lines and primary breast tumours. Further the overexpression of NOX4 in normal breast epithelial cells results in resistance to apoptosis and increased tumorigencity. This suggests the important role of NOX4 in breast carcinogenesis. Recently the involvement of NOX4 in breast cancer cell migration has been reported, but its role in regulation of cytoskeleton remodelling during breast cancer cell migration is largely unexplored. Also, NOX4 expression is induced by different growth factors and inflammatory cytokines like TNF-α, VEGF, TGF-β and PDGF etc. In that TNF-α is an important cytokine with known role in cancer cell migration and also it is highly expressed in breast carcinoma. It exerts diverse functions in cancer biology, such as it stimulates cancer cell survival and proliferation as well as promotes angiogenesis, tumour cell migration and invasion. Recently, Dominika et al reported the role of TNF-α in breast cancer cell migration. Interestingly, it has been identified as an important stimulator of NOX family during endothelial cell migration and adhesion. Also, TNF-α is shown to specifically stimulate NOX4 in smooth muscle, but how it is affecting NOX4 expression in breast cancer cells is still unclear.

Aims and Objectives

- To investigate the role of NOX4 in TNF-α induced breast cancer cell migration and invasion especially how NOX4 is affecting the cytoskeleton remodelling during cell migration and invasion.

Work Done

Owing to the important role played by ROS in the pathogenesis of several cancers including breast, modulation of the redox homeostasis may have greater therapeutic implications. In the present work, we studied the main differences in redox homeostasis of non-tumoral and tumor breast cell lineages. We did find significant discrepancies in ROS producing and scavenging activities between tumor and normal cells that might represent a vulnerability of cancer cells. We use the

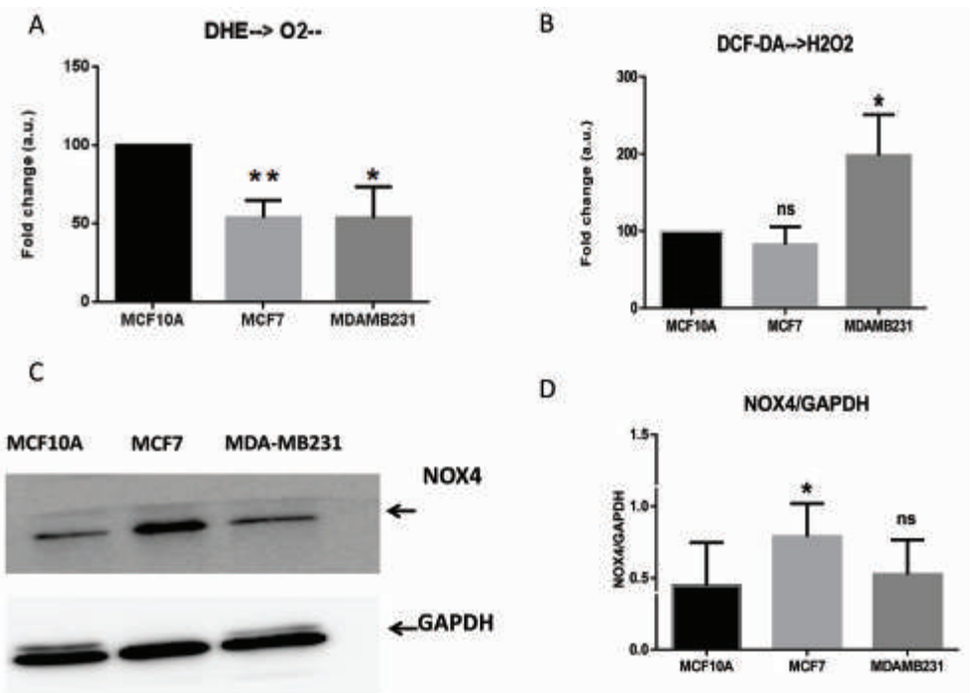

Fig. 1: (A) Endogenous superoxide (O2--) and (B) Hydrogen peroxide (H_2O_2) levels in MCF10A, MCF7 and MDA-MB231 cells. ROS were detected using specific probes by flow cytometry (FACS CALIBUR-BD Sciences, USA). The data was analyzed using Cell Quest software for determining the percentage of stained cells compared to unstained cell population. (C) Endogenous NOX4 protein expression by western blotting (D) Quantification of band intensities was carried out using Image J. Images are representative of three independent experiments Columns, mean from three different experiments; bars, SE. *, P < 0.05; **, P < 0.01; *** P< 0.001 versus control. These results are representative of three independent experiments.

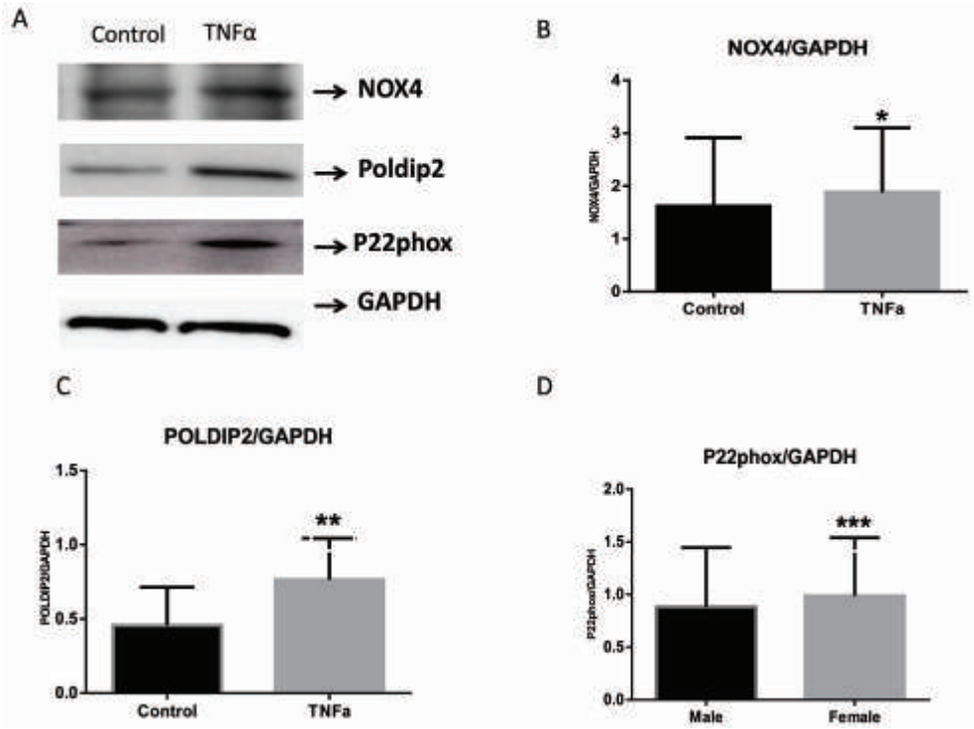

Fig. 2: (A) Expression of NOX4, Poldip2 and P22phox after TNFa treatment by western blotting, (B-D) Respective quantifications of band intensities that were carried out using Image J. MDA-MB231 cells were treated with TNF-α (20ng/ml) for 24h and western blotting was carried out using respective antibodies on MDA-MB231 whole cell lysates. Images are representative of three independent experiments. Columns, mean from three different experiments; bars, SE. *, P < 0.05; **, P < 0.01; *** P< 0.001 versus control. This result is representative of three independent experiments.

mammary epithelial cell line MCF-10A, and breast cancer cell lines; MCF-7 (less invasive) and MDA-MB-231 (highly invasive).

It is known that the redox status in tumors is usually different from that of normal tissue counterparts due in part to increased ROS production. The adaptive antioxidant capacity in certain cancer cells allows tumors to tightly control ROS levels while maintaining proliferative capacity. Thus, we first characterized the status of oxidative as well as nitrosative stress status in two breast cancer cells (MCF-7 and MDA-MB-231) exhibiting various degree of aggressiveness and normal mammary epithelial cell line MCF-10A. All the three cell lines showed undetectable to low endogenous nitric oxide (NO) as well as peroxynitrite (ONOO-) levels (data not shown), however, surprisingly the level of superoxide was more in MCF-10A cells and the level of H2O2 was more in MDA-MB-231 cells while MCF7 had significantly lower levels of both superoxide and hydrogen peroxide levels when compared to both MDAMB231 and MCF10A The increase in the levels of superoxide in MCF-10A cells could be attributed to its adaptive antioxidant capacity i.e. to the increased levels of manganese superoxide dismutase (Mn-SOD) or SOD2 which is considered to be a tumor suppressor gene, commonly deleted in human tumors and its over-expression causes inhibition of cell growth on several cell lines of epithelial cancer.

Next we determined the expression of endogenous NOX4 in the three cell lines and found that the expression of NOX4 was more in MCF-7 cells, however, the measurement of NOX activity will reveal its exact role in these cell lines. Tumour necrosis factor (TNF)-α is a key cytokine involved in cancer-related inflammation and act as a tumour-promotingfactor and is known to promote tumour cell migration and invasion. However, the mechanism by which TNF-α facilitates these events remains elusive. In this study, we have used TNF-α to study the involvement of NOX in breast tumor cell migration. NADPH oxidase 4 (NOX4) is a multisubunit enzyme and it requires the Poldip2 and P22phox proteins to perform its enzymatic activity. The expression of NOX4 and its regulatory subunits were checked in MDA-MB231 cells after 24hrs TNF-α treatment. A significant increase in NOX4 and its regulatory subunits Poldip2 and p22phox levels were observed after TNF-

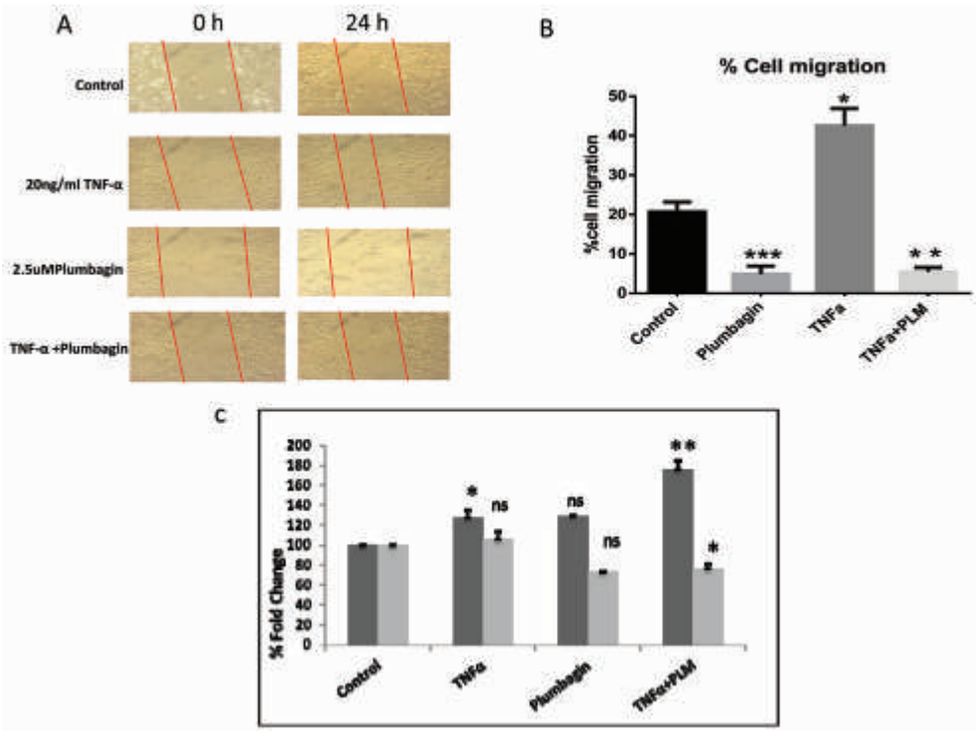

Fig. 3: (A) Determination of migration of MDA-MB231 cells. MDA-MB231 by scratch wound assay after NOX4 inhibition with Plumbagin. Cell monolayers were scratched by pipette and treated with Plumbagin (2.5mM) in the presence or absence of 20 ng/ml TNF-α. (B) Migration is expressed as % gap closure of TNF -α treated well. After incubation, MDA-MB231 cells were photographed (magnification, × 100) using Image pro plus and quantified using Image J software for above described experiments. (C) Endogenous superoxide ($O_2^{\cdot-}$) and Hydrogen peroxide (H_2O_2) levels in MDA-MB231 after treatment with Plumbagin in the presence and absence of 20 ng/ml TNF-α. ROS were detected using specific probes by flow cytometry (FACS CALIBUR-BD Sciences, USA). The data was analyzed using Cell Quest software for determining the percentage of stained cells compared to unstained cell population. Images are representative of three independent experiments. Columns, mean from three different experiments; bars, SE. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$ versus control. These results are representative of three independent experiments.

α treatment as compared to untreated control. In order to confirm the role of NOX4-ROS in breast cancer cell migration, Plumbagin, a plant derived bioactive naphthoquinone to inhibit the NOX4 activity was used and further its effect on breast cancer cell migration was seen by scratch-wound healing assay using MDA-MB231 cells. A significant decrease in the cell migration was observed in cells treated with Plumbagin compared to control cells treated with only TNF-α treated and untreated control cells, suggesting the role of NOX4 derived ROS in breast cancer cell migration. Since, NOX4 directly produces superoxide and H_2O_2 as a part of their enzymatic activity and Plumbagin is known to inhibit the superoxide producing activity of NOX4, the levels of superoxide and H_2O_2 in MDA-MB231 were checked further by flow cytometry. A significant increase in the level of superoxide was was observed in the cells after Plumbagin treatment compared with the cells treated with TNF-α alone and in the untreated controls, which were expected to be lower after plumbagin treatment. However, H_2O_2 levels were significantly decreased after Plumbagin treatment compared with the cells treated with TNF-α alone and in the untreated controls. The increase in superoxide level after Plumbagin treatment could be the result of alteration in the expression/activity of anti-oxidant enzymes such as Superoxide dismutases (SOD).

Future Research Plans

- Since one of the most important properties of cell migration is the ability of cells to fine-tune their cytoskeletal structure in response to changing environmental cues such as growth factor stimulation, our specific emphasis will be on understanding how NOX4 and cytoskeleton influence breast tumour cell migration and metastasis.

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Moving Fates Around: Understanding the Role of Endocytosis in Cell Fate Transitions

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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 the mechanism of regulation of components of the vesicular transport machinery in embryonic stem cells.
- To uncover components of the vesicular transport machinery that play a role in the maintenance of pluripotency.

Work Done

Uncovering a dual mechanism for the regulation of expression of endocytosis associated genes in mouse embryonic stem cells:

Using an *in silico* approach, we have identified a number of endocytic genes that are bound and repressed by the Polycomb repressive complex in mESCs. We have further uncovered a second mechanism by which these genes are kept repressed in mESCs by the action of members of the ESCC family of microRNAs (Fig. 1). One of the major endocytic genes regulated in this manner is Cav1, whose expression is almost undetectable in mESCs and is elevated upon differentiation. We also demonstrate that misexpression of Cav1 results in

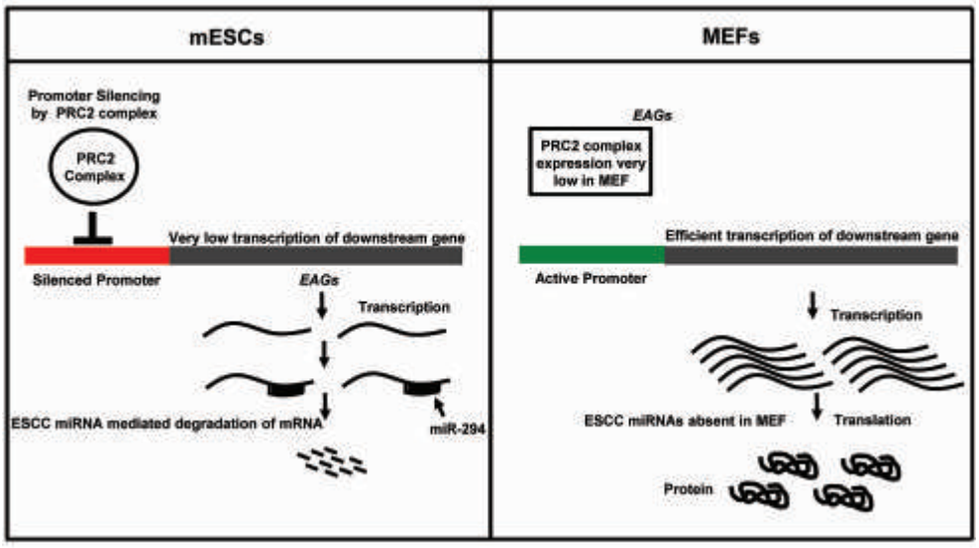

Fig. 1: Model showing the regulation of expression of endocytosis-asociated genes in embryonic stem cells: The expression of endocytosis associated genes (EAGs) are regulated by a dual mechanism involving the action of the Polycomb Repressive Complex and the ESCC family of microRNAs. Together, this dual action represses the expression of these genes in mESCs. This repression is relieved in differentiated cells such as MEFs, resulting in the expression of these genes.

a reduction in pluripotency of mESCs (Mote et al, Scientific Reports, 2017).

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. 2a). A number of controls were included in this screen. These included mock transfected cells that received only the

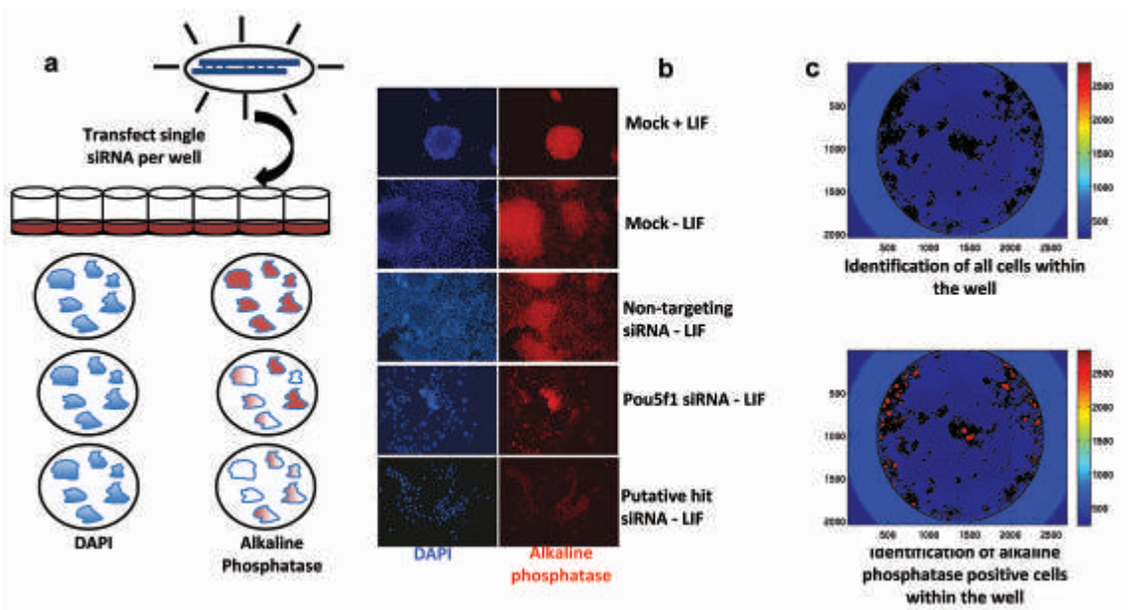

Fig. 2: Schematic of siRNA screen to knockdown genes involved in endocytosis in mouse embryonic stem cells: (a) Schematic of siRNA screen. mESCs were plated in 96 well plates. 24 hours post plating, individual siRNAs were introduced into each well at a final concentration of 50nM. At the time of transfection, 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.

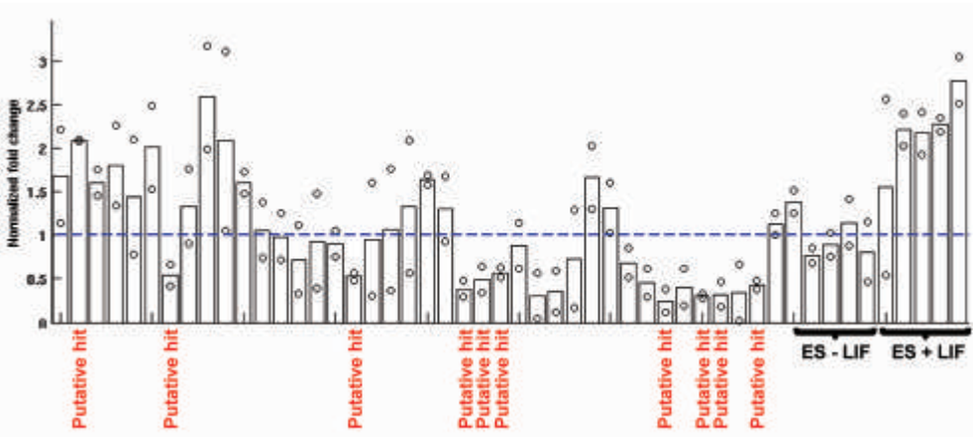

Fig. 3: Knockdown of genes involved in endocytosis and vesicular trafficking impact pluripotency of mouse embryonic stem cells: Graphs showing normalized fold change in Alkaline phosphatase staining compared to staining observed in mESCs grown under ES-LIF conditions. Each screen was repeated twice and 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.

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. 2b). 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. 2c). N2/N1 gives us the percentage of alkaline phosphatase positive cells within each well. This value was then normalized to the N2/N1 obtained from mock-transfected cells maintained in ES-LIF conditions. If the normalized value remained close to or equal to 1, then it indicated that the knockdown of that particular gene did not affect differentiation or pluripotency compared to the control. If the value was 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. 3), 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.

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Gene Regulatory Functions of Mammalian Long Noncoding RNAs [lncRNAs] During Quiescence-Proliferation Axis

Background

A large portion of the eukaryotic genome is transcribed into RNAs that do not code for proteins. These interesting molecules, formally known as noncoding RNAs (ncRNAs) that bypass the central dogma for flow of genetic information in cells, have been under constant scrutiny for their existence for several decades. Although the discovery and functional characterization of small ncRNAs have dominated the field of RNA biology over past several decades, long noncoding RNAs [lncRNAs] are the least explored emerging regulatory molecules. Although the function of a large number of lncRNAs is still not known, there is clear evidence for their importance in physiology, embryology and development with numerous novel gene regulatory functions, including their role in contribution to high degree of complexity observed in multicellular organisms. Various studies have revealed their active role in controlling multiple regulatory layers including chromosome architecture, chromatin modulation and epigenetic modification, transcription, RNA maturation, splicing and translation. Based on the current evidences, lncRNAs can perform their function by physically interacting with DNA, RNA and proteins, thereby regulating complex network of gene expression by acting as signals (for integrating spatiotemporal, developmental, and stimulus-specific cellular information), decoys (the ability to sequester a range of RNA-dependent effectors and protein partners), guides (for proper localization of chromatin-modifying complexes and other nuclear proteins to specific genomic loci to exert effects), and scaffolds (for bringing two or more proteins into discrete complexes).

Elucidating the different mechanism of action of lncRNAs will not only provide the basic biological understanding of cellular function but also a critical nexus for revealing the basis of lncRNAs in disease etiology and their use as targets in

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subsequent drug design. Most importantly, the fact that mammalian transcriptome comprises several thousands lncRNAs with diverse signatures, the question that whether all of them have biological purpose still stands unanswered. Thus, a comprehensive knowledge of their function would greatly facilitate our current understanding of various cell regulatory networks and disease mechanisms.

The long-term objective of this project is to understand the molecular basis of regulation and maintenance of cellular quiescence and proliferation. Using an inducible classic model for cellular quiescence, our laboratory address the regulation of gene expression and chromatin organization mediated through lncRNAs during cellular quiescence and proliferation.

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 pathologic 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.* Long noncoding RNAs [lncRNAs] have been implicated in regulating various cellular processes including terminal differentiation and senescence. However, their involvement in the regulation of cellular quiescence and cell-state transition has not been firmly established.

This proposal aims to answer the above-mentioned fundamental questions by utilizing an inducible classic model for cellular quiescence and cell cycle progression. *The focus of aim 1 is to identify and systematically characterize lncRNAs differentially expressed upon immediate entry and exit of cells into quiescence. This analysis will lead to the discovery of novel candidates required for the initiation and viability of the quiescent state of the cell and for the progression through different cell cycle stages. How do these lncRNAs regulate gene expression, what are the other protein partners they interact with to exert their function and finally, how are these lncRNAs temporally regulated will be dissected in aim 2.*

Aims and Objectives

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

Work Done

Understanding the molecular function of LNC11q

We have identified a novel lncRNAs, LNC11q that is induced at early response to growth factor stimulation in cells. Depletion of LNC11q using antisense oligos in HDFs resulted in slower S-phase progression indicating the role of LNC11q in cellular proliferation. 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.

LNC11q is required for proper execution of the cell cycle

The quiescent HDFs express extremely low levels of LNC11q, when induced with serum the LNC11q levels increase and start to reduce approx. 12hr of growth factor stimulation. To gain insight into the expression dynamics of LNC11q, we synchronized human U2OS cells to different stages of the cell cycle and analyzed its levels. We observed that LNC11q is upregulated during G1 phase of the cell cycle, further corroborating its upregulation in HDFs upon growth factor stimulation. Since, LNC11q depletion results in reduced proliferation in HDFs, to gain insight into the functional relevance of LNC11q in regulating S phase transition and cellular proliferation, we examined the effect of LNC11q depletion on cell cycle progression in synchronized U2OS cells. Briefly, U2OS cells were synchronized to G1/S boundary and further released in the presence and absence of LNC11q. Interestingly, cells depleted of LNC11q showed significantly slower progression than the control cells, indicating that LNC11q is required for proper progression of cells through S-

phase of the cell cycle. To further investigate the mechanism of action of LNC11q and the pathways regulated by is RNA, a detailed gene expression analysis is in progress.

HuR binding to its target mRNAs is reduced upon LNC11q depletion

In our previous experiments, we have identified HuR as one of the interacting partners of LNC11q. To investigate HuR binding region in LNC11q, we created 1kb length mutant clones and performed an in vitro RNA pull down assay for HuR. Our immunoprecipitation experiments suggest that HuR binds to approx. 2kb region of the exon 4 of LNC11q. To further understand the significance of LNC11q association with HuR, in quiescent cells induced to proliferate; we looked at their interaction during the course of quiescence exit and found that even though HuR levels do not change significantly, and LNC11q is upregulated at early stage, it interacts with HuR at later stages only. Additionally, in U2OS cells, LNC11q interacts with HuR significantly at G1/S stage of the cell cycle. To understand if LNC11q stabilizes HuR by associating with it in proliferating cells, we depleted LNC11q and looked for HuR levels. We did not see any significant difference in the levels of HuR upon LNC11q depletion, suggesting that LNC11q may not be required for HuR stability.

To further understand the functional significance of LNC11q and HuR interaction, we performed RNA immunoprecipitation using HuR antibody in the presence and absence of LNC11q and analyzed the enrichment of mRNA targets of HuR in the pull down samples. Interestingly, HuR binding with several of its mRNA targets was significantly reduced in LNC11q depleted cells. However, the levels of these target mRNAs were largely unaffected in LNC11q depleted cells. These data suggest that LNC11q is required for interaction of HuR and its target mRNAs. One possibility is that LNC11q stabilizes the binding of target mRNAs to HuR by interacting with them. To test this possibility, we checked if LNC11q shares partial complementarity with its target mRNAs using computational prediction tools. Among numerous complementarities, we narrowed down to candidate mRNAs containing at least 20-bp complementarity with LNC11q with greater than 90% identity. From the prediction we found approx. 24 mRNAs that met these criteria. This result suggests that HuR and LNC11q can bind to several shared target mRNAs. Further experiments to understand the functional significance of interaction of HuR with LNC11q and its activity during cellular proliferation are under progress.

Identification and characterization of novel lncRNAs during cellular quiescence and proliferation

We have generated a pipeline where we established the inputs as RNA-Seq data of diploid lung fibroblasts at early, mid and late stages of quiescence entry and exit. Additionally, we also incorporated earlier known annotations from RefSeq/GENCODE and the new annotations from MiTranscriptome assembly. The RNA-Seq data were aligned to the genome and assembled into transcripts by TopHat and Cufflinks, respectively. Additionally, with the known annotations, we considered transcripts with high conservation and diverse tissue expression. Finally, from this group we only selected the intergenic transcripts for further evaluation. In our initial analysis, we have focused on highly conserved and diversely expressed lncRNAs because it can provide exciting avenue for in vivo study of the role of lncRNAs in development and carcinogenesis.

From our RNA-seq data, we obtained etabol. 2500 differentially expressed transcripts, out of which 38 showed a significant GFOLD upon quiescence entry and 141 transcripts showed significant GFOLD upon quiescence exit. Additionally, we screened for etabol. 17000 candidates from databases, out of which 733 showed a cell cycle stage specific expression profile. To further corroborate active transcription of the lncRNAs, we intersected intervals surrounding the transcription start sites (TSSs) with ENCODE ChIP-seq data for H3K4me3, RNA PolII binding sites from 13 cell lines. Maximal enrichment of these marks at the TSSs of these genes but not at randomly shuffled control regions suggested that the assembled lncRNA possess actively regulated promoters.

So, finally, we have identified several previously uncharacterized lncRNAs that are differentialy expressed during quiescence entry and exit. Many of them also display a cell cycle stage specific expression pattern. Further cloning of these candidates and their functional characterization is in progress.

Studies on the effect of depletion and overexpression of the candidates in regulation of quiescence and proliferation

To gain insight into the functional relevance of the differential expression pattern of the lncRNA candidates, we designed antisense DNA oligos [LNA GapmeRs] and looked for the effect of their depletion on cellular phenotype.

In our primary functional screen for cellular quiescence, we selected 3 lncRNAs, lnc339, lnc503 and lnc1279 for our knockdown experiments. All these three lncRNAs are significantly upregulated in quiescent cells and their levels go down as soon as the quiescent cells are induced to proliferate. In order to understand the role of these lncRNAs in regulating cellular quiescence, we depleted these RNAs in HDFs and induced them to undergo quiescence. We observed a marked reduction of G0 population of the cells upon knockdown, indicating that these lncRNAs may be required or may facilitate the entry of cells into quiescence. Additionally, we also depleted these RNAs in quiescent HDFs and induced them to proliferate. The significant percentage of quiescent cells depleted of these RNAs failed to enter cell cycle, which further corroborates the importance of these lncRNAs in the maintenance of quiescent state of the cells. Further experiments to determine the role of these lncRNAs in regulating cellular quiescence is in progress.

In our primary functional screen for cellular proliferation, we have identified 115 candidates for G1, 25 for G1/S, 105 for S and 150 for G2/M specific expression dynamics. To understand the functional relevance of their cell cycle stage specific expression pattern, we depleted some of these lncRNAs and analyzed the cellular profile through flow cytometry. Depletion of several of these candidates displayed a series of cell cycle defects ranging from cell cycle stage specific arrest to apoptosis. Further experiments to determine their role in cell cycle progression is in progress.

Identification of protein interactors of the candidate lncRNAs in early stages of quiescence or proliferation

LncRNAs normally act together with specific proteins. In order to understand the molecular mechainsms underlying the cellular phenotype observed upon depletion of a specific lncRNA, it is important to identify specific protein targets that interact with the lncRNAs. Additionally, identification of interacting proteins would also help in understanding the biological function of the lncRNAs. To characterize the protein interactome of the lncRNAs during cellular quiescence and proliferation, we employ two approaches, in vitro biotinylated RNA pull down assay and MS2-TRAP assay. We performed RNA pull down experiments by incubating biotinylated LNC11q with nuclear extracts and further capturing it with streptavidin beads. We identified several proteins like HuR, AUF1, PTB, and Puf60 that specifically interact with LNC11q.

Additionally, we have generated stable cell lines that express MS2-binding protein tagged with YFP and HA. We have also prepared expression constructs of lncRNAs tagged with 24X MS2 repeats at their 3'ends. Co-expression of the MS2-tagged lncRNAs and YFP-MS2bp in mammalian cells and the affinity purification of the complex are in optimization stage. A large-scale experiment to affinity purify trans-binding factors associated with MS2-tagged lncRNAs is in progress.

Future Research Plans

- Understanding the mechanism of action of lncRNAs during quiescence and proliferation entry.
- Identification of protein interactors of the candidate lncRNAs in early stages of quiescence and proliferation.
- Studies on the effect of depletion or overexpression of the candidates in regulation of quiescence and cellular proliferation.
- Understand the temporal regulation of lncRNAs during the quiescence-proliferation axis.

Mohan Wani

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Studies on the Role of IL-3 in Regulating the Development of Th17 Cells

Background

Human rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammatory synovitis, production of autoantibodies and several pro-inflammatory cytokines, which together lead to destruction of joints. RA is a consequence of defect in both central and peripheral tolerance mechanisms leading to exaggerated Th17 cells responses, which contribute to the pathogenesis of RA. Also, IL-17 levels increased in serum and synovial fluid of RA patients. The exaggerated Th17 cells responses at the synovium are detrimental and promote inflammatory bone loss and massive cartilage damage by activating osteoclasts, synovial macrophages and fibroblasts.

IL-3, a cytokine secreted by Th cells stimulates the proliferation, survival and differentiation of hematopoietic cells. We have previously reported that IL-3 has an anti-inflammatory role and protects bone and cartilage damage in inflammatory arthritis. IL-3 also ameliorates collagen-induced arthritis (CIA) in mice by increasing the number of regulatory T (Tregs) cells. However, the role of IL-3 on development of Th17 cells is not yet delineated. In the present study, we evaluated the role of IL-3 on Th17 cell differentiation.

Aims and Objectives

- To evaluate the role of IL-3 in regulating the differentiation of Th17 cells from naïve T-cells.
- To investigate the role of IL-3 in development of pathogenic and non-pathogenic Th17 cells.
- To investigate the mechanism(s) of IL-3 action on Th17 cell differentiation.

Participants

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

Collaborators

Brig Yogesh Sharma, *AFMC, Pune*
Anil Ulemale, *Veterinary College, Shirwal*
Gyan C. Mishra, NCCS, Pune

Work Done

IL-3 inhibits the development of Th17 cells

To investigate the role of IL-3 on Th17 cells, we first examined the expression of IL-3Rα on these cells. For this, naïve $CD4^+CD25^-CD44^{lo}CD62L^{hi}$ T-cells isolated from spleen or lymph nodes of mice were stimulated with anti-CD3ε and anti-CD28 in the presence of Th17 polarizing conditions. At day 4, cells were analyzed for the expression of IL-3Rα. We observed that Th17 cells express IL-3Rα at both gene and protein levels. Further, naïve T-cells under Th17 polarizing conditions were incubated with different concentrations of IL-3. We observed that IL-3 dose-dependently inhibits the differentiation of Th17 cells (Fig. 1A and B). Previously, we have shown that IL-3 enhances $FOXP3^+$ Tregs; and as TGF-β is required for the differentiation of both Tregs and Th17 cells, we checked whether the decrease in Th17 cells is concomitant with increase in $FOXP3^+$ T-cells under Th17 skewing conditions. We noticed that IL-3 enhanced the percentage of $FOXP3^+$ T-cells (Fig. 1C). These results suggest that IL-3 regulates the developmental axis of Tregs and Th17 cells in the favor of Tregs.

IL-3 decreases the number of pathogenic Th17 cells

Th17 cells exist in both pathogenic and non-pathogenic states depending upon the co-production of other cytokines. Co-production of IL-17 with pro-inflammatory cytokines such as $IL\text{-}17^+TNF\text{-}\alpha^+$ and $IL\text{-}17^+IFN\text{-}\gamma^+$ in Th17 cells makes them pathogenic, whereas cells that co-produce anti-inflammatory cytokine like IL-10 along with IL-17 ($IL\text{-}17^+IL\text{-}10^+$) are non-pathogenic Th17 cells. In vitro activation of naïve T-cells in the presence of TGF-β1 and IL-6 results in the generation of non-pathogenic Th17 cells; and exposure to IL-23 induces a pathogenic phenotype. We examined whether IL-3 modulates pathogenic or non-pathogenic Th17 cells. Intracellular staining was performed to quantify the number of pathogenic and non-pathogenic Th17 cells. We observed significant decrease in percentage of pathogenic Th17 cells by IL-3 (Fig. 2A and B); however, no significant difference was observed in the number of non-pathogenic Th17 cells by IL-3 (Fig. 2C).

IL-3 regulates development of Th17 cells through STAT3

Next, we determined the mechanism of action of IL-3 on

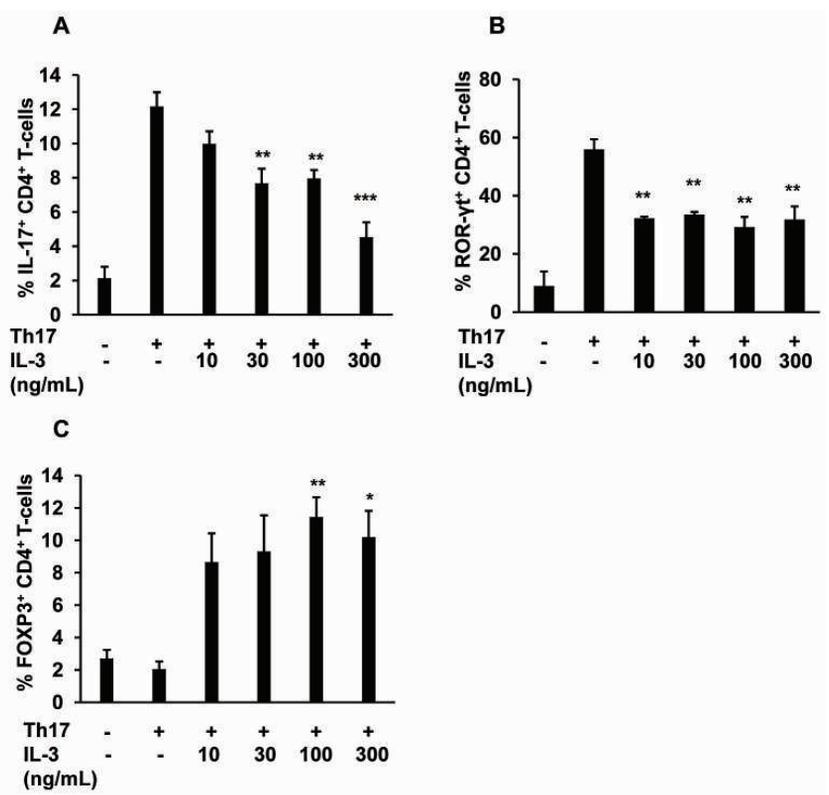


Fig. 1: Effect of IL-3 on differentiation of Th17 cells: Splenic $CD4^+CD25^-CD62L^{hi}CD44^{low}$ cells stimulated with anti-CD3ε (10 µg/mL) and anti-CD28 (2 µg/mL) mAbs were incubated with rhTGF-β (3 ng/mL), rmIL-6 (30 ng/mL) and rmIL-23 (50 ng/mL) along with anti-IFN-γ (10 µg/ml) and anti-IL-4 (10 µg/ml) antibodies in the absence and presence of different concentrations of IL-3. After 72 hrs, cells were analysed by flow cytometry for the percentage of $IL\text{-}17A^+$ (A), $ROR\text{-}\gamma t^+$ (B) and $FOXP3^+$ (C) $CD4^+$ T-cells. Data is representative of three independent experiments. *p < 0.05, **p < 0.01 and ***p < 0.001.

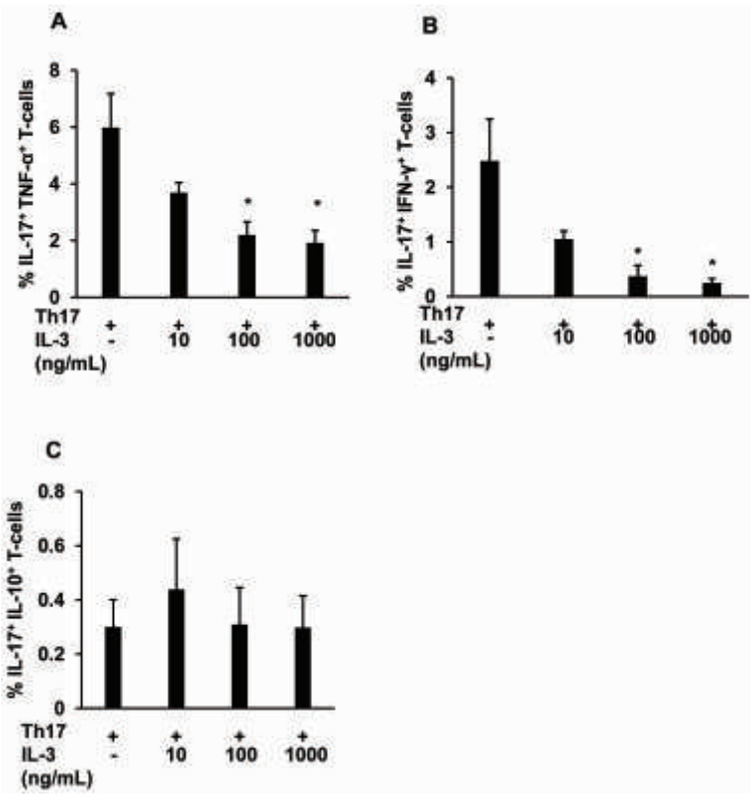


Fig. 2: Effect of IL-3 on pathogenic Th17 cells: Splenic $CD4^+CD25^-CD62L^{hi}CD44low$ cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated under Th17 polarizing conditions in the absence and presence of different concentrations of IL-3. After 72 hrs, PMA/ionomycin stimulated Th17 cells were analysed for pathogenic $IL\text{-}17^+TNF\text{-}\alpha^+$ (A), $IL\text{-}17^+IFN\text{-}\gamma^+$ (B) and non-pathogenic $IL\text{-}17^+IL\text{-}10^+$ (C) $CD4^+$ T-cells. Data is representative of three independent experiments. *p < 0.05, **p < 0.01 and ***p < 0.001.

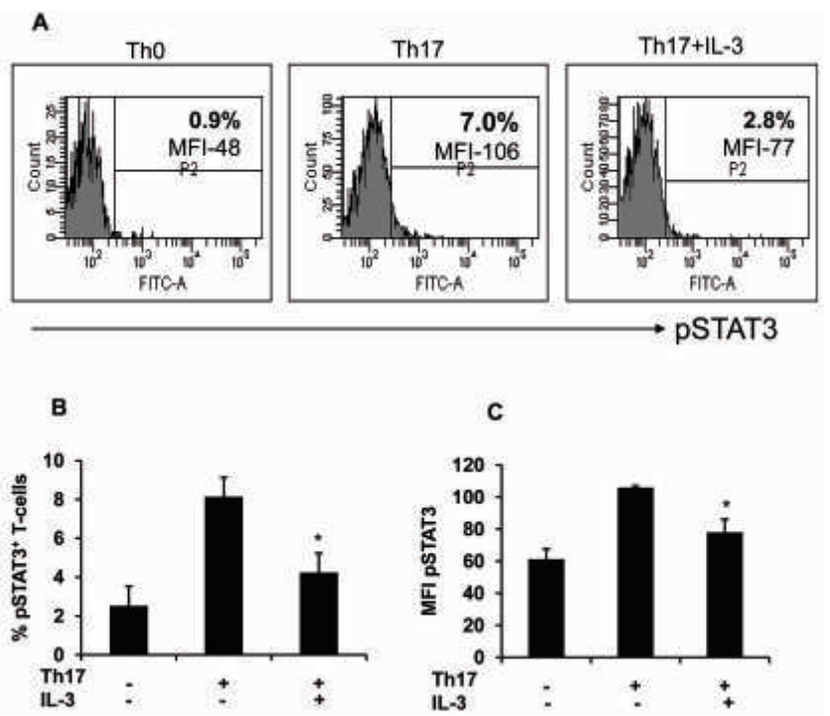
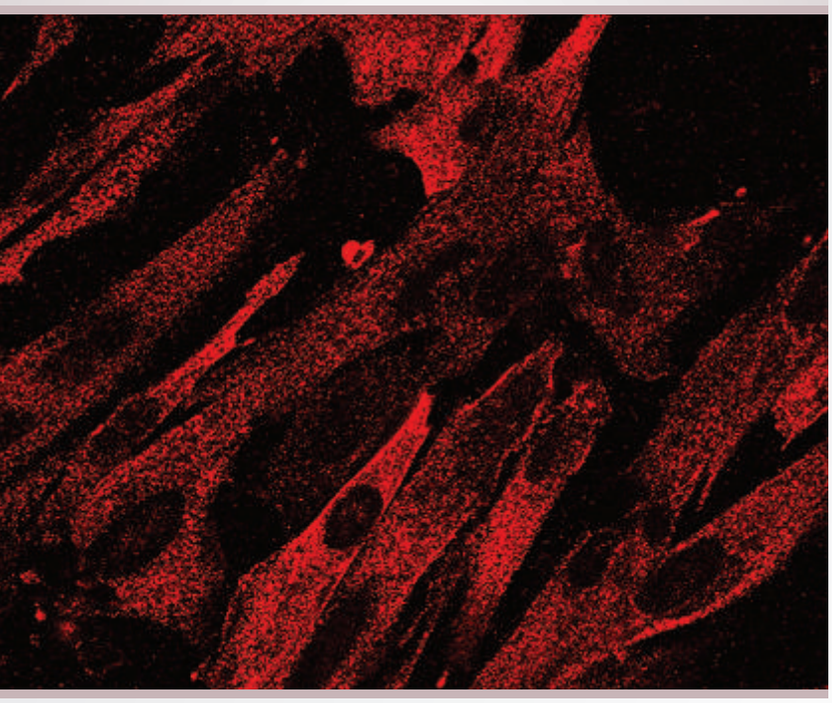


Fig. 3: Regulation of STAT3 activation by IL-3 in Th17 cells: Splenic $CD4^+CD25^-CD62L^{hi}CD44^{low}$ cells stimulated with anti-CD3ε and anti-CD28 mAbs were incubated under Th17 polarizing conditions in the absence and presence of IL-3 (100 ng/mL). After 72 hrs, cells were analysed for the expression of pSTAT3 by flow cytometry (A). Bar graph represents the percentage of pSTAT3 positive cells (B) and median fluorescence intensity of pSTAT3 (C) among $CD4^+$ Th17 cells. Data is representative of three independent experiments. *p < 0.05, **p < 0.01 and ***p < 0.001.

development of Th17 cells. IL-3 signaling is primarily mediated through the activation of JAK2 with subsequent phosphorylation and activation of STAT5 which also promotes the Treg cell differentiation. Th17 differentiation depends upon the activation of STAT3 which is a major transcription factor for Th17 differentiation and is antagonistic to STAT5. Cytokines such as IL-6, IL-21 and IL-23 phosphorylates STAT3, which regulates the expression of ROR-γt. To evaluate the effect of IL-3 on STAT3 phosphorylation, we incubated naïve $CD4^+$ T-cells stimulated under Th17 cell polarizing conditions with IL-3 for 3 days. We observed that IL-3 significantly inhibited the phosphorylation of STAT3 induced in Th17 cells (Figure 3A and B). These results suggest that IL-3 is a novel regulator of Th17 and Treg imbalance; and may inhibit Th17 cell-mediated immunopathology in CIA.

Future Research Plans

- We are investigating the in vivo role of IL-3 on regulation of Th17 cells and prevention of pathophysiology of RA using CIA mouse model.



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 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 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 -43 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 Scientists of the Institute for the conduct of animal experiments. Complete scientific support and advice is extended as per demand to the Scientists and their group members for the conduct of experiments under Institutional Animal Ethics Committee (IAEC) approved projects.

The total number of mice strains, inbred, outbred, and mutant and hybrids, being maintained at the Experimental Animal Facility stands at 56. 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*

Orbitrap Fusion Tribrid LC-MS/MS system

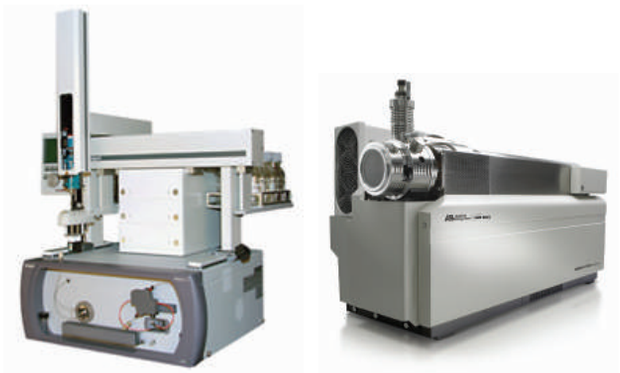
4800 LC-MALDI-TOF/TOF

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:

Orbitrap Fusion Tribrid LC-MS/MS system (Thermo Fisher Scientific) – This instrument, purchased through an extramurally funded project, was a new addition to the facility this year. It combines the best of quadrupole, ion trap and orbitrap mass analysis in revolutionary tribrid architecture to provide unprecedented depth of analysis and ease of use. The system in combination with nano flow LC module enables analyzing the most challenging low-abundant, high-complexity samples to identify more compounds faster, quantify more accurately and elucidate structures more thoroughly. This system is capable of multiple dissociation techniques viz. CID, HCD, and ETD with ion trap or orbitrap detection at any level of MSn maximize flexibility for research applications. The system performs a wide variety of analyses, from in-depth discovery experiments to characterization of complex PTMs to comprehensive qualitative and quantitative workflows. The number of samples analyzed is approximately 214 including 10 external samples from April-2017 to March-2018.

4800 LC-MALDI TOF/TOF system (AB Sciex) – This is a tandem time-of-flight MS/MS system combined with nanoLC and robotic spotter 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 98 including 25 external samples from April-2017 to March-2018.

4000 Q-Trap LC-MS/MS system (AB Sciex) – This 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. This system also is



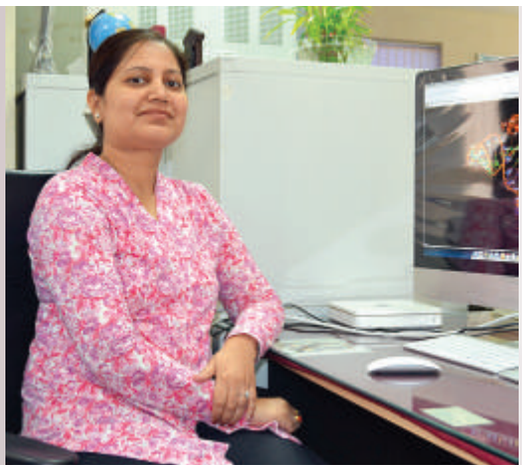
4000 Q-Trap LC-MS/MS

highly useable in the identification and quantification of metabolites in biological complex samples. The number of samples analyzed is approximately 62 including 26 external samples from April-2017 to March-2018.

Gas Chromatography Mass Spectrometry (GC-MS) system (Agilent) – This instrument, with new 7890B GC and 5977A MSD, provides unmatched sensitivity for ultra-trace analysis, and increased performance. It is highly suitable for the identification and analysis of metabolites specifically those which are volatile and semi-volatile in nature. GC-MS set-up is used for metabolomics applications for various diseases including cancer.

AGILENT GC-MS

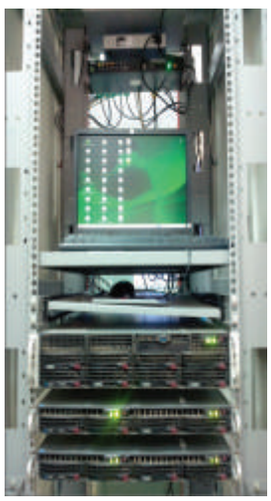
Inauguration of the new wing of the Proteomics Facility by Prof. Partha Majumdar (Chairman of the NCCS RAP-SAC).



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

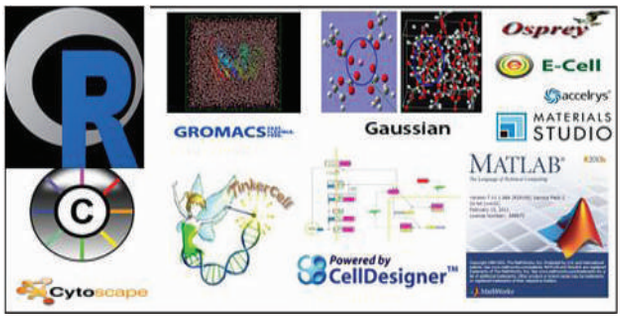
High End Desktop (4 in number)
HP workstations of Intel Core 2 Duo @3.00GHz with 8 GB of DDR2 memory, 320 GB of SATA storage and 19" LCD wide Display with Linux/Windows OS
HP Elite Desktop of Intel i7 processor, 3.4GHz with 16GB RAM, 2TB SATA storage and 21.1" LCD wide display with Windows 8.1 Professional 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: SVM^{light} 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 at the Bioinformatics and High Performance Computing Facility:

1. In-house "Applications of Computational Biology" training for graduate students. This was organized to help the students to develop a computational framework for gene survey of the biological sequences, which includes structure prediction, phylogenetic analyses, motif prediction, network modeling, molecular docking, protein-protein interaction etc. The workshop helped them to derive inferences about biological mechanisms and develop hypotheses for further experimental testing.

2. Training programmes conducted regularly for the students enrolled in the Ph.D. coursework.

   (Dates: 20.09.17, 27.09.17, 06.10.17, 01.11.17)

   This year, training was conducted for 33 students of the 2017 Ph.D. coursework, including students from ARI, NCCS and the Department of Biotechnology, SPPU.

   This training included: a) Different types of structure representation and implications – PyMol, Chimera.

   b) Surface calculation and implications: Hydrophobic, charge representation. c) Secondary structure prediction. d) Structure based alignment. e) Binding pocket prediction – Castp; Glycosylation, phosphorylation sites prediction. f) Modeller – homology modeling, threading. g) Energy Minimisation 8 Validation of models – Procheck, Whatif, Verify 3d. i) Auto dock VINA

3. A workshop on RNA-seq data analysis was also organised for students and project staff of NCCS from 20-22 December 2017.

Library

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 three hundred thirty-five books, and two hundred sixty-one NCCS Ph.D. theses. It subscribes to twenty scientific journals and thirty-four other periodicals in print form. The scientists 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 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).

The library has the Linux-based SLIM21 library software for its housekeeping operations 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.

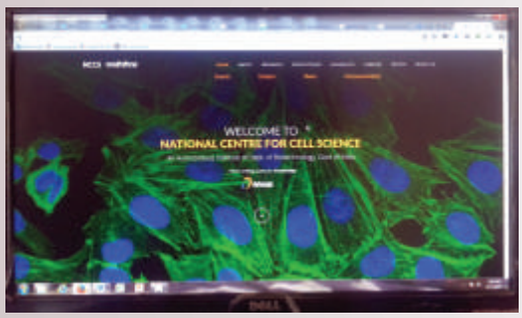
The library personnel are involved in providing library-related information for the NCCS website (Hindi & English), including library holdings, services, useful links and other relevant information. During the period under review, they have created a digital archive of the Ph.D. theses submitted by the 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. Krupasindhu Behera, *Technical Officer*

Mr. Rameshwar Nema, *Technical Officer*



Computer Section

Dr. M.V. Krishnasastry

(Facility In-Charge)

The computer section provides various computing and network infrastructure services to all scientists, technical and administrative users. This department provides secured network services, including the design and maintenance of campus- wide LAN / WAN solutions and internet / intranet solutions. It currently has two internet links viz., 100Mbps bandwidth from NKN and 10Mbps from Tata communications Ltd. The present network security system has been upgraded with the latest Sophos UTM firewall CR-1500XP and Sophos Antivirus for desktops, to provide a cohesive secured environment.

General Technical Support Services provided:

- Wired and wireless networking solutions & services to desktops, laptops and mobile phones.
- Internet connectivity, e-mail service to all scientists, staff and students.
- Computer hardware infrastructure procurement, installation and maintenance.
- Web services include design, maintenance and regular updating of the NCCS and intranet website, and its management and uploading of tenders on the CPP portal.
- User support services including software and hardware installations, printers, scanners and all other computer interfacing devices.
- Technical support in video conferencing / SKYPE / DROPBOX / VPN.
- Management of virtualised high-performance servers for hosting services like WWW, DHCP, DNS and Proxy.
- Network management of high-speed routers, switches and WL access points.
- Providing regular support for staff salary preparation, including TDS and EPF, and NPS deductions using the SARAL paypack software.

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. Chalking out specifications for core switch and access switches

A new core switch, i.e. Nexus 9300 from Cisco Systems, which is compatible with the current Cisco access switches, was found to be suitable for NCCS. This switch will have 3 software modules, namely Cisco Enterprise Management for LAN, CISCO Prime Infrastructure for complete lifecycle management of

cosnverged wired and wireless networks, and Cisco ISE for network device administration. The existing workgroup-based LAN environment will be converted to a domain-based environment with a single-sign-on feature.

2. New Sophos E-mail Gateway Security Appliance

A new E-mail gateway security appliance, (MX) ES1100, was installed and configured according to the NCCS security policy. This server has advanced features like antivirus, antispam, protection against spam and phishing attacks.

3. New Sophos Wifi Network connectivity in NCCS

New Sophos wifi network has been established by configuring a wifi ssid in Cyberoam firewall, and 30 access points were installed in all laboratories of NCCS. All scientists were given access to this wifi network on their new Dell laptops for browsing Internet/E-mail.

4. Stores & Purchase Management Server

A new virtual server having the Ubuntu 16.04 Operating System has been configured for hosting a stores & purchase management software. This software provides online access to data regarding budget allocation, indent, approvals, and material management. This system will have the option of exporting / importing data into it in CSV, XLS and text formats.

5. Renewal of Tata Internet Connectivity

The 10 Mbps (1:1) Internet Leased Line used for E-mail / DNS traffic from TATA Communications has been renewed for 1 year i.e. 2017-2018.

6. New Laptops, desktops and printers for scientists and staff

The Windows XP-based desktops were replaced with new desktops / laptops due to end of software support from Microsoft. Technical support was provided for preparing specifications for procurement from the GeM website, its installation, configuration and data transfer. This exercise included 35 new Dell laptops, 37 Acer desktops and 26 new Canon LBP6230BN laser printers.

Other Facilities

1) FACS Core Facility

The Team

- Dr. (Mrs.) L. S. Limaye *(Facility In-Charge)*
- Dr. Arunkarthick S. *(Assistant In-Charge)*
- Ms. Hemangini Shikhare *(Technician C)*
- Ms. Pratibha Khot *(Technician C)*
- Mr. Amit Salunkhe *(Technician C)*
- Ms. Ashwini Kore *(Technician B)*
- Mr. Dnyaneshwar Waghmare *(Technician B)*
- Mr. Atul Khirwale *(Operator provided by BD and posted in NCCS under BD-NCCS STEM CELL CoE)*

There are seven instruments in the FACS core facility. These are operated on a rotation basis by six dedicated operators.

The usage of the seven instruments for the period under consideration is summarized below:

IMMUNOPHENOTYPING & CELL CYCLE analysis

Equipment	Surface / Intracellular staining	DNA Cell cycle	CBA flex	CBA	Total Samples Acquired
FACS Calibur	2605	6015	---	---	8620
FACS Canto II (Old)	10230	---	---	---	10230
FACS Canto II (New)	6475	07	---	---	6482

STERILE SORTING

EQUIPMENT	SORTING	ACQUISITION **
FACS Aria II SORP	185	873
FACS Aria III SORP	447	1884
FACS Aria III Standard	295	831

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

Samples from extramural users:

As workload of outsider samples was increased, so since June 2012 NCCS had made a policy to charge the outsiders. For academic and research institute

charges are less and for private institutes / companies the charges are higher. The institutes like ARI, Sinhagad College of engineering, NCCS- Cipla collaborative project, Yakult Project utilized our facility from April 2017- March 2018. We had acquired 785 samples for Surface / Intracellular staining and DNA cell cycle analysis.

Activities of FACS core facility:

1. Canto-II training and examination:

The FACS facility organized training in batches on Canto-II during 2017-18, through which 19 students from NCCS received training. An examination for the trained students was conducted on 24-25th April 2017 by BD application specialists, with help from the FACS operators. 18 trained students appeared for the written and practical exam, out of which 15 students qualified the exam. The qualified students are now allowed to use the equipment without operator assistance.

2. The FACS Facility had arranged a demonstration of the BD FACS Melody instrument on 3rd and 4th October 2017 for NCCS students and FACS operators.

3. National Science Day: Through the open day organized at NCCS on the national science day on 28th February 2018, the facility operators Amit Salunkhe and Hemangini Shikhare presented a poster entitled "NCCS Flow Cytometry Core Facility". A demonstration of the FACS machines was also organized for the visitors.

Shifting of the FACS Facility: Under the supervision of Dr. L. S. Limaye, the FACS facility was shifted to a new space between June – July 2017, in stages without hampering the students' work. The new FACS facility was formally inaugurated by Prof. Partha Majumdar (Chairman of the NCCS RAP-SAC).

2) Imaging facility

The Team

- Dr. Jomon Joseph & Dr. Arunkarthick S. *(Facility In-Charge)*
- Mrs. Ashwini N. Atre (*Technical Officer A)*
- Mrs. Trupti Kulkarni (*Technician B)*

There are three confocal microscopes in the Confocal imaging facility of the Institute. These are operated on rotation basis by two dedicated operators.

The imaging facility has three scanning confocal laser microscopes, which includes Zeiss LSM 510 Meta, Olympus FV10i and Leica TCS SP5 models. All the systems are inverted microscopes and have a wide range of lasers. The systems can be used for doing FRET, FRAP, 3D imaging and reconstruction and live cell imaging, which are required for most cell biology research. All three instruments are used by in-house users as well as by users from neighbouring organizations.

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 (405 nm), multi-line Argon ion laser (458/477/488/514 nm), 543 nm He-Ne and 633 nm He-Ne. The spectral detector permits separation of upto eight emission signals, even if the fluorescence spectra are strongly overlapping.

ii) Leica SP5 II

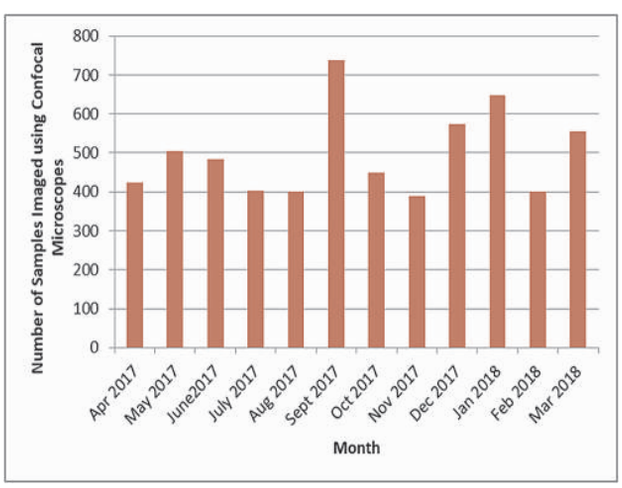
This is a high-end Broadband Confocal Laser Scanning Microscope with 3 PMT's, 2 Hybrid detectors and AOBS technology equipped with CO_2 incubator, fully motorized, automated and computer-controlled microscope Leica DMI 6000. The Lasers are Blue Diode Laser 405 nm, Argon ion Laser with 458/476/488/496/514 nm lines, DPSS 561 nm, He-Ne 594 nm and He-Ne 633 nm 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 FV10i

The FLUOVIEW FV10i microscope from M/s Olympus has a compact design and does not require a dedicated darkroom. This is an easy to use and self-contained confocal microscope equipped with four lasers [405, 473, 559 and 635 nm].

Usage of the Microscopes during 2017-18

The numbers of samples imaged during this year were approximately 6000 in-house, plus 120 samples received from various other institutes. The following graph shows the month wise details of number of samples imaged during last year in NCCS confocal microscopy facility.



Activities of Confocal Microscopy Facility:

1. Training Programs: The facility organized training in batches on both Zeiss and Leica confocal microscopes during April to May 2017 and October to November 2017. 10 students received training during the 3rd April 2017 to 4th May 2017 training batch, and 12 students received training in the second batch during 31st October 2017 to 24th November 2017. Trained students appeared for the written and practical examinations. The qualified students are now allowed to use the microscopes without operator assistance.

A hands-on training programme on Leica SP5II Confocal Microscope was also conducted on 7th & 8th December 2017 by the Leica company. Presentations were made and training on the Leica SP5II confocal microscope was given to 12 students.

2. Demonstration of Microscopes: The facility conducted a demonstration of two different microscopes during 2017-18. The Leica Company organized a demonstration of the 'Laser Scanning Confocal Microscope model SP8'. This instrument was installed at NCCS during 15th July 2017 to 25th July 2017. Many scientists and students checked the imaging capabilities of the SP8 confocal microscope using their samples. The Thermo FisherScientific Company conducted a demonstration of the Inverted Fluorescence Microscope, Model: Invitrogen Evos FL Auto on 8th November 2017.

3. National Science Day: Through the open day organized at NCCS on the national science day on 28th February 2018, the confocal microscopy facility arranged a demonstration of microscopes, for all the visitors. The features of the microscopes were explained to the visitors, with the help of power point slides and posters. They were also shown cell images and videos acquired with these microscopes.

3) DNA sequencing facility

The Team

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

The central sequencing facility of NCCS is located at the National Centre for Microbial Resource (NCMR) and houses two instruments from Applied Biosystems (3730 and 3730xl), along with all sequence and data analysis software. The facility offers services related to sequencing of plasmids, PCR products and cloned inserts; primer walking; and genotyping and fragment analysis, to researchers from NCCS and other organizations. This facility caters to the needs of research institutions and industrial clients across the country, for the identification of bacterial and fungal isolates. In addition, the facility serves as the back-bone of culture authentication and identification for NCMR's preservation activities.

Over the year 2017-18, nearly 47040 sequencing reactions were run on the machine. The facility provided support to the internal institutional research activity by delivering 13960 sequencing reactions. 567 services against payment were provided to 124 different academic and research institutions from 20 states across the country. Bacterial identification using 16S rRNA gene sequencing and fungal identification using the IT region sequence were mainly performed. For the identification of bioprospection cultures stored in the biobank at NCMR, 20359 cultures were processed. Also, 883 cultures were validated for general deposit in the culture collection during this year.

a) Name of the machine : ABI 3730XL DNA Analyzer.
b) Number of samples run on the machine during the said period (490 96-well plates) : ~ 47040.
c) No. of in-house users : 30
d) No. of extramural users benefited : 124 different institutions/universities from 20 states (Andhra Pradesh, Assam, Chhattisgarh, Delhi, Himachal, Gujrat, Rajasthan, Jammu, Karnataka, Kerala, Odisha, Punjab, Maharashtra, M.P., Tamilnadu, Telangana, Tripura, U.P., Uttarakhand and West Bengal).

4) IVIS Imaging System

The Team

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

The IVIS-Spectrum facility is a common central facility of NCCS. This instrument provides bioluminescent and fluorescent imaging of cells and whole small animals (especially mouse and rat) under in-vitro and in-vivo conditions. During 2017-18, more than 25-30 Scientists and Research Scholars of various laboratories of NCCS as well as collaborators from other organisations used the IVIS system. They used bioluminescence as well as fluorescence imaging in different strains of mice (NOD/SCID/ NUDE/ C57/Balb/C etc) and different type of tissue culture plates (i.e. 96 well, 24 well and 12 well etc).

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

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

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:

- High-sensitivity *in vivo* imaging of fluorescence and bioluminescence
- High throughput 6 mice) with 26 cm field of view
- High resolution (to 60 microns) with 3.9 cm field of view
- Dual 12-position emission filter wheels (24-position total) and 12-position excitation wheel

- A set of four filter pairs for fluorescent imaging come standard with the instrument, in addition to a set of four background filters for subtraction of tissue autofluorescence
- 25 x 25 cm alignment grid on the imaging platform ensures consistent accurate placement of animals for imaging
- Spectral imaging filters that acquire images at different wavelengths (ranging from 560 nm to 660 nm) facilitate 3D diffuse tomographic reconstruction and determination of the depth and location of a bioluminescent reporter
- Heated animal shelf (up to 40° C)
- NIST traceable absolute calibrations
- Class I Laser Product

IVIS Imaging System

5) Central Sterilization Facility

The Team

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

This facility is an infrastructure service department of the institute. It provides in-house services, such as washing, packing and sterilization of all the glassware and other research material, to all the research laboratories, the cell repository, the media section and the other service departments. It also supplies high-grade distilled water to all the sections of the institute. In addition to this, some technical staff members are also involved in the safe disposal of radioactive and biohazardous waste material.

NATIONAL CENTRE FOR
MICROBIAL RESOURCE (NCMR)

Establishment of Centre of Excellence for National Centre for Microbial Resource

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Background

The largest collection of bacteria of Indian origin housed at NCMR (formerly MCC) since 2009 is a valuable resource for many useful products and biotechnological applications. It became imperative that such collection be preserved for long-term at a permanent facility and efforts were initiated to meet the scientific and industrial standards and compliance. With more than 180,000 microorganisms (archaea, bacteria, fungi including yeasts) in its collection, NCMR is now the single largest culture collection in the world and has put India in the top three countries after USA and Japan. NCMR is the most unique culture collection as a major part of its collection is from diverse ecological niches in India, such as soils from Western Ghats, North East, mangroves, marine environment, industrial effluent polluted sites, and insect guts. It has played a vital role in the exploration and conservation of microbial diversity of India, which is now accessible to industry or individual researchers for exploitation for commercial and academic activities.

After due ordinances and approvals, Microbial Culture Collection (MCC) facility served till 31st March 2017, after which it was rechristened as a 'Centre of Excellence for National Centre for Microbial Resource (NCMR)' with effect from 1st April 2017. These major events have been chronologically depicted in Fig 1.

Recently, NCMR completed formalities for the acquisition of land for its permanent campus. The proposed site measuring 5068 m2 is located in Virbhadra nagar, Baner, which is 11 kms from Pune city centre and about 3 km from the current location of NCMR near the NH48 (Mumbai Pune bypass road) on the west. After several meetings with the team of architects the Design Basis Report was finalized, with a total build-up area of 126149 sq. feet. The proposed building plan include all the programs of NCMR like specialized labs, common labs, support area, preservation facilities, auditorium, administration, hostel, guest rooms, and residences of essential support staff. The design and building plans are now in the final stages and have been submitted to DBT for approval.

Aims and Objectives

The Mission of the Centre is to serve as a leading world class Microbial Resource Repository and provide authentic high-quality services for microbial preservation, characterization and authentication and supply to industry and academic institutions. The Centre is built on *"Service for Science, Science for Service"* model. It will also serve the nation in biodiversity conservation, biotechnological research and education by providing services of the highest international standards and conducting research in the related areas of microbial ecology and systematics, and human resource development. The Centre also serves as an International Depositary Authority (IDA) under the Budapest Treaty and is Designated as National Repository under Ministry of Environment & Forests & Climate Change under the Biodiversity Act 2002.

The specific objectives are as under:

- To complete characterization of the existing collection of 1.8 lakh strains so as to increase their utility for investigators.
- To develop an infrastructure to facilitate services of the highest standard, such as supply of authentic microbial cultures, identification of microorganisms, deposit of microorganisms, their long-term protection and other related areas.
- To serve as International Depositary Authority for deposit of Micro-organisms under the Budapest Treaty for protection of intellectual property rights.
- To serve as Designated National Repository under the Biological Diversity Act 2002 of India.
- To develop quality manpower with creative abilities in microbiology/ microbial biotechnology/technology management by providing both long and short-term training courses and workshops involving experts from across the globe.
- To undertake research in the relevant areas of microbial ecology and systematics so as to strengthen the services.

Work Done

More than ~200000 cultures collected from various ecological niches were preserved as safe deposit cultures. SOPs were custom designed and followed on routine basis for the periodic preservation and passaging. They have been categorized as below on the basis of their screening for different bio-active compounds:

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

1. Culture preservation status

All normal cultures are preserved in -80°C as per the SOP. In addition, all three star, re-fermented and a major proportion of normal cultures are also preserved in liquid Nitrogen (-196°C) (Table 1).

Table 1: Number of cultures preserved under each category (including pure and mixed).

Category	Preserved	Pure	Mixed
Normal	136159	100819	35340
Three star	9975	7938	2037
Re-fermented	1361	1079	282
Total	147495	109836	37659

2. Culture passage status

NCMR initiated the passage activity in later half of 2013 and has finished the 1st passage of all three star, re-fermented and 84686 normal cultures (Table 2).

3. Identification of cultures

All 7938 pure 'Three Star' cultures have been identified either by 16S rRNA gene sequencing or by MALDI-TOF mass spectrometry. In addition, all normal category cultures are presently being processed for identification by 16S rRNA gene sequencing or MALDI since September 2013. Since then, a total of 24,179 normal cultures have been identified (12,854 by 16S rRNA gene sequencing and 11,325 by MALDI-TOF MS analysis). The normal category cultures represent 146 different genera based on 16S rRNA gene sequencing (Table 3).

Table 2: Institute wise preservation status of microbial prospecting cultures at MCC

Institute	Processed	Preserved (-80 °C)	Preserved (Liquid Nitrogen)	1st Passage (-80 °C)
DU	24664	15235	9783	11880
GNDU	22907	15857	8910	6947
IBSD	27074	14018	10295	11673
IGIB	24421	17975	12839	9557
ILS	25778	13583	8602	4132
MSSRF	22377	11666	8690	7704
NCCS	25000	25000	10428	10628
NEERI	19834	12331	12231	12231
NIO	19691	10494	9258	9934
Total	211746	136159	91036	84686

Table 3. Genera observed for the normal category cultures based on the 16S rRNA gene sequencing

Achromobacter	*Curtobacterium*	*Mesorhizobium*	*Salinicoccus*
Acidovorax	*Deinococcus*	*Methylobacterium*	*Salinicola*
Acinetobacter	*Delftia*	*Microbacterium*	*Salinivibrio*
Aerococcus	*Devosia*	*Micrococcus*	*Salmonella*
Aeromicrobium	*Diaphorobacter*	*Mycobacterium*	*Serratia*
Aeromonas	*Dietzia*	*Neorhizobium*	*Shewanella*
Agromyces	*Dyadobacter*	*Nitratireductor*	*Shinella*
Albirhodobacter	*Dyella*	*Nocardioides*	*Sinomonas*
Alcaligenes	*Ensifer*	*Novosphingobium*	*Solibacillus*
Algoriphagus	*Enterobacter*	*Oceanimonas*	*Sphingobacterium*
Alishewanella	*Enterococcus*	*Oceanobacillus*	*Sphingobium*
Aminobacter	*Erwinia*	*Ochrobactrum*	*Sphingomonas*
Ancylobacter	*Erythrobacter*	*Olivibacter*	*Sphingopyxis*
Arthrobacter	*Escherichia/Shigella*	*Paenalcaligenes*	*Sporosarcina*
Azospirillum	*Exiguobacterium*	*Paenibacillus*	*Staphylococcus*
Bacillus	*Fictibacillus*	*Pantoea*	*Stenotrophomonas*
Brachybacterium	*Flavihumibacter*	*Paracoccus*	*Streptomyces*
Brevibacillus	*Flavobacterium*	*Parapedobacter*	*Tenacibaculum*
Brevibacterium	*Gordonia*	*Parapusillimonas*	*Terrabacter*
Brevundimonas	*Halobacillus*	*Phenylobacterium*	*Terribacillus*
Burkholderia	*Halomonas*	*Photobacterium*	*Thauera*
Camelimonas	*Hydrogenophaga*	*Planococcus*	*Thermomonas*
Caulobacter	*Idiomarina*	*Planomicrobium*	*Tsukamurella*
Cellulomonas	*Isoptericola*	*Promicromonospora*	*Variovorax*
Cellulosimicrobium	*Janibacter*	*Providencia*	*Vibrio*
Chelatococcus	*Jeotgalibacillus*	*Pseudocitrobacter*	*Virgibacillus*
Chitinophaga	*Jeotgalicoccus*	*Pseudoduganella*	*Vogesella*
Chryseobacterium	*Kaistia*	*Pseudogulbenkiania*	*Xanthomonas*
Chryseomicrobium	*Klebsiella*	*Pseudomonas*	*Yangia*
Ciceribacter	*Kluyvera*	*Pseudoxanthomonas*	*Yokenella*
Citricoccus	*Kocuria*	*Psychrobacter*	*Zhihengliuella*
Citrobacter	*Kurthia*	*Ralstonia*	*Zobellella*
Cobetia	*Leifsonia*	*Raoultella*	
Comamonas	*Leucobacter*	*Rheinheimera*	
Corynebacterium	*Lysinibacillus*	*Rhizobium*	
Cronobacter	*Lysobacter*	*Rhodococcus*	
Cryobacterium	*Marinomonas*	*Rothia*	
Cupriavidus	*Massilia*	*Rummeliibacillus*	

4. Cultures dispatched to Academia/Industry

As part of DBT's initiative to share the microbial cultures for screening of additional bioactive compounds, NCMR has supplied 4500 cultures to North Maharashtra University (NMU). In addition, at present DBT has sanctioned two other projects and NCMR is supplying cultures to these institutes.

5. Culture received from Piramal Life Sciences Pvt. Ltd.

Piramal Life Sciences, Mumbai has donated 28,294 cultures to NCMR. NCMR is in the process of preserving these cultures in -80 °C freezers and liquid Nitrogen (-196 °C) (Table 4).

Table 4: Distribution of cultures received from PLSL

Type of cultures	Total
Fungi	15139
Actinomycetes	7447
Eubacteria	5300
Marine actinomycetes	408
Total	28294

6. Services provided by NCMR

In addition to the preservation and supply of bioprospecting cultures, NCMR actively processes microbial samples received for identification and characterization services from researchers in academia and industry. Fig. 2 shows the demography of customers across the country to whom various services were offered by NCMR. Table 5 shows the summary of services offered by NCMR during the report period (2017-2018).

7. Supply of Cultures

NCMR is supplying lyophilized culture vials of many high demand cultures to its customers.

8. IDA/Patent deposits

In addition to the general deposits, NCMR is also receiving deposits for IDA from researchers in India and abroad. 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 12 vials are stored at NCMR at 4°C.

9. Cyanobacterial Culture Collection

In April 2015, NCMR started accepting cyanobacterial cultures in its collection. Since then, NCMR has received total 106 cyanobacterial cultures for preservation under general deposit.

10. Novel taxa described by NCMR Scientists

Over the past one year, NCMR scientists have described 12 novel taxa (Table 6).

Table 5: Summary of services offered by NCMR during the period of this report.

Services	Total
Preservation and Supply of Cultures	
General Deposits Accessioned (Bacteria)	601
General Deposits Accessioned (Fungi)	121
Cultures supplied (Bacteria)	456
Cultures supplied (Fungi)	89
Deposits under Budapest Treaty (IDA)	51
Safe deposits	20
Identification Services Provided	
16S rRNA gene sequencing as an identification service	102
DNA sequencing service for Research cultures (Internal)	10,430
MALDI	29,077
Phylogenetic analysis	12
Other Related Identification Services Provided	
FAME	57
DNA-DNA hybridization	26
% GC content	8
Polar lipid analysis	15
Genomic DNA Isolation /Purification	10
Phenotypic Characterization	15

Table 6: Novel taxa validly published during 2017-18 by NCMR scientists.

Sr. No.	Taxa	Scientist	Year
1	Reclassification of *Phycicola gilvus* and *Leifsonia pindariensis*	Dhiraj Dhotre	2017
2	*Mangrovibacter phragmitis*	Neetha Joseph	2017
3	*Candidatus* Phytoplasma asteris	Amit Yadav	2017
4	New subgroup 16SrXI-F phytoplasma	Amit Yadav	2017
5	*Lysinibacillus telephonicus*	Praveen Rahi	2017
6	*Aliinostoc morphoplasticum*	Prashant Singh	2017
7	*Westiellopsis ramosa*	Prashant Singh	2017
8	*Auricoccus indicus*	Om Prakash	2017
9	*Corynebacterium godavarianum*	Avinash Sharma	2017
10	*Nothophoma raii*	Rohit Sharma	2017
11	*Alanomyces indica*	Rohit Sharma	2017
12	*Microbacterium telephonicum*	Praveen Rahi	2018

Future Research Plans

Additional Services to be offered

NCMR plans to implement and/or extend certain services in the near future and needs approval of fees for the same.

- **Microbial genome sequencing and bioinformatic analysis:** Illumina Miseq platform has been successfully installed in the NCMR facility. NCMR is planning to utilize this platform to provide microbial genome sequencing and subsequent bioinformatic analysis services in the coming year.
- **Biolog based bacterial identification:** Biolog system for phenotypic characterization has been successfully installed in the NCMR premises and soon the service will be available to the custormers.
- **Anaerobic Culture Collection:** The facility for the cultivation of anaerobic microbes is now functional and we propose to start receiving anaerobic microorganisms for deposit in all the three categories, viz., general, safe and IDA.

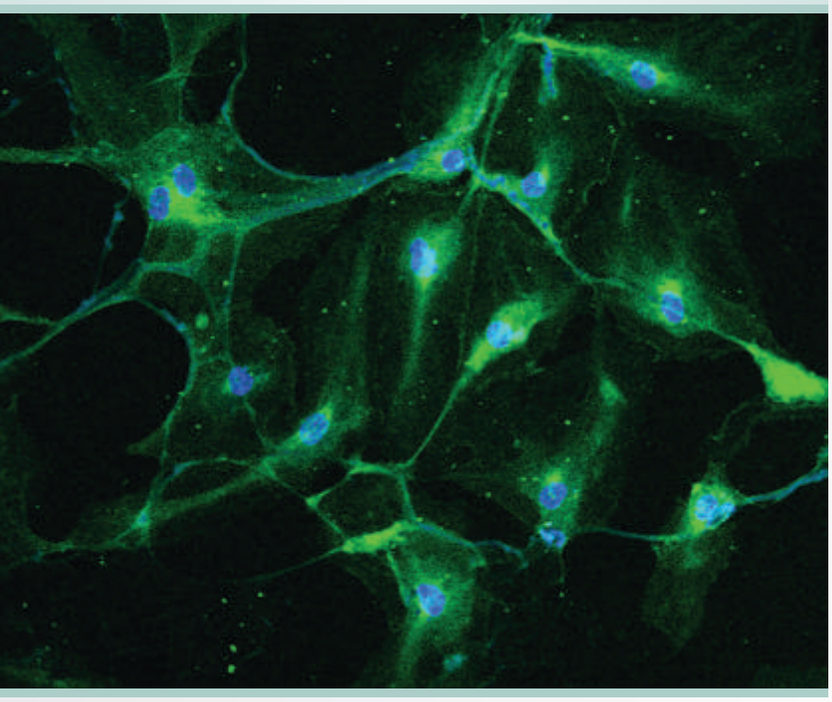
Supply of cultures to Industry

As part of DBT's initiative to share the microbial prospecting cultures for screening of additional bioactive compounds, DBT has sanctioned two projects and NCMR is supplying them these cultures. Apart from this, based on the expertise and infrastructure available in NCMR and NCCS, NCMR will provide customized services to various industries in the coming year.

Figures

Fig. 1: Major chronological events in the history of establishment of NCMR

Fig. 2: Distribution of services provided by NCMR across the country



Other Information

Publications / Book Chapters / Patents

Publications of NCCS faculty

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Books / Book Chapters / Reviews / Editorials (NCCS faculty)

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Publications (NCMR scientists)

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Books / Book Chapters / Reviews / Editorials (NCMR scientists)

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Patents resulting from research carried out by NCCS faculty members

- IL-3 in regulation of bone homeostasis

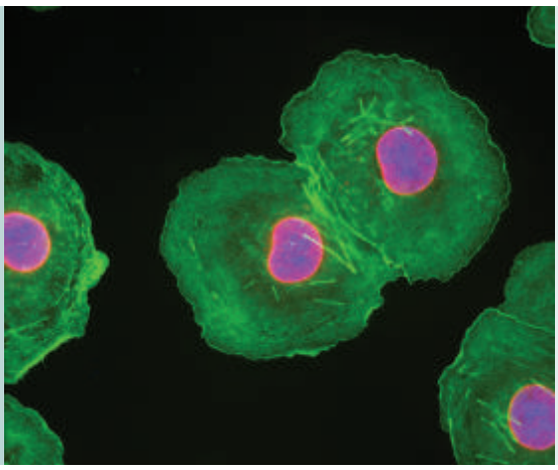
  Singh K., Mohan R. Wani. Patent Application No. 201721041260.

- Peptides that inhibit factor b, c2 and complement activation, and their uses

  Date of Grant: 17/10/2017

  (Patent No.: 288480; Patent application No.: 889/del/2010; Date of filing : 13.04.2010)

  Patentee: Department of Biotechnology, National Centre for Cell Science



Awards / Honours / Memberships

Awards / Honours / Memberships - NCCS Faculty

Sharmila Bapat

Awards / Honours

- TATA Innovation Fellowship 2017- 2020

Memberships

- Member, The EMT International Association (TEMTIA)

Samit Chattopadhyay

Honours

- ? Convener, Sectional Committee, Indian National Science Academy (INSA) (2017 onwards)

Memberships

- Research Council Member, CCMB, Hyderabad, 2017-2020.
- Research Council Member, IMTECH, Chandigarh, 2017-2020.

Radha Chauhan

Memberships

- Lifetime membership for Electron microscopy society India.
- Joint-secretary and founding member of Cryo Electron Microscopy and 3 Dimensional Image Processing Society of India (CEM3DIPSI)

Jomon Joseph

Awards / Honours

- International Travel Award from European Molecular Biology Organization for attending the Conference on 'Ubiquitin and SUMO: From molecular mechanisms to system-wide responses' held at Cavtat-Dubrovnik, Croatia, 15-19 September, 2017.

Memberships

- Life Member, Indian Society for Cell Biology
- Life Member, Society of Biological Chemists, India
- Life Member, Indian Society for Developmental Biologists

Janesh Kumar

Awards / Honours

- EMBO short-term fellowship (July – September 2017)

Gopal C. Kundu

Awards / Honours

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

Girdhari Lal

Memberships

- 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.
- Founding and Executing council member, Society of Inflammation Research, India.
- Member, Society of Mucosal Immunology (SMI), USA.

Nibedita Lenka

Awards / Honours

- Chairperson, Institutional Ethical Committee and Member, IC-SCR, OCT Therapies & Research Pvt. Ltd. Mumbai.
- Member, Institutional Committee for Stem Cell Research (IC-SCR), National Institute for Research in Reproductive Health (NIRRH), Mumbai.

Memberships

- Life Member, Indian Academy of Neuroscience.
- Active Member, International Society for Stem Cell Research (2005 – present).
- Member, Indian Society of Developmental Biology (2016-present).

Lalita S. Limaye

Memberships

- 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

Shekhar Mande

Awards / Honours

- BC Guha Memorial Award lecture of the Indian National Science Academy, New Delhi, 2017
- BK Bachhawat Memorial Award lecture of the National Academy of Science, India, Allahabad, 2017

Debashis Mitra

Awards / Honours

- J. C. Bose National Fellowship

Srikanth Rapole

Memberships

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

Shailza Singh

Memberships

- 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

Mohan Wani

Awards / Honours

- Member, Stem Cell Task Force, DBT (2018-21).
- Academic Editor, PLOS ONE (2017-18).

Awards / Honours – Students, Postdoctoral & Other Early-career Researchers, Technical Staff

- **Ananth P. Burada** (Dr. Janesh Kumar's group) : **The Newton-Bhabha PhD Placement fellowship** – to work in Prof. Elena Orlova's lab at the Department of Biological Sciences, University of London, London, UK, from June 2017 – October2017.

- **Dr. Avtar Meena** (former Ph.D. student of Dr. Bhat?): Named the 2017 Outstanding Junior Postdoc in the College of Graduate Health Sciences.

- **Debasish Paul** (Dr. M. Santra's group): **J. BIOSCIENCES best poster award;** The 2018 International Congress of Cell Biology (ICCB), 27-31 Jan 2018, Hyderabad, India.

- **Dhiraj Kumar, Amit Yadav, Mahadeo Gorain** (Dr. G.C. Kundu's group) : **'Best Poster Award'** '3rd International Conference on Translational Research: Applications in Human Health and Agriculture', 23-25 Sep. 2017, Amity University, Kolkata.

- **Dipti Athavale** (Dr. M.K. Bhat's group): **Best Poster Presentation Award & Certificate of Excellence,** World Congress on Cancer (WCC-2018), 3-5 February, 2018, Jaipur, India.

- **Dipti Athavale** (Dr. M.K. Bhat's group): **EMBO travel grant** to attend the EMBO workshop on Metabolic Disorders and Liver Cancer, 23-26 April 2017, Palma de Mallorca, Spain.

- **Jay Trivedi** (Dr. Debashis Mitra's group): 'Travel Merit Award' from the International Society for Antiviral Research. He presented a poster at the 30th International Conference on Antiviral Research, 21-25th May, 2017, Atlanta, USA.

- **Jyoti Kumari** (Dr. Janesh Kumar's group): **Fellowship** to work with Prof. Peter J. Peters at the Maastricht University, The Netherlands for 6-months (April – September 2017).

- **Kriti Chopra: NS Dasgupta Best Poster award,** 42nd Annual Indian Biophysical Society Meeting, 9-11 March 2018, IISER-Pune.

- **Dr. Lekha Rani** (Dr. M. Wani's group) : **Best Oral Presentation Award**
  The '19th Indo-US Cytometry Symposium & Workshop on Advanced Flow Cytometry and Applications', 17-18 February, 2018, NIRRH, Mumbai, India.

- **Dr. Lumbini Yadav** (Dr S.C. Mande's group): Selected for participation in the 68th Lindau Nobel Laureate Meeting scheduled in Germany from 24-29 June 2018.

- **Mahendra Kumar** (Dr. Krishnasastry's group): **Best Poster Presentation award**
  International Conference on Trends in Biochemical and Biomedical Research: Advances and Challenges, 13-15 February, 2018, BHU, Varanasi, India.

- **Pranay Ramteke** (Dr.Bhat's group) : **2nd Best Poster Presentation Award across all categories,** World Congress on Cancer (WCC-2018), 3-5 February, 2018, Jaipur, India.

- **Rahul Bodkhe** (Dr. Y. Shouche's group): Fulbright Nehru Doctoral Fellowship 2018

- **Raj Kumar Gour & Mahendra Kumar** (Dr. Krishnasastry's group): **Best Poster Presentation award**
  International Conference on Trends in Biochemical and Biomedical Research: Advances and Challenges, 13-15 February, 2018, BHU, Varanasi, India.

- **Ritika Kabra** (Dr. S. Singh's group): **Second Prize in Poster presentation** for, 'Miltefosine resistance reversal in Leishmania major by a synthetic peptide'; National Conference on Protein Structure and Dynamics in Health and Agriculture, 3-4 November 2017, New Delhi, India.

- **Satish Kumar: Best Paper Presentation award** at the World Research Journals Congress (WRJC-2018), Bangalore, India 10-13 January, 2018.

- **Shreyas Kumbhare** (Dr. Y. Shouche's group): **Early career scientist grant award** at the 7th FEMS Congress, Spain.

- **Siddharth Singh: Travel & Accommodation Grant** to attend the 'AMR DxC Summer School India 2017' from DBT, Govt. of India and University of Edinburgh, UK, at Bangalore; 4-7 July, 2017.

- **Siddharth Singh: International Travel Grant** from DST-SERB, Govt. of India & DBT, Govt. of India, to attend the 'Exploring Human Host - Microbiome Interactions in Health and Disease 2017' conference; Wellcome Genome Campus, 13-15 September, 2017, Cambridge, UK.

- **Sonal Patel** (Dr. S. Chattopadhyay's group) : **Best Oral Talk & Poster Awards,**
  CDAC-Accelerating Biology Conference, 9-11 January 2018, IISER- Pune, India.

- Surbhi Dhingra (Dr. Janesh Kumar's group): **EMBO Student Bursary** for participating in the EMBO practical course, CEM3DIP 2018, 18-29 March 2018, New Delhi, India.

- Tushar More (Dr. Rapole's group): **DST Travel award** to present a poster at the Metabolomics-2017 conference, 26-29 June 2017, Brisbane, Australia.

- Upasana Kapoor (Dr. N. Lenka's group): Abstract selected for oral presentation and travel award for attending the 41st Annual Meeting of the Japan Neuroscience Society, 2018 to be held in Kobe, Japan.

- Varun Haran M and Upasana Kapoor (Dr. N. Lenka's group): Selection/Short-listing of Project submitted for IIBR through Blind Review for oral presentation.

- Venkatesh Chanukuppa (Dr. Rapole's group): **Poster selected for oral presentation** at the 9th Annual Meeting of Proteomics Society, India, and the International Conference on Proteomics in Health and Disease, November 30-December 02, 2017, Institute of Life Sciences, Bhubaneshwar, India.

- Venkatesh Chanukuppa (Dr. Rapole's group): **Travel award** to attend the 9th Annual Meeting of Proteomics Society, India, and the International Conference on Proteomics in Health and Disease, November 30-December 02, 2017, Institute of Life Sciences, Bhubaneshwar, India.

Awards / Honours / Memberships - MCC Scientists

- All NCMR scientists are members of the Association of Microbiologist of India (AMI) and the Bergey's International Society for Microbial Systematics (BISMiS).
- Dr. Amit Yadav received Indian National Science Academy Fellowship under bilateral research program (2018) to work in DSMZ, Germany for 4 weeks.
- Dr. Avinash Sharma visited Beuth University of Applied Sciences under INSA-DFG International Scientific Collaboration and Exchange of Scientists Programme from July 2017 to October 2017 in Prof. Elisabeth Grohmann's Lab.
- Dr. Dhiraj Dhotre was invited as an expert for the First International workshop on "Systems Biology for Human and Plant Nutrition" at ICRISAT, Hyderabad, India on March 22-24, 2018.
- Dr. Kamlesh Jangid received the 2017 Albert Nelson Marquis Lifetime Achievement Award, Marquis Who's Who, USA.
- Dr. Kamlesh Jangid has been appointed as Associate Chief Editor of Soil Biology & Biochemistry, Elsevier® Science for three years beginning January 2017.
- Dr. Kamlesh Jangid was invited as an expert for the Indo-German Program on "Soil Protection and Rehabilitation for Food Security in India" by BAIF at Yavatmal, Maharashtra on January 5, 2018.
- Dr. Kamlesh Jangid has been appointed as Guest Associate Editor; Antimicrobials, Resistance and Chemotherapy; Frontiers in Microbiology, 2017.

Extramural Funding – NCCS Faculty

Radha Chauhan

- Reconstitution and structural studies on Nup93•Nup62•Nup54N•up58 quaternary complex 2015-2018. (DBT-basic sciences EMR funded).
- Structural and functional role of NPC in HIV infections 2016-2021. (DBT-Centre for excellence)

Samit Chattopadhyay

- Metabolic stress induced epigenetic changes in the transcriptional regulator gene SMAR1. 2017-2020 (DBT, India).
- Role of Nuclear Matrix Protein SMAR1 as Regulator of Suppressor T cell in Inflammatory Bowel Disease (IBD). 2017-2020 (DBT, India).
- Regulation of CD44 splicing by tumor suppressor SMAR1: Implications in cancer metastasis. 2016-2019 (DBT, India).

Jomon Joseph

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

Vaijayanti P. Kale

- "Identification of Aging-induced Epigenetic changes causing hematopoietic stem cell dysfunction: Rescue using in vitro niche technology-[BT/PR11928/MED/31/275/2014]"-Project sanctioned by DBT in Feb. 2017 for 3 years.

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).
- Structure and function of a novel family of AMPA receptor auxiliary subunits. 2018 – 2021. (DST-DAAD).

Gopal C. 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).
- Antigen-specific regulatory CD8 T cell vaccination to control food allergy and establishment of oral tolerance. From Department of Biotechnology, Government of India. (2017-2020).

Nibedita Lenka

- Nibedita Lenka. NER Twinning Project, DBT (2016-2019).
- Nibedita Lenka. ICMR Assigned Validation studies (2018).

Lalita S. Limaye

- BT/PR12696/MED/31/287/2014: "Studies on generation of induced pluripotent stem cells from umbilical cord tissue derived adult stem cells -Project in collaboration with AFMC sanctioned by DBT in Feb. 2017 for 3 years.
- BT/PR11928/MED/31/275/2014: Identification of aging-induced epigenetic changes causing hematopoietic stem cell dysfunction: Rescue using in vitro niche (IVN)-technology- Project sanctioned by DBT in Feb. 2017 for 3 years.
- BT/PR23620/MED/31/368/2017: "Molecular analyses of extra-cellular vesicles isolated from bone marrow-derived mesenchymal stromal cells treated with specific signaling modifiers and assessment of their effects on the fate of hematopoietic stem cells"- Project in collaboration with SSBS ,Pune sanctioned by DBT in March 2018 for 3 years.

Amitabha Majumdar

- WT-DBT India Alliance intermediate fellowship (IA/I/13/2/501030); 01.02.2014 – 31.01.2019

- SERB ECR grant (ECR/2016/000429); 15.03.2017 – 14.03.2019

Shekhar Mande

- International Associated Laboratory in the area of Systems Immunology 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. 2012-2018 (DBT).
- Center for Excellence in Biomolecular Structure and function on host-pathogen interactions in TB infections. 2016-2021 (DBT).

Debashis Mitra

- Debashis Mitra and Shekhar C. Mande. Cellular Stress Proteins in HIV infection: Biochemical and Functional Characterization Under the Center of Excellence in Biomolecular Structure and Function on Host-Pathogen Interactions (2016-2021) at NCCS, Department of Biotechnology, Govt. of India.

Srikanth Rapole

- 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 (Twining project- DBT).
- Identification of Ring – Finger E3 ubiquitin ligases involved in NF-κB pathway activation and deciphers the molecular mechanism (DST-SERB).

Vasudevan Seshadri

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

Anjali Shiras

- Developing bank of human induced pluripotent stem cells for drug screening and disease modelling. 2017-2020 (Department of Science and Technology, India).

Yogesh Shouche

- Establishment of Center of Excellence for "National Center for Microbial Resource (NCMR), April 2017 to March 2020, Department of Biotechnology, Ministry of Science & Technology, Government of India.
- Industry-sponsored project from various industries like Tata Steel, Tata Chemicals, Unilever India, Bhoruka Charitable Trust etc.

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

- Mechanism of breast cancer stem cell radio-resistance: Role and epigenetic regulation of Nrf2-Keap1- (2015103701RP01892-BRNS) Funding Agency: Board of Research in Nuclear Sciences (BRNS).

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

- Regulation of development of pathogenic T-helper 17 cells in collagen-induced arthritis'. funded by Science and Engineering Research Board (SERB), DST, New Delhi for the period 2018-2021.

Extramural Funding – NCMR Scientists

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. (SERB, 2017)

Om Prakash and Dhiraj Dhotre

- Study of biomethanation potential of landfill methanogens and their prospective role in global climate change, carbon sequestration and generation of bioenergy Rs. 28.3 Lakh (DBT, 2017-2019)

Praveen Rahi

- Population structure of root-nodulating bacteria associated with pea cultivated in different agroclimatic regions of India. Rs. 24.36 Lakh (SERB, 2015)

Rohit Sharma, OmPrakash and Dhiraj Dhotre

- Development of Efficient and low-cost biotechnology for colour removal of biomethanated spentwash from distillery Rs 36.5 Lakh (DBT, 2017-2019)

Research Students of NCCS awarded with Ph. D. Degrees

(01.04.2017 – 31.03.2018)

No.	Research Scholar	Title of the Thesis	Date of award of Ph.D. (dd.mm.yyyy)	Research Guide
1	Swapnil Kamble	Validation of molecular stratification in high grade serous ovarian adenocarcinoma.	19.04.2017	Dr. Sharmila Bapat
2	G.N.V.R. Chandrika	Targeting mammalian target of rapamycin (m TOR) pathway for suppression of invasion in human glioblastomas.	21.04.2017	Dr. Padma Shastry
3	Avneesh Kumar Gautam	Structural-functional analysis of Kaposica, the complement regulator of Kaposi's sarcoma-associated herpes virus (KSHV/HHV-8).	26.04.2017	Dr. Arvind Sahu
4	Phalguni Rath	Enhanced reprogramming towards human induced pluripotent stem cell and defining conditions for lineage-specific differentiation.	19.05.2017	Dr. Anjali Shiras
5	Swastik Phulera	Structural and functional studies on redox related proteins from Mycobacterium tuberculosis.	27.05.2017	Dr. Shekhar Mande
6	Pompom Ghosh	Studies on the role of CC chemokine ligand 5 (CCL5) in tumour-stroma interaction leading to tumorigenesis and metastasis.	07.07.2017	Dr. Gopal Kundu
7	Mr. Deepak Khuperkar	Novel role for RanGTPase in intercellular transport.	17.07.2017	Dr. Jomon Joseph
8	Surbhi Chouhan	Hyperglycemia-induced alterations in on progression of hepatocellular carcinoma and impact chemotherapeutic outcome.	24.07.2017	Dr. Manoj Kumar Bhat
9	Rohan Kulkarni	Creation of in vitro niches (IVNs) to modulate stem cell functions in vitro.	04.09.2017	Dr. Vaijayanti Kale
10	Sapna Deore	To investigate the role of EBA175 RVI sequences in invasion, immune responses and infection.	08.09.2017	Dr. M. V. Krishnasastry
11	Mahendra Jamdhade	Proteomics analysis of glycosomes from *Leishmania donovani*.	13.09.2017	Dr. Milind Patole
12	Vineeta Mandlik	Network modulatory dynamics of inositol phosphorylceramide synthase in Leishmania: A chemical biology approach.	19.09.2017	Dr. Shailza Singh
13	Sapana Jalnapurkar	Modulation of mesenchymal stem cell cultures to study their effect on the hematopoietic stem cell functions.	25.09.2017	Dr. Vaijayanti Kale
14	Milsee Mol J.P.	Immunomodulatory synthetic signaling circuit targeting CD14, TNF and EGFR pathways in Leishmaniasis.	15.12.2017	Dr. Shailza Singh
15	Mangesh Suryavanshi	Molecular characterization of oxalic acid etabolizing bacteria: implications for human health.	27.12.2017	Dr. Yogesh Shouche
16	Neeraja Kulkarni	Role of chemokine receptor CCR6 in the differentiation and function of Th17 and Treg cells during autoimmune colitis.	28.12.2017	Dr. Girdhari Lal
17	Kanupriya Singh	Studies on the role of interleukin-3 in regulation of RANKL and OPG expression in osteoblasts.	01.01.2018	Dr. Mohan Wani

No.	Research Scholar	Title of the Thesis	Date of award of Ph.D. (dd.mm.yyyy)	Research Guide
18	Sarojini Singh	Role of nuclear factor E2-related factor 2 (Nrf2) mediated antioxidant mechanisms against hyperglycemia induced oxidative stress in diabetic cardiomyopathy.	02.01.2018	Dr. Sandhya Sitasawad
19	Snehal Joshi	Studies on regulation of bone metastasis by interleukins.	04.01.2018	Dr. Mohan Wani
20	Kedar Limbkar	Evaluation of the effect of oral feeding of nutraceuticals belonging to the class of poly-unsaturated fatty acids on the haematopoiesis and thrombopoiesis of mice.	04.01.2018	Dr. Lalita Limaye
21	Ankita C. Dhenge	Studying the role of valproic acid and polyunsaturated fatty acids in differentiation of megakaryocytes and biogenesis of platelets.	04.01.2018	Dr. Lalita Limaye
22	Sandip Ashok Sonar	Cellular and molecular mechanism of blood-brain barrier to control inflammation and autoimmunity.	24.01.2018	Dr. Girdhari Lal
23	Saurav Paul	Natural killer cell subsets and their role in modulating the effector CD4 T cell response in cancer.	24.01.2018	Dr. Girdhari Lal
24	Nandaraj Taye	Role of SMAR1 in the Wnt signaling pathway.	12.02.2018	Dr. Samit Chattopadhyay
25	Ashwin V. Khandare	Studies on immunomodulators, immunopathology and interaction of mouse malarial parasite and parasite components with immune system.	13.03.2018	Dr. Prakash Despande
26	Vikrant Piprode	Role of interleukin-3 in pathophysiology bone remodeling.	20.03.2018	Dr. Mohan Wani

Teaching, Training and Outreach

Teaching and Training

Talks delivered by & hands-on activities / training conducted by NCCS faculty

Scientist	Topic / Symposium	Class / Department	Institution	Date
Sharmila Bapat	(i) "Science for human welfare" (ii) "Recent Concepts in Cancer Biology"	B.Sc, M.Sc Students of all Life Science Disciplines	ASM's CSIT, Junior College in Pimpri	14 Feb. 2018
Sharmila Bapat	"Cancer Stem cells in tumor heterogeneity : therapeutic implications"	B.Sc, M.Sc Students of all Life Science Disciplines	Co-organiser and speaker at one-day symposium on "Stem Cells & Cancer - India 2018" at Somaiya Vidyavihar Campus, Mumbai	05 Feb. 2018
Sharmila Bapat	2 talks - (i) "Cancer Development, Drug Resistance and Cancer Stem Cells" (ii) "Cancer Metastases"	B.Sc, M.Sc Students of Life Science Disciplines, Biotechnology and Bioinformatics	Convener and speaker at the 2-day Science Academies Lecture workshop on 'Advances in Cancer Biology' at Sri Ram Narayan Kedia Government Degree College, Desaipet, Banswada, Telangana	27-28 Feb. 2018
Sharmila Bapat	"Cancer Metastases"	Clinical and medical practitioners	ICMR funded ROME workshop for conducted by the Moving Academy of Medicine	07 March, 2018.
Dr. Gopal C. Kundu	Cancer Awareness in India Shirpur, Dhule, Maharashtra	XI		06 Jan. 2018
Nibedita Lenka	To be or not to be, the Stem Cells at the crossroad	B.Tech	IIT, Bhubaneswar	26, Oct. 2017
Nibedita Lenka	Embryonic Stem Cells and Cell fate decision	M.Sc. Biotechnology	Utkal University, Bhubaneswar	01 Nov. 2017
Nibedita Lenka	Stem Cells from the perspective of Development and Disease	T.Y.B.Sc. & M.Sc. Biotechnology	Ravenshaw University, Cuttack	02 Nov. 2017
Amitabha Majumdar	Outreach activity talk	School students	Muktangan	27 March, 2018
Amitabha Majumdar	Talk on Nobel prize in Physiology / Medicine 2017	Bachelors students	IIT Bombay	25 Oct. 2017
Shekhar C. Mande	Title of Presentation: Three dimensional and Nature	Junior College - INSPIRE Camp	SV University, Tirupati.	08 July, 2017
Shekhar C. Mande	Mapping protein flexibility	Bachleor's Course	Dempe College, Panaji, Goa organized as a part of Lecture Workshop of the Science Academies	27 Apr. 2017
Shekhar C. Mande	Systems view of Biology	Bachleor's Course	Dempe College, Panaji, Goa, organized as a part of Lecture Workshop of the Science Academies	28 Apr. 2017
Shekhar C. Mande	Protein Structure and Function: Overview	Bachleor's Course	Goa University, Goa, organized as a part of Lecture Workshop of the Science Academies	13 Dec. 2017
Shekhar C. Mande	Architectures of Protein Structures: Form, Stability and Functions	Bachleor's Course	Goa University, Goa, organized as a part of Lecture Workshop of the Science Academies	14 Dec. 2017
Anjali Shiras	Generation of Human Induced Pluripotent Stem Cell-lines using non-coding RNA based Strategy.	B.Sc. and M.Sc Biotechnology	DY Patil College of Medicine, Kolhapur;	25 Apr. 2017

Scientist	Topic / Symposium	Class / Department	Institution	Date
Anjali Shiras	Stem Cells and Cancer	M.Sc Biotechnology	Somaiya Vidyavihar; Mumbai	05 Feb. 2018
Yogesh Shouche	NGS Technology	M.Sc. Microbiology	Savitribai Phule Pune University	13, 22 March
Yogesh Shouche	Deep sphere life and space microbiology	M.Tech. Earth Sciences	Indian Institute of Science, Bangalore	3-6 Oct. 2017
Deepa Subramanyam	Hands on training on DNA and protein isolation.	8^{th}, 9^{th} standard	Students selected as part of the REACH project from Muktangan Exploratory Science Centre	02 Dec. 2017 -20 Jan. 2018
Deepa Subramanyam	Stem Cells - a panacea to use with a little caution	TEDx talk.	FLAME University (Bachelors Students)	04 Oct. 2017

Classes taught by NCCS faculty for the JRF coursework (2017)

(for Ph.D. students registered with the S.P. Pune University)

Scientist	Module
Dr. Arunkarthick	Advances in bioimaging
Dr. Manoj K. Bhat	Advances in cancer biology
Dr. Radha Chauhan	Advances in structural biology (course coordinator)
Dr. Radha Chauhan	Advances in bioimaging
Dr. Radha Chauhan	Quantative methods
Dr. Jomon Joseph	Science communication
Dr. Jomon Joseph	Advances in cell biology
Dr. Jomon Joseph	Advances in molecular biology and genetic engineering
Dr. Janesh Kumar	Advances in molecular biology and genetic engineering
Dr. Janesh Kumar	Quantitative methods
Dr. Janesh Kumar	Advances in structural biology
Dr. Gopal C. Kundu	Advances in applied immunology - Tumor Immunology and Targeted Therapy
Dr. Girdhari Lal	Advances in applied immunology - Transplantation Immunology & Tumor Immunology
Dr. Lalita Limaye	Quantitative Methods - Flow cytometry
Dr. Amitabha Majumdar	Stem cells, development and neurobiology
Dr. Shekhar Mande	Advances in structural biology
Dr. B. Ramanamurthy	Laboratory animal experimentation and ethics
Dr. Srikanth Rapole	Advances in proteomics
Dr. Arvind Sahu	Advances in applied immunology
Dr. Arvind Sahu	Advances in proteomics
Dr. Manas Santra	Advances in cancer biology
Dr. Manas Santra	Advances in proteomics
Dr. Manas Santra	Quantitative methods
Dr. Vasudevan Seshadri	Science Communication
Dr. Vasudevan Seshadri	Translation and control
Dr. Vasudevan Seshadri	Microarray, Qunatitative PCR and NGS
Dr. Anjali Shiras	Advances in molecular biology and genetic engineering (Hallmarks of Cancer)
Dr. Shailza Singh	Computer Applications and Bioinformatics
Dr. Nishant Singhal	Stem cells, development and neurobiology - Pluripotency and Reprogramming
Dr. Deepa Subramanyam	Stem cells, development and neurobiology - Stem Cell Biology
Dr. Vidisha Tripathi	Advances in molecular biology and genetic engineerings
Dr. Vidisha Tripathi	Science communication
DR. Mohan Wani	Advances in applied immunology

Classes taught by NCMR Scientists for the JRF Coursework (2017)

(for Ph.D. students registered with the S.P. Pune University)

Name of Scientist	Subject / Topic
Dr. Kamlesh Jangid	Concept of Biodiversity and Ecology
Dr. Kamlesh Jangid	Concept of diversity (α, β & γ), calculation of diversity indices (richness and evenness) and rarefaction analysis
Dr. Kamlesh Jangid	Collection of ecological samples for community analysis
Dr. Amit Yadav	Techniques used in microbial ecology
Dr. Praveen Rahi	Modern approaches to study microbial diversity: Omics in diversity analysis (metagenomics + metatrancriptomics + metaproteomics)
Dr. Praveen Rahi	Modern approaches to study microbial diversity: Omics in diversity analysis (metagenomics + metatrancriptomics + metaproteomics)
Dr. Rohit Sharma	What is Microbial Systematics? concept of Identification, Nomenclature and Classification. The Species concept
Dr. Amaraja Joshi	Phenotypic characterization in systematics (morphological, biochemical, chemotaxonomy, etc.)
Dr. Dhiraj Dhotre	Molecular and computational tools for phylogenetic analysis (Molecular chronometers, concept of Tree-building, algorithms and model fitting)
Dr. Dhiraj Dhotre	Molecular and computational tools for phylogenetic analysis (Molecular chronometers, concept of Tree-building, algorithms and model fitting)
Dr. Dhiraj Dhotre	Whole genome comparisons for microbial systematics
Dr. Kamlesh Jangid	Conservation and bioprospecting of Microbial Resources

Other Talks Delivered by NCMR Scientists

Invited talks were delivered by NCMR scientists at various scientific meetings/ universities/ colleges/ within and outside India during the duration of this report. This list includes classroom lectures given by the faculty as part of their teaching responsibilities at various departments of SP Pune University.

Dr. Amaraja Joshi

- "Bacterial Classification and Chemotaxonomy" under lead college activity at Yashavantrao Chavan Institute of Science, Satara on 9th October, 2017.

Dr. Avinash Sharma

- "National Centre for Microbial Resource; A microbial reservoir at National Centre for Cell Science, Pune" at BIODIVERS 18 from 27th to 29th January, 2018.
- "Life at Extreme Environments" at Graphic Era University, Dehradun (December 2, 2017)
- "Invasive establishment of allochthonous microbial communities during world's largest bathing festival (KumbhMela)" at the Humboldt Colloquium "Germany and India – Partners in Education and Research", 23 to 25 November 2017 in Bengaluru.

Dr. Dhiraj Dhotre

- Delivered a talk "Human microbiome analysis" at the omics workshop in IWSA, Vashi, Navi Mumbai on March 2017
- "Microbial genomics and its application" in SP College, Pune, India in January 2018
- Conducted two days' workshop on Basic Bioinformatics at St Xavier's College, Mapusa, Goa, January 2018.
- Delivered a talk in lecture series conducted B.N. Bandodkar college of Science, Thane, India on "Human Microbiome: An Indian perspective" in February 2018.
- Conducted a four days' workshop on "Bioinformatics, an introductory approach" at Department of Microbiology, SP Pune university on March 2018
- Invited a resource person to conduct a 3 days workshop during March 21-23, 2018 at IASST, Guwahati, India on "NGS and Microbiome analysis".

Dr. Kamlesh Jangid

- Conducted one-day session on "Isolation and Identification of Microbes" and "PCR and Gel Electrophoresis" as part of faculty development program workshop on "Green Technology in industrial Waste minimization" at GIET, Gunupur, Odisha on January 20, 2018.
- "Advances in microbial cultivation strategies & computational analysis of microbial communities" at Sanjivani College of Pharmaceutical Education and Research, Kopargaon, India on November 16, 2017.
- "Characterizing structural and compositional diversity in gene libraries using K-shuff" at the 2nd Global Soil Biodiversity Conference, Nanjing, China on October 18, 2017.
- "Microbial isolation and identification" at the Advanced Workshop on Microbial Technology for Clean Environment. Tripura University, Agartala, India on September 21, 2017.
- "Microbiology in daily life" talk for Citizen Science initiative at Venezia Co-operative Housing Society, Pune, India on August 29, 2017.

Dr. Praveen Rahi

- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) applications in microbial ecology at Department of Biotechnology, Panjab University, Chandigarh (21 March 2017).

Dr. Shrikant Pawar

- "Wonders of Microbiology" at the 'Second Saturday talk series' at IUCAA in December 2017.
- "We and Microbiology" at Kaliedoscope under the SUEXSA (St. Ursula's Ex Students Association) in December 2017.
- Invited as a Resource person for open forum: 'An interview session of Eminent Alumni - Vividha 2018' at Modern College, Ganeshkhind, Pune, January 2018.
- Conducted two days workshop on Basic Bioinformatics at St Xavier's College, Mapusa, Goa, January 2018.

Workshops / Hands-on Training Programmes Conducted

Hands-on Training Workshops on Basic Cell Culture Technology

Organized by the Cell Repository, for early career researchers from all over India

15 – 18 May 2017

9 – 12 October 2017

Workshop on Network modeling and Protein-protein Interaction

15, 16 June, 2017

In-house hands-on workshop for research scholars (Ph.D. students) of NCCS, organized by the High Performance Computing Facility

Lecture and hands on session on "Gene annotation"

September 2017

Conducted by NCMR-NCCS: More than 50 M.Sc. second year students from Abasaheb Garware College, Pune, attended the session conducted by Dr. Dhiraj Dhotre.

PFMS-EAT Module Workshop

7-8 September, 2017



NCCS Confocal Microscopy Training31st October to

24th November 2017

In-house training for research scholars (Ph.D. students) of NCCS {confirm with facility report}

Microscopy training program

7, 8 December 2017

Training given by experts from Leica. {confirm with facility report}

Workshop on 'RNA-seq Data Analysis'

20 – 22 December 2017

In-house training for research scholars (Ph.D. students) and project staff of NCCS, organized by the High Performance Computing Facility

In-house Worshop in Hindi

20 December, 2017

सूचना प्रौद्योगिकी से संबंधित विभिन्न सॉफ्टवेयर की जानकारी तथा सरकारी कामकाज में मोबाइल एवं कंप्यूटर के माध्यम से ऑफलाइन / ऑनलाइन टाइपिंग, ट्रांसलेशन एवं डिक्टेशन देना।

Conducted by Shri. Rajendra Verma, Assistant Director, Hindi Teaching Scheme, Pune

(Talk and demonstration about IT-related software, & the use of cell phones & computers for offline / online typing, translation & dictation in Hindi and other regional languages)


Workshop on Microbial Identification and Preservation

8 – 13 January 2018

Hands-on training workshop organized by the NCMR-NCCS : 16 participants including Ph.D students, scientists from government institutes and industries, and faculty members from colleges and universities from across India were trained.

Workshop on Microbial Genomics

15 – 19 January 2018

Hands-on training workshop organized by the NCMR-NCCS: 44 participants including Ph.D. students, scientists from government institutes and industries, faculty members from colleges and universities across India were trained.

Outreach

1] National Science Day at NCCS

28 February, 2018

a) Popular Science Public Talk

'Genesis and Relevance of Ancient Indian Science and Technology: An Archaeological Perspective'

- Prof. Vasant Shinde

(Vice-Chancellor, Deccan College, Pune)

b) 'Innovative Ideas in Biological Research' competition - Presentations by the shortlisted teams

c) Open day with exhibits & displays (open to all)

2] Prelude to the National Science Day at NCMR

26 February, 2018

a) Public Talk :

'Mangalyaan - India's Mission to Mars: Towards Mars...A Perspective'

by Dr.H.N.Suresh Kumar* (Senior Scientist, ISRO Satellite Centre)

b) Science-based Essay and Blog writing competition (open to all)

3] Other Public Talks

a) 'The mechanisms of intracellular transport and regulation of neuronal functions'

- by Prof. Nobutaka Hirokawa

University of Tokyo, Japan; President, HFSP; President, IFCB

22 May 2017

b) 'The 2017 Nobel Prizes : A Curtain Raiser to the Award Ceremony'

4th December, 2017

(organized in association with IUCAA)

'And the Nobel Prize in Physics 2017 goes to ... Gravitational Waves'

- Prof. Sanjeev Dhurandhar (Emeritus Professor, IUCAA, Pune)

'Making the invisible visible: The power of cryo-electron microscopy'

- Dr. Janesh Kumar (NCCS, Pune)

'Tick-Tock: The Circadian clock'

- Dr. Amitabha Majumdar (NCCS, Pune)

Q & A

c) 'Creativity and Life in Science'

8th February, 2018

- by Dr. Amit Ghosh. (D.Sc.(hc),FNAS, FAS, FBRS)

J. C. Bose Distinguished Chair Professor, National Academy of Sciences, India

Emeritus Scientist, National Institute of Cholera and Enteric Diseases (NICED), Kalyani.

Jointly hosted by NCCS (NCMR) and Association of Microbiologists of India (AMI) Pune Unit

d) Popular Science Lecture

'History of X-ray crystallography'

Special invited talk delivered by Dr. Shekhar Mande at the Indian Institute of Technology, Kharagpur

20 February 2018.

4] Exhibitions

a) 5th Bharatiya Vigyan Sammelan (BVS) and Expo

11 - 14 May, 2017

Display of posters and exhibits at the Fergusson College, Pune

b) India International Science Festival 2017

13-16 October, 2017

Display of posters and exhibits at the IISF 2017 in Chennai

5] Edu-Bridge

'Edu-Bridge' (NCCS, Pune - JBCS, Wardha) - An ongoing programme where the faculty members of NCCS teach the fundamental concepts of science through lectures & hands-on activities to students of the Jankidevi Bajaj College of Science (JBCS), Wardha. 'Edu-Bridge' was inaugurated on 22nd September, 2015.

6] The Microbial Science Outreach Initiative (MSOI)

The Microbial Science Outreach Initiative was started by Dr. Shouche's group this year, with the aim of engaging undergraduate students and the public in appreciating the fascinating world of microbes present around us. It is an attempt to demystify the academic language of science and approach the audience with the hope that correct awareness among the public should only lead to a better appreciation for these little creatures. The initiative aspires to provide an open platform for stimulating ideas and wider engagement, which are crucial for the spread of science. Under this initiative, screenings of documentaries, publishing blogs on microbial science and interviews of scientists are regularly conducted.

Documentaries Screened

- Seven Wonders of the Microbe World
- Origin of Life
- World of Bacteria (DeutscheWelle)
- Life in Hell (Extremophiles)
- DNA - Playing God
- Charles Darwin & the Tree of Life
- Creating Synthetic Life
- DNA Genesis - The Children of Adam
- In the minds of Plants
- The Evolution of Size - a perspective through the gut
- Virus Evolution
- Western Ghats - Monsoon Mountains
- Vaccines - Calling the Shots

Interviews

- Prof. Samir Brahmachari
- Prof. Madhav Gadgil
- Prof. K.C. Malhotra
- Prof. P. Balaram

Blogs

- Human Microbiome: Discovering your true inner self
- Lonar Lake: The key to understanding the process of life is gradually dying
- Poop- A pronounced Salvager (Fecal Microbiome Transplant)
- Life in extremes! (Extremophiles)
- Science Day 2018 Blog Writing Competition Winning Blogs – 6 nos

7] Popular Science Articles

आक्रमक सूक्ष्मजीव – Written by Dr. Yogesh Shouche for 'Bhavtaal'.

8] Atal Incubation Centres (NITI Aayog) - Outreach Event

31 July, 2017

Organized jointly by the Atal Innovation Mission (AIM) and NCCS, to create awareness about setting up of AICs with support from the Atal Innovation Mission of NITI Aayog, Govt. of India.

Dr. Shekhar Mande with
Mr. Ramanathan Ramanan,
Mission Director, Atal Innovation Mission

9] International Women's Day & World Kidney Day

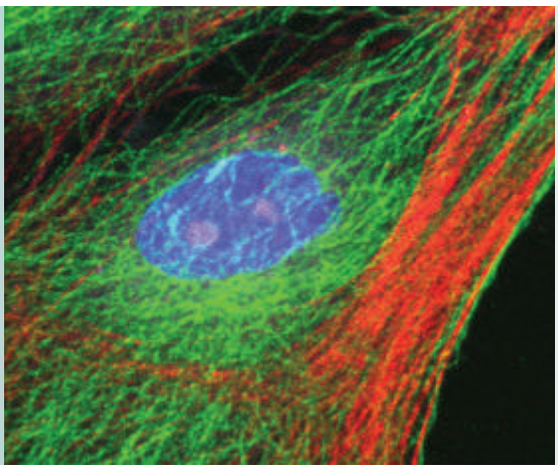
8th March, 2018

Creating awareness about organ donation

In candid conversation with Prof. Saroj Ghaskadbi and Dr. Surendra Ghaskadbi, about their personal experiences with receiving and donating a kidney.

10] Visits by students to NCCS & NCMR

More than 300 hundred students from various colleges visited NCCS and NCMR during the report period. The students were informed about the activities, facilities and services of the NCCS and NCMR, and were shown and explained the high-end techniques and facilities used in research.



Participation in Conferences / Meetings / Workshops

Participation by the NCCS Faculty

Sharmila Bapat

- "Stem Cells & Regenerative Medicine" Invited Talk at DBT Strategy Meet, Delhi, India from 29-30 April, 2017.
- "Cancer Stem cells in tumor heterogeneity: therapeutic implications" Invited Talk at the "Oral Cancer Science Symposium", held during the 6th World Oral Cancer Congress of the International Academy of Oral Oncology (IAOO) at Bangalore, India from 17th - 20th May, 2017.
- "Phenotypic plasticity along the Epithelial-Mesenchymal Axis in ovarian cancer" Invited Talk at the one-day symposium on "Epithelial-Mesenchymal Transition – New Advances in Cancer Development" at IISC, Bangalore on 6th October, 2017.
- "Cellular Phenotypes in Cancer" Invited white board talk at Guha Research Council, Kochi, Kerala from 2nd - 6th December, 2017.
- "Phenotypic plasticity along the Epithelial-Mesenchymal Axis in ovarian cancer" Invited Talk at the 8th International EMT meeting (TEMTIA) at MD Anderson Cancer Centre, Houston, USA held from 7th – 10th December, 2017.
- "Cancer Stem cells in tumor heterogeneity therapeutic implications" Co-organiser and speaker at one-day symposium on "Stem Cells & Cancer – India 2018" at Somaiya Vidyavihar Campus, Mumbai on 5th February, 2018 on behalf of NCCS, along with Sathgen Biotech, Mumbai.
- "Cancer Development, Drug Resistance and Cancer Stem Cells" and "Cancer Metastases" Convener and speaker at the 2-day Science Academies Lecture workshop on 'Advances in Cancer Biology' at Sri Ram Narayan Kedia Government Degree College, Desaipet, Banswada, Telangana, on February 27th–28th, 2018.
- "Cancer Metastases" Invited Talk at the ICMR funded ROME workshop for clinical and medical practitioners conducted by the Moving Academy of Medicine, Pune on 7th March, 2018.
- "Novel Transcripts and Proteins" in Tumors Invited Talk at the Cancer Meeting on Mechanisms of Neoplasia and Precision Medicine in Oncology at National Centre for Biological Sciences, Bengaleru on 4th May, 2018.

Manoj K. Bhat

- Glucose Induced Changes in Cancer Cell Growth and Drug Sensitivity. 86th Conference of Society of Biological Chemists Emerging Discoveries in Health and Agricultural Sciences, JawaharLal Nehru University, New Delhi, November 16-19th 2017.
- Integrative Medicine for Health Care: Emerging Scientific Knowledge and New Therapies; JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, February 23- 24th , 2018.
- A Symposium on Recent Trends in Biology, Department of Zoology, (UGC- Centre of Advanced Study), Savitribai Phule Pune University, Pune, March 23-24th 2018.

Talks Delivered

- Invited: Title: Cancer and Hyperglycemia: Glucose Induced Changes in Cancer Cell Growth and Drug Sensitivity:
- 86th Conference of Society of Biological Chemists Emerging Discoveries in Health and Agricultural Sciences, JawaharLal Nehru University, New Delhi, November 16-19th 2017.
- Plenary: Cancer and Metabolic disorders: An Overview of Ongoing Work: Integrative Medicine for Health Care: Emerging Scientific Knowledge and New Therapies; JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, February 23- 24th, 2018.

- Invited: Title: Cancer and Metabolic disorders: An Overview of Ongoing Work: A Symposium on Recent Trends in Biology, Department of Zoology, (UGC- Centre of Advanced Study), Savitribai Phule Pune University, Pune, March 23-24th 2018.
- Invited: Title: Cancer and Metabolic disorders: An Overview of Ongoing Work: Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, February 8th 2018.

Radha Chauhan

- Electron microscopy based structural analysis of the central transport channel of the nuclear pore complex. Electron microscopy society India annula meeting. July 17-21, 2017. Mahabalipuram, India.
- Is my protein ready for crystallization? NCL-TTP membrane protein workshop, Aug 18, 2017. NCL, Pune India

Jomon Joseph

- Invited Speaker: 'Annulate Lamellae: Exploring new functions of an old organelle' at the Satellite meeting of International Congress of Cell Biology on 'Dynamics within and across the confined cellular space', held at IISER Pune, 2-3 February, 2018.
- Invited Speaker: 'Involvement of a nucleoporin in the coupling of miRNA-induced silencing complex with the target mRNA' at the Indo-Israel work shop on 'Recent advances in molecular genetics with new biomedical insights' at INSA, New Delhi, 12-13 February, 2018.
- Keynote Address: 'Use of Microscopy in Biological Research' in 'National Workshop on BIOANALYSIS' held at St. Aloysius College, Mangalore, on 21 February, 2018.

Janesh Kumar

- Dealing with detergents: SPA-CryoEM of Membrane proteins; Invited talk; M4I, Maastricht University; Maastricht, Netherlands, August 2017.
- Ionotropic Glutamate Receptors: Structure, Function and Regulation; Invited talk; M4I, Maastricht University; Maastricht, Netherlands, September 2017.
- Structural Insights into Functions of Delta Glutamate Receptors; Invited Talk; 11th Biophysics Paschim Meeting, 4th November 2017, Indian Institute of Science Education and Research; Pune, India.
- Making the Invisible Visible: The Power of Cryo-Electron Microscopy; Curtain Raiser to The Noble Prize in Chemistry 2017; 4th December 2017, National Centre For Cell Science, Pune, India.
- The Power of Cryo-Electron Microscopy: Making the Invisible Visible; Science Academies' Lecture Workshop on Role of Three-Dimensional Structures in Biological Function; 13 -15, December 2017, Goa University, Goa, India.
- Insights into Modulation of Kainate Receptor Functions by Neto1; Science Academies' Lecture Workshop on Role of Three-Dimensional Structures in Biological Function; 13 -15, December 2017, Goa University, Goa, India.
- Structural Insights into Enigmatic Delta Glutamate Receptors; Science Academies' Lecture Workshop on Role of Three-Dimensional Structures in Biological Function; 13 - 15, December 2017, Goa University, Goa, India.
- Conformational Changes Underlying GluK3-subtype Kainate Receptor Functions; Science Academies' Lecture Workshop on Role of Three-Dimensional Structures in Biological Function; 13 -15, December 2017, Goa University, Goa, India.
- Glutamate Receptors in Neonatal Brain Injury: Where has Evidence and Basic Sciences Taken us so Far; Invited Talk; Pediatric Research and Education Society of India-Defining Excellence (PRESIDE)-Annual Meeting, 16-17, December 2017, Armed Forces Medical College, Pune, India.
- Structure, Function and Regulation of Glutamate Receptor Ion Channels; Invited Talk; Sakura Science Plan for Indian Administrators and Young Scientists, 21-27, January 2018, Japan Electron Optics Laboratory (JEOL), Tokyo, Japan.
- Fluorescence Detection Size Exclusion Chromatography (FSEC) based Screening For Membrane Protein Expression and Purification; Invited Talk; Workshop on Protein Expression, Purification and Sub-Cellular Fractionation, 26 - 27, February 2018. National Centre For Cell Science, Pune, India.
- Novel Insights into Structure and Dynamics of Kainate Receptor Ion Channels; Invited Talk; 42nd Annual Meeting of the Indian Biophysical Society (*IBS*), 9 - 12, March, 2018, Indian Institute of Science Education and Research; Pune, India.

Gopal C. Kundu

- National Seminar on Recent Development in Biological Science and Chemical Science, Garhbeta College, West Bengal, 1st April, 2017 (Invited Talk).
- Science on the Swan International Conference, One Health: Perth, Australia, 2-4th May, 2017 (Invited Talk).
- Cancer and Cancer Stem Cell Workshop; Curtin University, Perth, Australia, 5th May, 2017.

- Nano and Materials Summit, Goa, Organized by Springer Nature, 18-19th May, 2017.
- EU-Inno Indigo Project Meeting and Mini Symposium, University of Oslo, Norway, 6-7th June, 2017.
- Work in Progress, National Centre for Cell Science, Pune, 23rd June, 2017.
- National Conference, Centre for Bioinformatics, Pondicherry University, Puducherry, 9th August, 2017.
- CME on Molecular Oncology, Dept. of Surgical Oncology and Human Genetics, Sri Ramachandra University, Chennai, 23rd August, 2017.
- 2nd Intl. Conference on Nutraceuticals and Chronic Diseases, Goa, 1-3rd September, 2017.
- 4th International Conference on Angiogenesis Research. Dr. Saroj Gupta Cancer Centre, Kolkata, 22nd September, 2017.
- 3rd International Conference on Translational Research, Amity University, Kolkata, India, 23-25th September, 2017.
- Indian Breast Cancer Conference (IBCC), Jaipur, 7-8th October, 2017.
- International Conference on Osteopontin Research, The Renaissance Harborplace Hotel, Baltimore, USA, 28th October-2nd November, 2017.
- Tumor Biology Department, Research in Progress Seminar, Moffitt Cancer Center, Tampa, Florida, USA, 3rd November, 2017.
- Seminar Series, Dept of Hematology and Oncology, University of Alabama, Birmingham, USA, 6th November, 2017.
- Indian Society of Translational Research (ISTR), NER Workshop, IASST, Guwahati, 21st November, 2017.
- Indian Society of Nano Medicine (ISNM) Conference, Trivandrum, 6-8th December, 2017.
- Seminar on Cancer Awareness in India, J B Science College, Wardha, 15-16th December, 2017.
- World Congress on Cancer, Mahatma Gandhi Medical College & Hospital, Jaipur, 3-5th February, 2018.
- Talk at Intas Pharmaceuticals, Ahmedabad, 7th February, 2018.
- 7th International Conference on Translational Cancer Research (ICTCR), Westin Hotel, Chennai, 8-11th February, 2018.
- Trends in Biochemical and Biomedical Research: Advances and Challenges (TBBR-2018), BHU, Varanasi, 13-15th February, 2018.
- Indian Association for Cancer Research (IACR) Conference, Bose Institute, Kolkata, 23-25th February, 2018.
- National Conference, Krishna Institute of Medical College, Karad, 6-7th March, 2018.
- National Cancer Conference, GCRI, Gujarat, 16-17th March, 2018.

Girdhari Lal

- Lal G (2018) Education, discipline and territorial restriction of immune cells are necessary for the healthy body. National Seminar on Trends in Biology held at Department of Zoology, S. P. Pune University, Pune on 23rd March. *(Invited Talk)*.
- Sonar SA and Lal G (2018) Effect of peripheral inflammation on the transmigration of immune cells across the blood-brain barrier. National Symposium on Trends in Biochemistry in Post-Genomic organized at Department of Biochemistry and Molecular Biology, Pondicherry University on 27-28th February 2018. *(Invited Talk)*.
- Lal G (2018) Role of CCR6 in the differentiation and function of Th17 and Tregs during inflammation and autoimmunity. Molecular Immunology Forum 2018 held on 14-16th January at Diveagar, Maharashtra. *(Invited Talk)*.
- Kulkarni N, Meitei HT, Lal G (2017) Chemokine receptor CCR6 signaling in the differentiation and function CD4 T cells during gut inflammation and autoimmunity. 44th Annual Conference of the Indian Immunology Society (IIS), IMMUNOCON 2017 held at Ahmedabad from 14-16th December. *(Invited talk)*.
- Lal G (2017) The non-chemotactic function of chemokine receptor in inflammation and autoimmunity. Society of Inflammation Research (SIR) inaugural conference (SIRCON) 2017 held on 29th October at Bangaluru. *(Invited talk)*.
- Lal G (2017) The location of cellular meeting controls the alloimmunity, autoimmunity and immunity. Invited talk on World Immunology Day on 29th April at Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth (Deemed University) Tathawade, Pune. *(Invited talk)*.
- Lal G (2017) Adoptive cellular therapy: Exploiting the potential of bad and good cells is new good. XXIII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology and National Conference on Challenges in Livestock and Poultry productions with Biotechnology held on 17-19th April 2017 at Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra. *(Invited talk)*.

Nibedita Lenka

- S. Krishnan, Varun Haran M, D. Rangasamy, N. Lenka. LINE1 Mediated Loss of Function Genetic Screening Unveils Tollip and its Novel Role in Myogenic Cell Fate Modulation. Indian Society of Developmental Biologists' (InSDB) Biennial Meeting, June 2017, Indian Institute of Science and Educational Research (IISER), Pune, Maharashtra (Invited Speaker).
- N. Lenka. Wnt rescues Stat3 deficiency during early neurogenesis. XXXV Annual Meet of Indian Academy of Neurosciences (IAN) and International Conference on Translational Neurosciences and its Application in Protection of Mental Health, Oct. 2017, Ravenshaw University, Cuttack, Odisha (Invited Speaker).
- N. Lenka. Deciphering a Novel Role of Tollip Underlying Mesodermal Cell Fate Choice during Embryonic Stem Cells Differentiation. 44th Annual Conference of the Indian Immunology Society (IIS)- Immunocon, Dec. 2017, Nirma University, Ahmedabad, Gujarat (Invited Speaker).

Amitabha Majumdar

- Presented talk at Indian Drosophila meeting 2017 at IISER Bhopal.

Shekhar Mande

- Structural basis of electron transfer mechanisms in *M. tuberculosis*, 11-July-2017, 45th National Seminar on Crystallography, Banaras Hindu University, Varanasi.
- Structural characterization of low oxygen sensing transcription factor of *M. tuberculosis* 13-Aug-2017, IISc, Bangalore Elettra Awareness Programme Meeting, IISc, Bangalore.
- Three dimensional structures and Biotechnology, 16-Aug-2017, University of Hyderabad, Hyderabad. UGC Human Resources Development Centre, UoH, Hyderabad.
- TLS and Normal Modes, 19-Aug-2017, Indian Institute of Science, Bangalore IUCr Crystallographic Computing School, Indian Institute of Science, Bangalore.
- Deciphering the structural basis of redox processes in *M. tuberculosis* 27-Aug-2017, University of Hyderabad, Hyderabad GIAN Workshop on Protein Structure and Drug Discovery, University of Hyderabad, Hyderabad.
- Overview of protein crystallography and its applications 28-Aug-2017, University of Hyderabad, Hyderabad GIAN Workshop on Protein Structure and Drug Discovery, University of Hyderabad, Hyderabad.
- Structural basis of hypoxic gene regulation in *M. tuberculosis* 30-Oct-2017, Queensland University of Technology, Brisbane, Australia 13th Indo-Australian Conference: Omics in Health.
- Structural basis of redox homeostasis in *M. tuberculosis* 14-Nov-2017, Banaras Hindu University, Varanasi, BC Guha Memorial Award Lecture of the Indian National Science Academy, New Delhi.
- Structural understanding of electron transfer proteins of *M. tuberculosis*, 21-Nov-2017, Bose Institute, Kolkara, 100 years celebrations of the Bose Institute: Acharya JC Bose and beyond.
- Prediction of the substrates of Serine/Threonine protein kinases (STPKs) of *Mycobacterium tuberculosis* and analysis of the resultant network, 23-Jan-2018, Indian Institute of Technology Madras, Chennai, 1st IBSE International Symposium, From genotype to phenotype: computational approaches to understand biological sytems.
- Structure based drug design, 12-Feb-2018, Tezpur University, Tezpur, Seminar cum workshop on computer aided drug design for human pathogens, Tezpur University Tezpur.
- Structural basis of hypoxic gene regulation in *Mycobacterium tuberculosis*, 11-Mar-2018, IISER, Pune. 42nd Annual meeting of the Indian Biophysical Society, Indian Institute of Science Education and Research, Pune.
- 3-dimensional structure-based predictions of the phosphosites of Serine/Threonine kinases of *Mycobacterium tuberculosis*, 13-Mar-2018, IISER, Pune, Workshop on archiving and enriching structural data in biology, 42nd meeting of the Indian Biophysical Society, Indian Institute of Science Education and Research, Pune.

Debashis Mitra

- Cellular factors and signalling pathways: novel targets in the fight against HIV/AIDS, 5th October 2017, Regional Centre for Biotechnogy, Faridabad.
- Cellular Stress proteins in HIV/AIDS, International Conference celebrating 100 years of Zoology, University of Calcutta, 1st – 3rd February 2018, Kolkata.
- Cellular stress proteins and signalling pathways as potential targets in the fight against HIV/AIDS, 6th International Conference on Molecular Signalling (ICMS 2018), University of Hyderabad, 8th-10th February 2018, Hyderabad.
- Host factors as potential targets in the fight against HIV/AIDS, Foundation Day, Department of Bioscience and

Bioengineering, Indian Institute of Technology, Jodhpur, 15th-16th February 2018, Jodhpur.

Srikanth Rapole

- NCD-CAPomics meeting cum exchange visit under Inno Indigo project organised by CQM, July 3-10, 2017 at Madeira, Portugal.
- Workshop on fundamentals of mass spectrometry-based proteomics organized by SPPSPTM, October 6-7, 2017 at NMIMS, Mumbai.
- MS-INDUSCON 2017 organized at International Centre, November 6-10, 2017, Dona Paula, Goa.
- Education day program on 'Proteomics and Its Applications for Biological Research' organized by Institute of Life Sciences, November 29, Bhubaneshwar.
- 9th Annual Meeting of Proteomics Society, India and International Conference on Proteomics in Health and Disease organized by Institute of Life Sciences, November 30-December 02, 2017, Bhubaneshwar.
- Indian myeloma congress organized by Myeloma congress, February 9-11, 2018, Manekshaw Convention Center, New Delhi.
- International symposium on targeted proteomics organized by Indian Institute of Technology, Bombay from 24-28 February, 2018.
- Identification of Candidate Cancer Biomarkers/Targets using Proteomic and Metabolomic Approaches. Invited talk at NCD-CAPomics meeting cum exchange visit under Inno Indigo project organized by CQM, July 3-10, 2017 at Madeira, Portugal.
- Basics of developing mass spectrometric application for the proteomics research. Invited talk at workshop on fundamentals of mass spectrometry based proteomics organized by SPPSPTM, October 6-7, 2017 at NMIMS, Mumbai.
- Identification of Candidate Cancer Biomarkers/Targets using Proteomic and Metabolomic Approaches. Invited talk at MS-INDUSCON 2017 organized at International Centre, November 6-10, 2017, Dona Paula, Goa.
- Mass Spectrometry based Quantitative Proteomics. Inaugural talk at education day program on 'Proteomics and Its Applications for Biological Research' organized by Institute of Life Sciences, November 29, Bhubaneshwar.
- Proteomic and metabolomic profiling towards candidate markers for Breast Cancer. Invited talk at 9th Annual Meeting of Proteomics Society, India and International Conference on Proteomics in Health and Disease organized by Institute of Life Sciences, November 30-December 02, 2017, Bhubaneshwar.
- Indian data: Proteomics in myeloma – The road ahead. Invited talk at Indian myeloma congress organized by Myeloma congress, February 9-11, 2018, Manekshaw Convention Center, New Delhi.
- Quantitation and validation of new targets and biomarkers for Multiple Myeloma. Invited talk at international symposium on targeted proteomics organized by Indian Institute of Technology, Bombay from 24-28 February, 2018.

Arvind Sahu

- Biosafety practices for laboratory, Institute of Bioinformatics and Biotechnology (IBB), S. P. Pune University, Pune, October 4, 2017.
- Complement: role in immune protection against viral infections, Unité des Aspergillus, Institut Pasteur, Paris, November 2, 2017.
- Complement; complementing immune protection against viral infection, Guha Research Conference, Kumarakom, Kerala, December 4, 2017.
- Role of complement in viral illness: does it help fight flu, PRESIDE-Annual Meeting-2018, Department of Pediatrics, Armed Forces Medical College, Pune, December 16, 2017.
- In silico identification of CCP sequence motifs allow identification of novel complement regulators, Molecular Immunology Forum, Diveagar, Maharashtra, January 15, 2018.
- Role of complement during pandemic influenza A(H1N1)2009 virus infection, India EMBO Symposium on RNA viruses: Immunology, pathogenesis and translational opportunities, Faridabad, March 28, 2018.

Manas Santra

- Indo-US Conference on Transcription, Chromatin Structure, DNA Repair and Genomic Instability, Bangalore, March 6-10, 2018.
- An Interdisciplinary Approach to Biological Sciences (IABS) conference 2018 at Kolkata February 1 – 3, 2018.
- Ubiquitin and SUMO: From molecular mechanisms to system-wide responses, 15 – 19 September 2017, Cavtat-Dubrovnik, Croatia.
- Delivered talk on March 8, 2018, Indo-US Conference on Transcription, Chromatin Structure, DNA Repair and Genomic Instability, Bangalore. Title: F-box protein FBXO31: an important gatekeeper for maintaining genomic integrity.

Vasudevan Seshadri

- Attended the 9th RNA group meeting in Varnasi, India, between October 26-28, 2017.
- Attended the Society fo Biological Chemists meeting in New Delhi, between November 16-19, 2017. Emerging Discoveries in Health and Agricultural Sciences.
- One of the coordinators for the workshop cum training program on "Ribosome and Translation Techniques" conducted by Tejpur University, Under DBT-Unit of excellence program for NE between Nov-25-26, 2017.
- Gave an oral presentation titled "Import of human Argonaute 2-miRNA complex into Plasmodium falciparum and regulation of the parasite gene expression" at the International Congress of Cell Biology, at Hyderabad, between 26-31 January, 2018.
- Invited Speaker at Recent Trends in Biology 2018" at Department of Zoology, S.P Pune University, Pune. Title of the talk was "Role of RNA-protein Interactions in cellular decision making processes", 23rd - 24th March 2018.

Anjali Shiras

- Glioma Stem Cells Signaling; ISNOCON; 7th April; 2018; AIMS, New Delhi, India
- International Congress on Cell Biology; Jan 26th -30th, 2018; Hyderabad, India.

Yogesh Shouche

- Microbial Diversity: Characterization and sustainable utilization at Conference on Himalayan Biodiversity: Characterization and bioprospection for sustainable utilization" University of Kashmir, Srinagar, September 18-19, 2017.
- Human Gut Microbiome in health and disease at One day Symposium at Center for Infectious Disease Research Indian Institute of Science, Bangalore, November 23, 2017.
- Microbiome: Forensic implications at International Conference to convey a sincere tribute to the pioneer of DNA Fingerprinting in India A.P.J. Abdul Kalam at Institute of Forensic Science & Criminology, Bundelkhand University, Jhansi, 22nd – 24th February, 2018.
- Human Microbiome: Indian perspective at Indo-US workshop on Genomics and Bioinformatics to Explore Human Microbial Ecology in Health and Diseases from 6th-8th September, 2017 New Delhi.
- Human Microbiome: Indian perspective at Microbiology in the New Millennium: from Molecules to Communities" at Bose Institute Kolkotta Oct 27-29, 2017.
- Human microbiome the: The second geonome at International Symposium on " Emerging Biological Trends in 21st Century " P.P. Savani University(private), Surat 5th November 2017.
- Molecular methods in cataloguing microbial, animal and plant biodiversity at Annual Symposium of DNA Society of India Guwahati 16th November 2017.
- Bacterial profiling of Panchagavya 12th Annual Conference of Tadvidya Sambhasha Ayurveda – XII at Mumbai November 18,2017.
- Talk on metagenomics at INSPIRE internship science camp to be held at SASTRA, Thanjavur, between December 26th – 30th, 2017.
- Search for life outside earth International Conference on 'BIOSCIENCE AND BIOTECHNOLOGY' Bharathiyar University, Coimbtore December 16-18, 2017.
- Microbial diversity and metagenomics at *"Biosciences -- An avenue for a better tomorrow"* at VES College, Chembur on 17th February 2018 Search for extraterrestrial life at Science day celebrations at Warna Science and Innovation center Warnanagar February 28,2018.
- Search for extrateresterial life at "Advences in Life Sciences" KKHA Arts, SMGL Commerce & SPHJ Science College Neminagar, Chandwad, January 19-20, 2018.
- Human Microbiome: A new avenue for entrepreneurship for biotechnologists at "Industry-Academia Conclave 2018 (IAC 2018)" at Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Mumbai-Bangalore Highway, Tathawade, Pune February 23-24, 2018.
- Metagenomics and biofertilizers at Two days National Level Conference on Frontiers in Biofertilizers and Biopesticides at Rajmata Jijau Shikshan Prasarak Mandal's Arts Commerce & Science College Bhosary, 09th February 2018.
- Translating Science to Business: Case of Microbiome at Microbial Technology for Sustainable Development Dayanand College, Solapur February 2, 2018.
- Talk on Metagenomics and human microbiome BIOTIKOS, the student-run association of Biotechnology department at SASTRA University, Thanjavur, March 7, 2018.

Shailza Singh

- Plenary lecture on "Signaling framework for Synthetic circuit immuno-modulation in Leishmaniasis: A Structural Perspective for Drug Delivery Systems" at SBCADD 2018, Alagappa University, Karaikudi, 20th-23rd February 2018.
- Invited talk on "Systems framework for Synthetic biology

inspired therapy in Leishmaniasis" at Cross-Talk in Omics 2017, Centre for Bioinformatics, School of Life Sciences, Pondicherry University, Pondicherry, August 9, 2017.

Sandhya Sitaswad

- Targetting mitochondrial oxidative stress as novel therapy in Diabetic Cardiomyopathy' (Invited talk); Symposium on "Molecular Medicines for Lifestyle Disease: Emerging Targets and Approaches" (MMLD 2017), CDRI, Lucknow, India, 20th to 21st of November,2017

Deepa Subramanyam

- Regulation of Embryonic Stem Cell Pluripotency by Intracellular Trafficking / Invited talk/ Wellcome Researchers Meeting, Warwick/ March 15-16, 2018.
- Regulation of Embryonic Stem Cell Pluripotency by Intracellular Trafficking / Invited talk/ ICCB Satellite Meeting on Stem Cells and Disease Biology, inStem, Bangalore/ Feb 2-3, 2018.
- Regulation of Embryonic Stem Cell Pluripotency by Vesicular Trafficking / Selected talk/ International Congress of Cell Biology, Hyderabad/ Jan 27-31, 2018.
- Regulation of Embryonic Stem Cell Pluripotency by Vesicular Trafficking / Invited talk/ Annual Meeting of the Indian Society of Developmental Biologists, IISER Pune/ June 2017.

Vidisha Tripathi

- Understanding the role of long noncoding RNAs in cellular proliferation; Invited talk; 9th RNA Group Meeting; BHU, Varanasi, India, Oct 26-28, 2017.

Mohan Wani

- 2nd Annual Symposium on "Cell and Gene Therapy" at Christian Medical College (CMC), Vellore, September 7-8, 2017.
- Guha Research Conference (GRC) 2017 meeting at Kumarakom, Kerala, December 2-6, 2017.
- Molecular Immunology Forum (MIF) 2018 at Diveagar, Raigadh, January 14-16, 2018.
- "Recent advances in animal cell culture technology" at Institute of Veterinary Biological Products, Pune, May 29, 2017.
- "Role of IL-3 in regulation of pathophysiology of bone homeostasis" during National Conference on Biotherapeutics and Bioanalytical Techniques" at Dr. D. Y. Patil College of Pharmacy, Pune, November 27, 2017.
- "Regulating the pathophysiology of bone remodeling" during Guha Research Conference-2017 at Kumarakom, Kerala, December 4, 2017.
- "Mesenchymal stem cell therapy for repair of bone and cartilage damage" at Fergusson College, Pune, February 23, 2018.
- "New concepts in osteoimmunology" at Nagpur Veterinary College, Nagpur, March 6, 2018.

Participation by Other Scientists, Students and Staff of NCCS

- Abir Mondal; Molecular Insight of Exosomes Mediated Angiogenesis in Glioblastoma (Abir Mondal, Aman Sharma, Prashant Phulpagar and Anjali Shiras), International Congress of Cell Biology, 27th-31th January, 2018, Hyderabad, India.
- Abir Mondal; Molecular Insight of Exosomes Mediated Angiogenesis in Glioblastoma (Abir Mondal, Aman Sharma, Prashant Phulpagar and Anjali Shiras), EMBO practical course: Extracellular Vesicles: From biology to Biomedical Application, 8th-14th April, 2018, Heidelberg, Germany.
- Aftab Alam; SMAR1 mediated MHC1 regulation by modulation of calnexin expression in cancer and infection. 3rd EACR conference on Cancer Genomics, 25th June, 2017 – 28th June, 2017, Cambridge, UK.
- Ananth Prasad Burada; EMBO course on image processing for Cryo-Electron Microscopy, University Of London, 5 - 15 September 2017, London, U.K.
- Ananth Prasad Burada; Structural insights into Ionotropic Glutamate receptors (Ananth Prasad Burada, Elena Orlova, Peter J Peters and Janesh Kumar), 42nd Annual Meeting of the Indian Biophysical Society, 12 - 13, March, 2018, IISER-Pune, India.
- Ancy Abraham, Snehal Gulhane and Devesh Kumar Choukikar participated in Stem Cell and Cancer Conference, Mumbai on 5th Feb. 2018.
- Ankita Deb, Resistin accelerates breast cancer proliferation by inducing metabolic changes in tumor microenvironment. Deb A, Bhat MK. World Congress on Cancer (WCC), 3rd to 5th February 2018, MGUMST Hospital, Jaipur, India.
- Anshul Assaiya; CryoEM sample preparation at IIT Delhi, 13-14 April 2017, Delhi, India.
- Anshul Assaiya; Membrane Protein crystallization techniques at National Chemical Laboratories Pune 18 August 2017, Pune India.

- Anshul Assaiya; Structural and Functional studies on Drosophila Ionotropic Receptors (Anshul Assaiya and Janesh Kumar), NGN meeting 2017, 13-14 October 2017, Pune, India.
- Anshul Assaiya; GIAN Course 2018: Recent Advancements in Biophysical Techniques and Virology at IIT Roorkee, 15-21 April 2018, Roorkee, India.
- Anshul Assaiya; GIAN Course 2018: Recent Advancements in Biophysical Techniques and Virology at IIT Roorkee, 15-21 April 2018, Roorkee, India.
- Apoorva Parulekar; SMAR1 inhibits stem cell traits by repression of hTERT in colorectal cancer. International Congress of Cell Biology, 27th January, 2018 – 31st January, 2018, Hyderabad, India.
- Arpankumar Choksi; Role of SMAR1 in tumor metabolism via regulation of PKM alternative splicing. International Congress of Cell Biology, 27th January, 2018 – 31st January, 2018, Hyderabad, India.
- Arya Ghate; Complement activation peptide C5a skews macrophage polarization by promoting a proinflammatory phenotype (Ghate, A. and Sahu, A.), Microbiology in the New Millennium: From Molecules to Communities, 27-29, October, 2017, Kolkata, India.
- Bhavnita Soni and Shailza Singh (2017) Systems biology of IL6 in Leishmaniasis (2017) Bharatiya Vigyan Sammelan, Fergusson College, Pune, 11th to 14th May 2017.
- Bhavnita Soni and Shailza Singh (2017) Systems Studies Unveil Role of IL6 in macrophage polarization during *L. major* infection. National Conference on Protein Structure and Dynamics in Health and Agriculture, New Delhi. 3/11/2017 to 4/11/2017. Journal of Proteins and Proteomics 2017, Volume 8:4, Pg: JPP27-28. (Poster presentation).
- Debasish Paul was selected for J. Bioscience Best poster award in International Congress of Cell Biology, Hyderabad, January 26-31, 2018.
- Deepti Tomar: Presented Poster at "Frontiers in Cancer Science 2017". 6th to 8th November, 2017, Singapore.
- Dipti Athavale, Role of glucose in regulation of PCSK9. Athavale D, Chouhan S, Pandey V, Bhat MK. EMBO workshop: Metabolic Disorders and Liver Cancer, 23-26th April 2017, Palma de Mallorca, Spain.
- Dipti Athavale, Cause and consequences of hypercholesrolemia in hepatocellular carcinoma (HCC). Athavale D, Chouhan S, Pandey V, Mayengbam SS, Singh S, Bhat MK. World Congress on Cancer (WCC), 3rd to 5th February 2018, MGUMST Hospital, Jaipur, India.
- Divya Kumari; Identification and Functional Characterization of Glioma Stem Cells-Derived Exosomal Cargo in Glioma Pathogenesis (Divya Kumari and Anjali Shiras), International Congress of Cell Biology, 27th-31st January, 2018, Hyderabad, India.
- Garima Pandey attended "1st International Conference on Recent Trends in Bioengineering (ICRTB 2018) at MIT School of Bioengineering Sciences and Research, Pune, February 17, 2018.
- Garima Pandey attended the workshop on " RCB Bioimaging School 2018" at Regional Centre for Biotechnology, Faridabad, New Delhi, March 19-24, 2018.
- Gulshan; Role of long non coding RNA SOX2OT in Glioblastoma development and progression (Gulshan, Nameeta Shah and Anjali Shiras), 9th RNA Group Meet, 26th - 28th October, 2017, BHU, Varanasi, India.
- Indrasen Magre: Participated cell biology and physics of morphogenesis (Biophymorph2017) – 41st Mahabaleshwar seminar on modern biology, held at Fountainhead Leadership Centre, Alibaug, India, 28 February - 4 March 2017.
- Indrasen Magre: Travel award from the International Congress of Cell Biology for attending its annual meeting at Hyderabad, 27-31 January 2018.
- Jay Trivedi, Afsana Parveen, Ashoke Sharon and Debashis Mitra, Identification of HSP-90 inhibitors as potential anti-HIV molecules, 30th International Conference on Antiviral Research organised by International society for Antiviral Research, 21-25 May 2017, Atlanta, GA, USA.
- Kailash Gupta and Debashis Mitra, Differential Regulation and Function of Cellular HSP40 Isoforms during HIV-1 Infection, International congress of cell biology, 27th-31st January 2018, CCMB, Hyderabad.
- Kapoor, U., N. Lenka. Plumbagin: Putative Mechanisms of Action mediating Cells Cycle, Metabolism, and Apoptosis in Glioblastoma cells. 7th International Conference on Translational Cancer Research, *February 8-11*, 2018, IIT Madras, Chennai (Selected for Oral Presentation – delivered by student).
- Kriti Chopra, Phylogenetic Analysis of the Nuclear Pore Complex Illustrates Divergent Evolution from Fungi to Metazoans. 42nd annual meeting of the Indian biophysical society. 9th-11th March, 2018. IISER Pune India.
- Kruthika Iyer, Priyanka Chaudhary and Debashis Mitra, Regulation of Cellular HSP70 protein during HIV-1 Infection,

International congress of cell biology, 27th-31st January 2018, CCMB, Hyderabad.

- Dr. Kusum Dhakar (N-PDF) Skill Development Course on Mass Spectrometry and Proteomics Techniques organized by National Chemical Laboratory-CSIR, Pune, India during 1st Nov-17th Nov 2017.
- Dr. Lekha Rani, Research Associate presented the paper entitled "IL-3 alleviates the disease activity in collagen-induced arthritis by reducing T-helper 17 cells" and received "Best Oral Presentation Award" during 19th INDO-US Cytometry Symposium and Workshop on Advanced Flow Cytometry and Applications held at Mumbai, February 17-18, 2018.
- Madhuri More; Study of tumor progression in ovarian cancer – Poster Presentation at International Conference of Cell Biology, Hyderabad, India, Jan 2017.
- Madhuri More; Understanding stemness, cellular plasticity and drug resistance in ovarian cancer – Poster Presentation at Stem Cell and Cancer Conference, Mumbai on 5th Feb. 2018.
- Mahadeo Gorain: Best Poster Award in 3rd International Conference on Translational Research: Application in Human health and Agriculture held during 23rd-25th September 2017 at Amity University, Kolkata.
- Meenakshi Setia; Identification of protein interactions of a novel oncogenic lincRNA Ginir and its role in regulation of mitotic cell division (Meenakshi Setia, Suchismita Panda, Navjot Kaur) and Anjali Shiras) 9th RNA Group Meet, 26th-28th October, 2017, Varanasi, India.
- Meenakshi Setia; Role of a novel non-coding RNA Ginir in regulating mitotic cell division through interaction with proteins of Kinesin family (Meenakshi Setia, Suchismita Panda and Anjali Shiras), International Congress of Cell Biology, 27th-31st January, 2018, Hyderabad, India.
- Misha K.R.: Participated in 9th RNA Meet at BHU, Banaras, 26-28 October 2017.
- Misha K. R.: Participated the International Congress of Cell Biology meeting at Hyderabad, 27-31 January 2018.
- Mohsina Anjum Khan; Human Induced Pluripotent Stem Cells 2nd-7th April, 2018, Vellore, India.
- Navvya Premraj; GIAN Course 2018: Recent Advancements in Biophysical Techniques and Virology at IIT Roorkee, 15-21 April 2018, Roorkee, India.
- Nikhat Firdaus Khan (Technical Officer): Presented posters about NCCS at the 5th Bharatiya Vigyan Sammelan (BVS) and Expo at the Fergusson College, Pune (11 - 14 May, 2017), & at the India International Science Festival 2017 in Chennai, 13-16 Oct, 2017.
- Padghan P, Pathak P and Lal G (2017) CCR6 affects the class switch recombination in B cells during homeostasis and gut inflammation. 3rd Mini-symposium on Cell Biology held at National Centre for Cell Science (NCCS), Pune on 23rd May. *(Poster presentation)*.
- Parul Dutta was selected for oral presentation in Indo-US Conference at Bangalore, March 6 – 10, 2018.
- Pathak M, Padghan P and Lal G (2018) CCR9$^+$ dendritic cells (DCs) promote regulatory CD4$^+$ T cell differentiation during gut inflammation. International congress of Cell Biology 2018 held in Hyderabad, on 27th-31st January. *(Poster presentation)*.
- Pathak M, Padghan P and Lal G (2017) CCR9+ dendritic cells (DCs) promote regulatory CD4+ T cells during gut autoimmunity. 3rd Mini-symposium on Cell Biology held at National Centre for Cell Science (NCCS), Pune on 23rd May. *(Poster presentation)*.
- Pavan Kumar M S; Investigating the Role of RNA Binding Proteins in Pluripotency and Differentiation (Pavan Kumar M S and Anjali Shiras), 9th RNA Group Meet, 26th - 28th October, 2017, BHU, Varanasi, India.
- Pavan Kumar M S; Investigating the Role of RNA Binding Proteins in Pluripotency and Differentiation (Pavan Kumar M S and Anjali Shiras), International Congress of Cell Biology, 27th - 31st January, 2018, Hyderabad, India.
- Poulomi Banerjee: Participated in 9th RNA Meet at BHU, Banaras, 26-28 October 2017.
- Poulomi Banerjee: Participated in the International Congress of Cell Biology meeting, Hyderabad, 27-31 January 2018.
- Prachi Deshmukh: Selected for short talk, 9th RNA Meet at BHU, Banaras, India, 26-28 October 2017.
- Prachi Deshmukh: Travel award from the International Congress of Cell Biology for attending its annual meeting at Hyderabad, 27-31 January 2018.
- Prajakta Shinde, Poster title: Optimal Generation of Dendritic Cell from Apheresis Samples of Multiple Myeloma Patients for Use in Cancer Immunotherapy. (Prajakta Shinde, Sameer Melinkeri, Vaijayanti Kale, Lalita Limaye). Poster presentation in the International Society for Experimental Hematology (ISEH)-46th annual scientific meeting held Goethe University Frankfurt, Germany from 24th August to 27th August 2017.
- Pranay Ramteke, Crosstalk between molecular and metabolic programming induced due to hyperglycaemia

and breast cancer. Ramteke P, Mayengbam S S, Deb A, Singh A, Deshmukh B, Bhat M K. World Congress on Cancer (WCC), 3rd to 5th February 2018, MGUMST Hospital, Jaipur, India.

- Prashant Phulpagar; A Non-Canonical Cytoplasm-to-Nucleus Transport of miR-1 and miR-25: A Two-Way Route (Prashant Phulpagar, Raksha Ganesh, Manoj Kumar Gupta, Anjali Shiras, Ravi Sirdehmukh), 9th RNA Group Meet, 26th-28th October, 2017, Varanasi, India.
- Prashant Phulpagar; A Non-Canonical Cytoplasm-to-Nucleus Transport of miR-1 and miR-25: A Two-Way Route (Prashant Phulpagar, Divya Naik, Raksha Ganesh, Anjali Shiras, Ravi Sirdehmukh), International Congress of Cell Biology, 27th-31st January, 2018, Hyderabad, India..
- Pratibha Bharti; AMPA receptor modulation by its auxiliary protein CKAMP44 (Pratibha Bharti, Rajesh Vinnakotta, Monika Jaiswal and Janesh Kumar), 45th National Seminar on Crystallography, 9 - 12 July, 2017, Varanasi, India.
- Pratibha Tiwari and Ashwani Kumar participated in IUCr-Computational Crystallography workshop organized by Molecular Biophysics Unit (MBU), IISc, Bangalore during Aug, 15-20 2017.
- Pratibha Tiwari, Ashwani Kumar, Md. Yousuf Ansari participated in 42nd meeting of Indian Biophysical Society at IISER, Pune during March 9-11, 2018.
- Pratibha Tiwari and Md. Yousuf Ansari participated in workshop on "Practical Protein Crystallography using PX beamline at Indus-2 Synchrotron" held at RRCAT, Indore during March 27-28, 2018.
- Priyanka; Mechanistic insights into LPS regulated cancer progression: Fine tuning of tumor suppressor SMAR1, International Congress of Cell Biology, 27th January, 2018 – 31st January, 2018, Hyderabad, India.
- Rahul Bodhke (SRF) Oral presentation at 17th International Celiac Disease Symposium, New Delhi. 8th - 10th Sept 2017.
- Raj Kumar, Mahendra Kumar, Conference: Trends in Biochemical & Biomedical Research: Advances and Challenges" - Feb 13-15, 2018, Department of Biochemistry, Institute of Science, BHU, Varanasi.
- Rajashri Shende; Mep1p, a metalloprotease secreted from *Aspergillus fumigatus* conidia aids in immune evasion by cleaving complement components (Shende, A., Wong, S.S.W., Rapole, S., Beau, R., Ibrahim-Granet, O., Monod, M., Gührs, K-h, Latgé, J.P., Madan, T. Aimanianda, V. and Sahu, A.) Microbiology in the New Millennium: From Molecules to Communities, 27-29, October, 2017, Kolkata, India.
- Rajesh Vinnakota; Modulation of AMPA and Kainate Receptor Functions by their Auxiliary Subunits (Rajesh Vinnakota, Pratibha Bharti, Jyoti Kumari, Monika Jaiswal and Janesh Kumar), NGN Meeting 2017, 13-14, October 2017, IISER-Pune, India.
- Rajesh Vinnakota; Amino-terminal, ligand binding, and C-terminal domain interactions affect the gating properties of AMPA and Kainate receptors (Designing chimeric toolbox for various interaction studies) (Rajesh Vinnakota and Janesh Kumar), 42nd Annual Meeting of the Indian Biophysical Society, 12 - 13, March, 2018, IISER-Pune, India.
- Richa Pant; Deciphering the role of nuclear matrix binding protein SMAR1 in adipogenesis: its implication in obesity associated cancers. International Congress of Cell Biology, 27th January, 2018 – 31st January, 2018, Hyderabad, India.
- Ritika Kabra and Shailza Singh (2017) Lipid trafficking through lipid transport proteins in leishmaniasis, Bharatiya Vigyan Sammelan, Fergusson College, Pune, 11th to 14th May 2017*(Poster presentation)*.
- Ritika Kabra and Shailza Singh (2017) Poster presentation: Miltefosine resistance reversal in *Leishmania major* by a synthetic peptide. National Conference on Protein Structure and Dynamics in Health and Agriculture, New Delhi. 3/11/2017 to 4/11/2017.
- Rutuja Kuhikar, Poster title: Resveratrol accelerates and enhances in vitro generation of red blood cells derived from Hematopoietic stem cells. (Rutuja Kuhikar, Vaijayanti Kale and Lalita Limaye). Poster presentation in the International Congress of Cell Biology (ICCB), organized by CSIR-Centre for Cellular & Molecular Biology (CCMB), Hyderabad, India, during 27th-31st January 2018.
- Sagar Varankar, Madhuri More, Ancy Abraham, Snehal Gulhane and Devesh Kumar Chaukikar participated in 3rd Mini-Symposium on Cell Biology organied by NCCS, Pune on 23rd May 2017.
- Sakalya Chavan: Participated the International Congress of Cell Biology meeting at Hyderabad, 27-31 January 2018.
- Samruddhi Zende; Human Induced Pluripotent Stem Cells 2nd-7th April, 2018, Vellore, India.
- Gulshan; Role of long non coding RNA SOX2OT in Glioblastoma Pathogenesis (Gulshan, Nameeta Shah and Anjali Shiras), 10th Annual Conference Of Indian Society Of Neuro-Oncology, 5th - 8th April, 2018, New Delhi, India.
- Sangita Niranjan, Exploring Structural and Biochemical basis of Nup155 in NPC assembly. EMBO Practical course

CEM3DIP 2018: of macromolecular assemblies and cellular tomography. 18-29th March 2018. IIT New Delhi India.

- Satish Kumar (Teacher fellow) Attended the 3rd International World Research Journals Congress (WRJC-2018) held at Bangalore, India January 10 to January 13, 2018, presented my Ph.D. work entitled "Metagenomics and metabolomics guided exploration of Lonar Lake ecosystem".
- Sheetal Kadam, Meghana Kanitkar, Kadambari Dixit, Rucha Deshpande Vaijayanti Kale: 'Curcumin rescues high glucose and diabetes-induced Endothelial Progenitor Cell (EPC) dysfunction in-vitro and in-vivo'; Presented at International Diabetes Summit 2017, Pune, 10-12 March, 2017.
- Shreyas Kumbhare (SRF) Presented poster at the 7th FEMS congress, Valencia, Spain, July 2017.
- Shubhanath Behera attended "1st International Conference on Recent Trends in Bioengineering (ICRTB 2018) at MIT School of Bioengineering Sciences and Research, Pune, February 17, 2018.
- Shyamananda Singh Mayengbam, LDL and HDL cholesterol increase colorectal cancer proliferation by enhancing glucose uptake. Mayengbam SS, Deshmukh B, Singh A, Athavale D, Ramteke P, Bhat MK. World Congress on Cancer (WCC), 3rd to 5th February 2018, MGUMST Hospital, Jaipur, India.
- Dr. Siddharth Kumar Singh (N-PDF) Participated in "The AMR DxC Summer School India 2017" on "Antimicrobial resistance" by DBT, Govt. of India and University of Edinburgh, United Kingdom at Bangalore during 04/07/2017 to 07/07/2017.
- Dr. Siddharth Kumar Singh (N-PDF) Presented Poster on "Attenuating host-pathogen cross-talk using recombinant surface adhesins of probiotic origin" in Exploring Human Host-Microbiome Interactions in Health and Disease conference, at Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom during 13/09/2017 to 15/09/2017.
- Dr. Siddharth Kumar Singh (N-PDF) Presented Poster on "Molecular docking based knowledge-driven choice of monomers for protective encapsulation of small molecules" in National seminar on Technological interventions in food processing and preservation at Amity University, Jaipur on 17/11/2017.
- Dr. Siddharth Kumar Singh (N-PDF) Attended National workshop on "Network analysis in biology" at ICMR-National Institute for Research in Reproductive Health, Mumbai during 26/02/2018 to 27/02/2018.
- Snehal Gulhane participated in International Conference of Cell Biology, Hyderabad, India, Jan. 2018.
- Sonali Jathar; Regulation of cellular quiescence by long noncoding RNAs (lncRNAs) (Sonali Jathar and Vidisha Tripathi), 9th RNA Group Meeting; BHU, Varanasi, India, Oct 26-28, 2017.
- Sophia Fernandes, Poster title: Differentiation of human induced pluripotent stem cells of Indian origin to neural and hematopoietic lineages (Sophia Fernandes, Vaijayanti Kale and Lalita Limaye). Poster presentation in the International Society for Experimental Hematology (ISEH)-46th annual scientific meeting held Goethe University Frankfurt, Germany from 24th August to 27th August 2017.
- Srinadh Choppara Delivered oral presentation entitled "AKT promotes the degradation of tumor suppressor FBXO31 by APC/C complex and prevents the senescence induction" from 27-31 January, 2018 in CCMB-ICCB conference 2018..
- Surbhi Dhingra; Structural Investigations into Heteromeric Kainate Receptor Functions (Surbhi Dhingra, Peter Peters and Janesh Kumar), Young Scientist Research Symposium, 9 Nov. 2017, IIT-Madras, India.
- Surbhi Dhingra; Membrane Protein crystallization techniques at National Chemical Laboratories Pune 18 August 2017, Pune India.
- Surbhi Dhingra; Structural Insights into Heteromeric Kainate Receptor Functions (Surbhi Dhingra, Peter Peters and Janesh Kumar), NGN Meeting, 13-15th Oct. 2017, IISER-Pune, India.
- Surbhi Dhingra; "Structural Insights Into Heteromeric GluK2/GluK5 Kainate receptors", (Surbhi Dhingra, Peter Peters and Janesh Kumar), 42nd Annual Meeting of the Indian Biophysical Society, 9-11 March 2018, IISER-Pune, India.
- Surbhi Dhingra; "Structural Investigations into Functional Mechanisms of Heteromeric Kainate Receptor", (Surbhi Dhingra, Peter Peters and Janesh Kumar), EMBO Practical Course CEM3DIP2018: Of Macromolecular Assemblies and Cellular Tomography, 18-29 March 2018, IIT-Delhi, India.
- Surbhi Dhingra; EMBO Practical Course CEM3DIP2018: Of Macromolecular Assemblies and Cellular Tomography at IIT Delhi, 18 - 29, March, 2018, New Delhi, India.
- Surbhi Dhingra; EMBO Practical Course CEM3DIP2018: Of Macromolecular Assemblies and Cellular Tomography at IIT Delhi, 18 - 29, March, 2018, New Delhi, India.
- Tushar H. More presented a poster entitled "Comprehensive quantitative metabolomic profiling to investigate metabolic alterations in invasive ductal carcinoma of the breast" at

Metabolomics-2017 conference, 26-29 June 2017, Brisbane, Australia (Received DST travel grant).

- Varun Haran M, N. Lenka. Differential influence of USP, a novel Wnt target gene, during mesoderm induction and subsequent differentiation into its derivatives. 3rd Mini-Symposiumin Cell Biology, May 23, 2017, NCCS, Pune (Selected for Oral Presentation - delivered by Student).
- Varun Haran M, N. Lenka. Differential influence of USP, a novel Wnt target gene, during mesoderm induction and subsequent differentiation into its derivatives. Indian Society of Developmental Biologists' (InSDB) Biennial Meeting, June 2017, Indian Institute of Science and Educational Research (IISER), Pune, Maharashtra.
- Varun Haran M and U. Kapoor. Novel Stem Cell therapies against Glioblastoma. IIBR – 2018, February 28, 2018, NCCS, Pune (Selected for Oral Presentation through Blind Review).
- Venkatesh Chanukuppa presented a poster entitled "Quantitative proteomic analysis towards new targets and biomarkers for Multiple myeloma" at Institute of Life Sciences, November 30-December 02, 2017, Bhubaneshwar (Poster selected for Travel award and Oral presentation).
- Vibhuti Kumar Shah; Studies on chromatin remodeling protein SMAR1 in CD4+ memory T cell differentiation. International Congress of Cell Biology, 27th January, 2018 – 31st January, 2018, Hyderabad, India.
- Vikas Ghattargi (PA) Poster presentation titled 'The Resistome and Mobilome of *Enterococcus faecium* strains' November 2017 IndoFrench Conference 2017, Bangalore.
- Vikas Ghattargi (PA) Poster presentation titled 'Genomic Investigation of Probiotic and Non-probiotic Species' in FEMS Spain 2017.
- Vikas Ghattargi (PA) Poster presentation titled 'Comparative genome of *Enterococcus faecium* strains' in FEMS Spain 2017.
- Vikram Kumar; A miRNA-lncRNA regulatory network mediates Replication Stress Response (Vikram Kumar, Supraja Ranganathan, Vidisha Tripathi), 9th RNA Group Meeting; BHU, Varanasi, India, Oct 26-28, 2017.
- Vipul V. Nilkanth participated in the India EMBO Symposium titled 'Big Data in Biomedicine' from 25-27 February, 2018 in New Delhi.
- Vivek Arora; Investigating the molecular mechanisms elucidating the cellular transformation functions of a pair of novel lncRNA Ginir and Giniras (Vivek Arora, Meenakshi Setia, Suchismita Panda and Anjali Shiras), International Congress of Cell Biology, 27th-31st January, 2018, Hyderabad, India.

Events Organized

Conferences / Symposia / Other Events Organized by NCCS

3rd Mini-symposium on Cell Biology (students' symposium)

23 May, 2017

Nobutaka Hirokawa

Lolitika Mandal

M.R.S. Rao

Rishikesh Narayanan

Science Academies' Summer Research Fellows' Symposium

13 July, 2017

Mini-Symposium on Cell Biology (Swarnajayanti Fellows' Mini-Symposium)

October 26, 2017

Mid-career Life Scientists' Meeting *(organized in association with IISER-Pune)*

15, 16 November, 2017

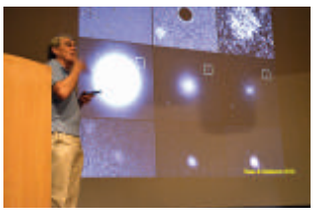
Technology Showcase of Promising Innovations in Pune

(organized in association with IKP Knowledge Park, Hyderabad, for participants from NCCS, neighboring academic/research institutions and the industry)

27 November 2017

Talk by Nobel Laureate, Richard Henderson

22 January, 2018




Extramural Events Organized

a) 4th International Conferenceon Angiogenesis Research — 22 September, 2017
Co-organized with the Dr. Saroj Gupta Cancer Centre, Kolkata.

b) 3rd International Conference on Translational Research — 23-25 September, 2017
Co-organized with the Amity University, Kolkata.

c) Symposium on Stem Cells and Cancer — 05 February, 2018
Co-organized with Sathgen Biotech & K. J. Somaiya College of Engineering, Mumbai.

NCCS Foundation Day

26th August, 2017

Foundation Day Oration:

'Another look at traditional geometric art:
Pattern morphogenesis, link with quasicrystals, contribution to a revival'

Jean-Marc Castera

French artist with a background in mathematics

Felicitation of employees who have completed 20 years of service


Mr. Vijay Khambayat


Mr. Z. D. Walkoli


Mr. Dilip Thorat

Talks by Other Invitees

- 'Novel approaches towards designing 3d microenvironment - inspired biomimetic porous materials for tissue regeneration'
  Dr. Ganesh Ingavle - University of Brighton, England
  12th April, 2017

- 'Hippo Signaling in Breast Cancer Progression'
  Dr. Madhura Kulkarni - Cancer Science Institute, Singapore
  17th April 17, 2017

- 'Chronic Lymphocytic Leukemia- Novel Growth Regulatory Mechanisms'.
  Prof. Subbarao Bandada - Dept. of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, USA
  27th April, 2017

- 'Cancer Stem Cell: A mother bee to target'
  Dr. Shrikant Anant
  Professor and Vice Chair of Research, Dept. of General Surgery, Univ. of Kansas Cancer Center, USA.
  16th May, 2017

- 'Investigating the interplay between cell cycle and gene expression'
  Dr. Kedar Natarajan
  Wellcome Trust Sanger Institute (Sanger Institute) and European Bioinformatics Institute (EBI).
  17th May, 2017

- 'Beyond Cell-Cell Adhesion – Understanding Non-canonical Roles of E-cadherin'
  Anup Padmanabhan
  Mechanobiology Institute, National University of Singapore
  5th June, 2017

- 'Ubiquitination in the regulation of inflammation and cancer'
  Dr. Venuprasad K. Poojary.
  Baylor Institute for Immunology Research (BIIR), Baylor Scott and White Research Institute (BSWRI), Dallas, USA.
  14th June, 2017

- 'Multigene families are central to severe malaria'
  Dr. Suchi Goel
  IISER, Tirupati, India
  4th July, 2017

- 'Investigating Molecular Mechanisms at a Systems Level: Oscillations, transitions and cycles'
  Dr. Ullas Kolthur-Seetharam
  Dept. of Biological Sciences, Tata Institute of Fundamental Research, Mumbai.
  17th July 2017

- 'Opportunities for Indo-Swiss collaborative research and postdoc opportunities'
  Maitree Dasgupta (Head Academic Relations & Projects, Swissnex, Consulate General of Switzerland)
  Gaganjot Kaur (Manager Academic Relations and Projects, Swissnex, Consulate General of Switzerland)
  18th July, 2017.

- 'New advances in our understanding of the "fantastic" RNase L in host-pathogen interaction and immune signaling'
  Dr. Shuvojit Banerjee
  Dept. of Cancer Biology, Cleveland Clinic Foundation, USA
  27th July, 2017

- 'Deciphering the Gene Regulatory Code of Brain Development and Function'
  Dr. Vijay Tiwari
  Group Head, Epigenetic Regulation of Development and Disease, Inst. of Molecular Biology (IMB), Centre of Excellence for Life Sciences, Boehringer Ingelheim Foundation, GERMANY
  28th July, 2017

- 'Targeting viral tropism and inflammation in the host to improve H5N1 and H1N1 induced virulence and mortality'
  Dr. Smanla Tundup
  Univ. of Virginia, and University of Chicago, USA.
  1st August, 2017

- 'Forebrain development – from early patterning to human disorders'
  Dr. Achira Roy
  Seattle Children's Research Institute, Seattle, USA.
  14th September, 2017

- 'Early mesoderm developmental cues differentiate pluripotent stem cells into muscle / cardiac bi-potent progenitor pool'
  Dr. Ramkumar Sambasivan
  InStem, Bangalore
  15th September, 2017

- 'Our gut microbiota: friend or foe?'
  Dr. Parag Kundu
  Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore.
  19th September, 2017

- 'Bridging the Gap between Genome Science and Clinical Practice'
  Prof. Samir K Brahmachari FNA, FASc, FNASc, FNAE, FTWAS, FESPM
  Chief Mentor, Open Source Drug Discovery; Academy Professor, AcSIR;
  Founder Director, CSIR-IGIB; Former Director General, CSIR;
  20th September, 2017

- 'Dense core secretory vesicle biogenesis and their role in physiological functions'
  Dr. Bhavani Sahu
  University of Minnesota and University of Cambridge
  26th September, 2017

- 'Resolving the 3D structure of the Type VII secretion system in Mycobacterium tuberculosis; Setting up a Cryo-EM lab'
  Prof. Peter J. Peters
  Professor of Nano Biology, Maastricht MultiModal Molecular Imaging Institute (M4I), Maastricht University, The Netherlands
  12th October, 2017

- 'Gene regulation in the malaria parasite Plasmodium falciparum'
  Dr. Shruthi Vembar
  Academic Consultant
  25th October, 2017

- 'Human Pluripotent Stem Cell Cultures - Promises and Caveats'
  Prof. M.M. Panicker
  National Centre for Biological Sciences, Bengaluru
  27th October, 2017

- 'Contributions of altered axonal lysosome transport to Alzheimer's disease pathology'
  Swetha Gowrishankar
  Dept of Cell Biology, Yale school of medicine.
  9th November, 2017

- 'Systems approach to cancer, infectious disease, the environment, and education'
  Prof. Nitin Baliga
  Senior Vice President and Director, Institute for Systems Biology, Seattle, USA.
  8th December, 2017

- 'Regulation of mechanosensitive genes and microRNAs to target atherosclerosis'
  Dr. Sandeep Kumar
  Emory University, USA
  14th December, 2017

- How to obtain rights in intellectual property
  Dr. Chitra Arvind
  IPR consultant and Lawyer
  27th December 2017

- 'Emerging concepts in normal breast heterogeneity and its impact on tumor/metastasis characterization'
  Prof. Harikrishna Nakshatri
  Marian J. Morrison Professor in Breast Cancer Research, Indiana University School of Medicine, USA
  3rd January, 2018

- 'Ayurveda: where the past is still present and also getting ahead of the curve'
  Prof. Rama Jayasundar
  Department of NMR, All India Institute of Medical Sciences, New Delhi
  10th January 2018

- 'Multi-OMICs analysis of Biological Systems'
  Dr. Tulika Srivastav
  School of Basic Sciences, IIT Mandi.
  15th January, 2018

- Analysing the barriers to progress in electron cryomicroscopy
  Prof. Richard Henderson (Nobel Laureate in Chemistry 2017)
  MRC Laboratory of Molecular Biology, Cambridge, UK
  22nd January, 2018

- 'Remodeling of DNA replication forks- Implications in tumorigenesis and chemoresistance'
  Dr. Arnab Ray Chaudhuri
  Principal Investigator and Group Leader, Dept. of Molecular Genetics, Erasmus University Medical Center, The Netherlands.
  24th January, 2018

- 'Stress Granules mediate metabolic adaptation by directing local remodeling of cellular architecture and resources'
  Dr. Daniel Kaganovich
  Hebrew Univ. of Jerusalem, Israel
  25th January 2018

- 'Gene silencing therapy for human neurodegenerative disease'
  Prof. Don Cleveland
  University of California at San Diego, USA
  1st February 2018

- 'Alternative modes of epithelial polarity'
  Professor Daniel St Johnston
  Director, The Gurdon Institute, University of Cambridge, UK
  1st February 2018

- 'Compartmentalization and stress response'
  Prof. Anne Spang
  Biozentrum, University of Basel, Switzerland
  6th February, 2018

- 'Molecular regulation of normal and malignant stem cells'
  Dr. Michael Clarke
  Stanford Institute for Stem Cell& Regenerative Medicine, Stanford University, USA
  6th February, 2018

- 'ER Membrane Protein Complex: A Transmembrane Stabilizing Factor for a Partially Destabilized Membrane-Penetrating Non-Enveloped Virus'
  Dr. Parikshit Bagchi
  Department of Cell and Developmental Biology, University of Michigan Medical School, USA
  6th February, 2018

- 'Temporal persistence of a primed receptor conformation underlies synergistic interactions in GPCR signaling'
  Dr. Tejas Gupte
  Dept. of Genetics, Cell Biology and Development, University of Minnesota, USA
  8th February, 2018

- 'Creativity & Life in Science'
  Prof. Amit Ghosh
  J.C.Bose Distinguished Professor; Emeritus Scientist; National Institute of Cholera & Enteric Diseases, Kolkata
  8th February, 2018

- 'The curious case of non-junctional E-cadherin – Repurposing Cell Adhesion Receptor for Actomyosin Cortex Regulation'
  Dr. Anup Padmanabhan
  Mechanobiology Institute, National University of Singapore
  12th March, 2018

- 'Shape does matter: Cell-geometry regulates response to TNFa -signaling'
  Dr. Aninda Mitra
  Mechanobiology Institute, National University of Singapore, Singapore &
  FIRC Institute of Molecular Oncology (IFOM), Milan, Italy
  16th March, 2018

Technical Seminars

- 'Quantitative Pathology Solutions for ImmunoHisto Chemistry (IHC)Applications and High Content Analysis for cell-based assays'.
  Research & Innovation seminar by PerkinElmer.
  20th April, 2017

- 'Introduction to the Orbitrap Fusion instrument series – Instrument features and application examples'
  Martin Zeller, Product specialist, CMD Life Science, Thermo Fisher Scientific (Bremen) GmbH
  18th May, 2017

- 'In vivo-like, physiological conditions for cell-based assays during live cell imaging'
  Dr. Christian Leibold, Director Global Sales, ibidi GmbH, Germany.
  13th July, 2017

- 'Cryo-EM work flow'
  Ben Lich, Business Development manager, FEI, Eidenhoven, The Netherlands
  6th October, 2017

- 'Quantitative Microscopy of Fixed and Live Cells'
  (with a demonstration of Cytation – Automated Cell Imaging Mutimode Reader)
  Dr. Peter Banks, Scientific Director, Bio Tek Instruments Inc., USA
  7th November 2017

- 'Droplet Digital PCR'
  M/s. Bio-Rad Laboratories (India) Pvt. Ltd., Mumbai
  14th December 2017

- 'Deep Interrogation in Biology and Disease: A Mass Cytometric Approach'
  Boon-Eng The, Field Application Scientist-Proteomics, Fluidigm Corporation
  6th March, 2018

Other Happenings at NCCS

1] Discussions with eminent scientists

Prof. Nobutaka Hirokawa
President, Human Frontier Science Program
22 May, 2017

Nobel Laureate, Richard Henderson
22 January, 2018

2] Exploring collaborations with the Armed Forces Medical College (AFMC), Pune

9th August, 2017

3] 32nd DeLCON Nodal Officer's meeting

23, 24 October, 2017

4] Lecture on fire safety & firefighting demonstration

26th May, 2017

Conducted by Mr. Jhilewar (PMC Fire officer, Aundh); Organized by Mr. Suresh Basutkar (Technical Officer)

5] International Day of Yoga

21st June, 2017



6] Sports

2017: Prize distribution for the Sports events held in 2017 - by judoka, Ms. Prajakta Dhadwe-Patil (15 Aug. 2017)




2018: Sports Events : February 2018.

Prize distribution by Nikhil Kanetkar (national badminton champion) & Shilpa Chillal (national judo champion) : 01 March, 2018



7] Cultural Programme

01 March 2018






MINISTRY OF FINANCE
GOVERNMENT OF INDIA

Economic Survey 2017-2018

08 CHAPTER

Transforming Science and Technology in India

the determinants and life-course of biological pathways and disease. India already has a strong foundation of life science research institutes[2] which together can make significant contributions in this area.

[2] TIFR, IISc, IISERs, Center for Cellular and Molecular and Integrative Biology, National Center for Cell Science,

Kiran Mazumdar Shaw
Researchers @NCCS_Pune develop new mechanism to rejuvenate #stemcells from older donors, making them useful for bone marrow transplantation

Indian scientists develop mechanism to rejuvenate aged stem cells
thehindubusinessline.com

Kiran Mazumdar Shaw

8:04 AM

Tweet

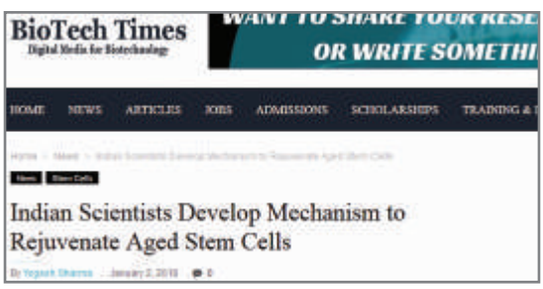
Principal Scientific Adviser, Govt. of...
@PrinSciAdvGoI

Replying to @SarahHyder @madhankrish and 3 others

Some institutions doing an exemplary job in connecting science, society through communication. Time to tip hat. Some are: @India_Alliance @NCBS_Bangalore @NCCS_Pune etc. @iiscbangalore @IITKanpur etc. (do add). Also Sc.journalism alive and well.

20/08/18, 7:10 PM from Sholinganallur, India

5 Retweets 30 Likes



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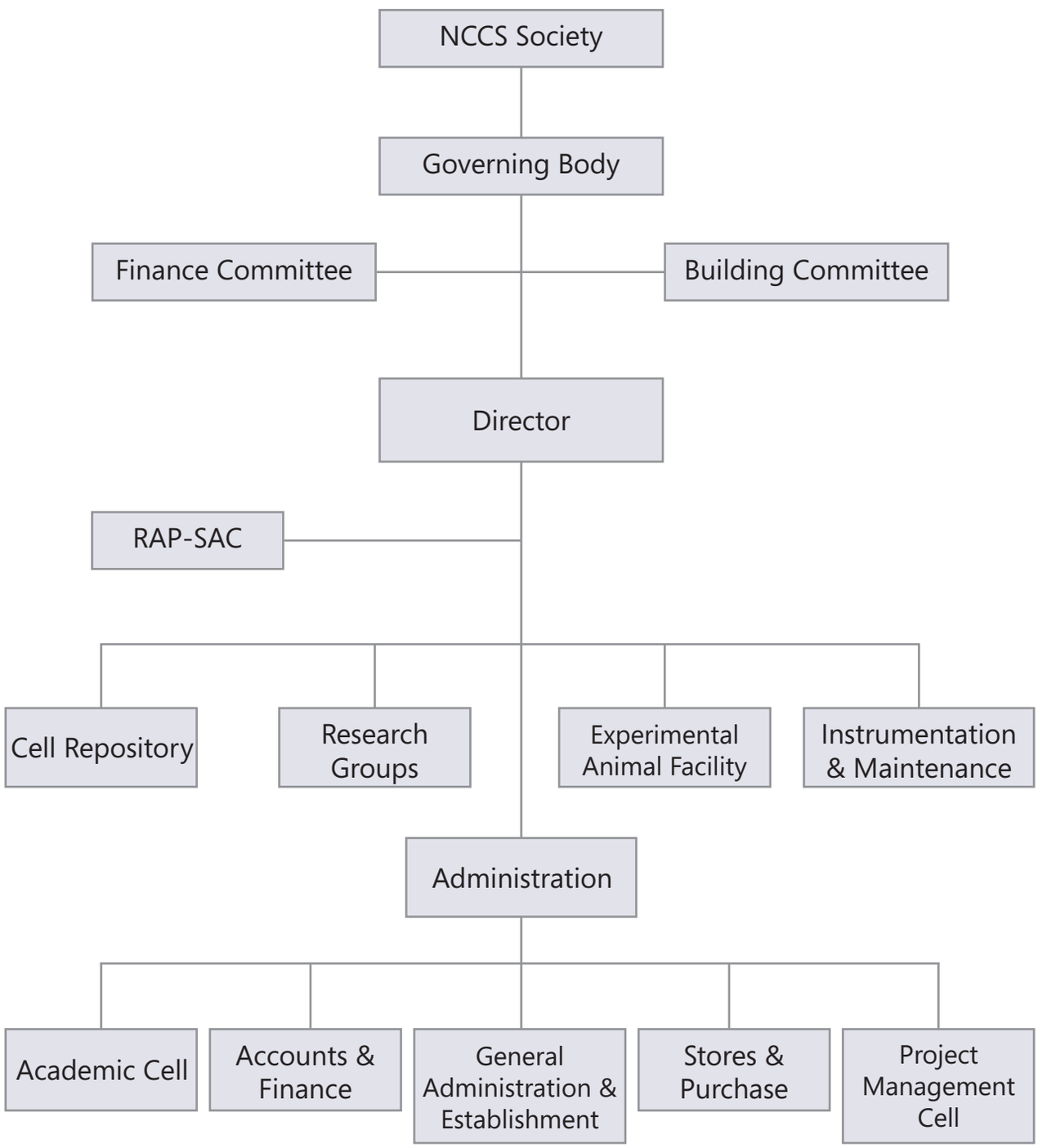
Indian Scientists Develop Mechanism to Rejuvenate Aged Stem Cells

NCCS Organization



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5. **Dr. Sukhanand Sopan Bhosale** — Member
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   Ganeshkhind, Pune - 411 007

10. **Shri. A. C. Pendhari** — Convener
    Tech. Officer 'C' (Maintenance)
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    Ganeshkhind, Pune - 411 007

NCCS Research Area Panels - Scientific Advisory Committee (RAP-SAC) Members

1. **Prof. Partha Majumdar** — Chairman
   Distinguished Professor
   National Institute of Biomedical Genomics,
   Kalyani 741251, West-Bengal, India

2. **Prof. Madan Rao** — Member
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3. **Prof. Upinder Bhalla** — Member
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4. **Dr. Amitabha Mukhopadhyay** — Member
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5. **Prof. R. N. K Bamezai** — Member
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6. **Dr. Debasisa Mohanty** — Member
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7. **Prof. Dipshikha Chakravorthy** — Member
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8. **Prof. Roop Malik** — Member
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9. **Prof. Maneesha S. Inamdar** — Member
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10. **Dr. Vineeta Bal** — Member
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11. **Prof. Rajiv Sarin, MD** — Member
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12. **Dr. B. Ravindran** — Member
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13. **Dr. Malini Sen** — Member
    Principal Scientist
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14. **Prof. Gourisankar Ghosh** — Member
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15. **Dr. Ranjan Sen** Member

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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	:	35
Administrative Staff	:	42
Technical Staff	:	74
Total	:	151

Reservation Policy

NCCS follows the Government of India orders on reservation matters. For direct recruitments, respective rosters are followed, with reservation as follows: 15% for SC, 7.5% for ST and 27% for OBC, on an All India Basis by Open Competition. Liaison officers have been nominated to ensure compliance with the reservation orders issued in favour of SC/ST/OBC. NCCS also follows the Government of India reservation policy for physically handicapped candidates.

Right to Information Act 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. V. S. Shinde, Officer 'C' (Administration) as the CPIO and Dr. Jomon Joseph, Scientist 'F', 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) and Institutional Committee for Stem Cell Research (IC-SCR)

Disciplinary Matters

The Centre follows CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the Centre.

Vigilance Matters

Vigilance reports

Monthly reports on vigilance-related matters at NCCS were sent to the DBT in their prescribed format from April 2017 till March 2018.

Vigilance awareness week 2017

As declared by the CVC, New Delhi, the vigilance awareness week was observed in NCCS from 30th October 2017 to 4th November 2017. The theme this year was "My vision: Corruption-free India." On this occasion, a pledge of integrity was taken by all staff members of NCCS on 30th October. Dr. Manoj Bhat, acting Director, NCCS, dictated the oath. Banners showing the commemoration of the vigilance awareness week and information to help curb corruption were displayed near the NCCS gate and in the auditorium, respectively. A talk by Mr. D. P. Boraste, Additional SP, Anticorruption Bureau, Pune, was organized in the NCCS auditorium on 3rd November, 2017, to sensitize the staff of NCCS on the topic of anticorruption. Dr. Limaye, CVO, NCCS, introduced the speaker to the audience.

Mr. Boraste delivered a very informative talk, wherein he explained the functioning of the anticorruption bureau. The talk

was punctuated with attention-grabbing succinct video clips, through which he very effectively conveyed to the masses the adverse consequences and ill effects of corruption on the society. The clips also demonstrated the indirect ways in which people can demand bribes, and further made people aware of their rights and how they could make a complaint against such people. Mr. Boraste also discussed in detail the ways and means to prevent corruption, and the importance of the contribution of every individual to this process, so that our dream to make India free of corruption could become a reality one day. The talk was followed by a very interactive session of questions and answers. The talk was attended by a large number of staff members. The event concluded with a vote of thanks and presentation of a floral bouquet to Mr. Boraste.

Implementation of the 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 orders of the Government of India to use the Official Language in day-to-day official work.

The Hindi fortnight was celebrated with much enthusiasm by holding various competitions for the staff & students of NCCS, including 'Hindi essay writing', 'general knowledge', 'Hindi handwriting & dictation' and 'Hindi elocution'. During the Hindi fortnight, Shri. Jayant Sahastrabuddhe, Organising Secretary, Vijnana Bharati, was invited to deliver a special talk on the topic titled 'Vivkeanand: Ek Vaidynanik Drashta', which was organized on 11th September, 2017 to commemorate 125 years of Swami Vivekanand's lecture at Chicago. Shri. Sahastrabuddhe enlightened the audience with Swami Vivekanand's philosophy and his scientific views, in Hindi.

Taking into consideration the enthusiastic and overwhelming response received over the last two years, each of these competitions was organized separately for 'Hindi Bhashi' & 'Non-Hindi Bhashi' participants this year as well, with separate cash prizes and certificates awarded to winners from both categories, for each competition. Dr. Rajendra Shrivastav, Chief Manager (OL), Bank of Maharashtra, Pune, graced the Hindi Day function held on 21st September, 2017, as the Chief Guest. On this day, the fifth issue of 'Meemansa' (Hindi patrika) was released at the hands of Dr. Rajendra Shrivastav, Dr. G. C. Mishra (former Director of NCCS), Dr. Shekhar C. Mande (Director, NCCS) and Dr. Shailza Singh (Scientist & Chief Editor-Meemansa).

Shri. D. P. Boraste, Addl. S.P., Anti-Corruption Bureau, Pune delivered a lecture on Vigilance Awareness in Hindi during the Vigilance Awareness Week, with this entire programme also being compered in Hindi by Dr. Lalita Limaye, Scientist & CVO. Shri. Rajendra Prasad Varma, Assistant Director, Hindi Teaching Scheme, Pune, was invited to conduct a workshop on the use of software and apps for typing, translation and dictation in Hindi for use in Official Language on 20th December, 2017. A proposal to hold a scientific conference in Hindi, in association with ARI and CSIR-NCL, Pune, was formally made, and the necessary course of action was initiated in the year 2017-18.

Shri. Jayant Sahastrabuddhe delivering a talk on 'Vivkeanand: Ek Vaidynanik Drashta' - 11th September, 2017

Release of the **fifth issue** of **'Meemansa'** Dr. Rajendra Shrivastav (Chief Manager, Bank of Maharashtra), Dr. G. C. Mishra (former Director of NCCS), Dr. Shekhar C. Mande (Director, NCCS) and Dr. Shailza Singh (Chief Editor-Meemansa) - 21st September, 2017

National Centre for Cell Science
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